# The effects of climate change on decomposition in Dutch peatlands: an exploration of peat origin and land use effects

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**Photography, unless mentioned otherwise:** Maikel Aragon van den Broeke
**Layout and printing:** Ridderprint BV, Ridderkerk, the Netherlands

**ISBN:** 978-94-6299-281-8

This thesis should be cited as: Brouns, K. (2016) The effects of climate change on decomposition in Dutch peatlands: an exploration of peat origin and land use effects. PhD thesis. Utrecht University, Utrecht, the Netherlands

# The effects of climate change on decomposition in Dutch peatlands: an exploration of peat origin and land use effects

De effecten van klimaatverandering op veenafbraak in Nederlandse veengebieden:
een verkenning van de invloeden van veen-origine en landgebruik

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor
aan de Universiteit Utrecht
op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op woensdag 24 februari 2016
des ochtends te 10.30 uur

door

Karlijn Brouns

geboren op 9 augustus 1984 te Dordrecht

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This study was supported by the research program Knowledge for Climate (Kennis voor Klimaat), hotspot 'shallow waters and peat meadow areas' project code HSOV1a.

# Contents

# Chapter 1

# General introduction

## 1.1 Introduction

In wet and acidic conditions, net primary production can exceed the decomposition of organic matter, leading to the formation of peat. Peatlands cover only 3% of the earth's land surface but hold approximately one third of the world's soil organic carbon (450-550 Pg C), which is nearly the same amount as in the atmosphere (Gorham, 1991; Parish et al., 2008). No other terrestrial ecosystem is so efficient at absorbing and storing carbon, year after year, century after century. However, land use change (e.g. reclamation) and climate change can turn this large carbon sink into a carbon source (Laiho, 2006), thereby generating a positive feedback on climate change. Currently, about 3 Pg of carbon are annually emitted from peatlands due to drainage, fires and exploitation. This emission is equivalent to more than 10% of the global fossil fuel emissions (Parish et al., 2008). In the Netherlands, peatland drainage for agriculture causes a net carbon emission from these organic soils (Veenendaal et al., 2007; Coenen et al., 2013) and 1-2 $cm \cdot yr^{-1}$ of soil subsidence (Schothorst, 1977; Van den Akker et al., 2007). Climate change is expected to stimulate peat decomposition and associated subsidence rates in these drained peatlands (Witte et al., 2009).
This Ph.D. dissertation describes a number of experimental studies and a report on stakeholder workshops in case study areas relevant in the context of the consequences of future climate change for drained peatlands in the Netherlands. This research was funded by the National Programme Kennis voor Klimaat (Knowledge for Climate) and carried out in collaboration with other scientific institutions and stakeholder organisations. In this chapter, the research program Kennis voor Klimaat is introduced (§1.2), followed by a description of the history of Dutch peatlands and current environmental challenges in these areas (§1.3). Relevant aspects of the process of peat decomposition are described (§1.4), as well as the effects of expected climate change on this process (§1.5). Finally, the objectives, research questions, hypotheses and outline of this Ph.D. thesis will be given (§1.6).

## 1.2 Kennis voor Klimaat

Kennis voor Klimaat (Knowledge for Climate, 2008-2014) is a large, interdisciplinary research programme initiated and largely financed by the Dutch national government for the development of knowledge and services for adaptation measures to a changing climate in the Netherlands. The research programme focused on eight important societal areas, called hotspots, e.g. the port of Rotterdam, the southwest delta, dry rural sandy areas, shallow waters and peat meadow areas, etc. (Kennis voor Klimaat, 2014). In each hotspot, various scientific studies were performed and their results were integrated and discussed in workshops with local stakeholders, ultimately leading to 'options for regional climate adaptation strategies'. The research presented in this thesis was carried out in the hotspot 'shallow waters and peat meadow areas'. The dilemma regarding the peat meadow

region is that continuing drainage to support current agricultural practices will maintain the characteristic peat meadow landscape, at least for the coming centuries, but will also aggravate peat oxidation and subsidence, which will ultimately result in the complete disappearance of the peat layers. Impeding drainage would most likely lead to the opposite: conservation of peat and carbon storage in newly formed peat, accompanied by the gradual loss of the characteristic agricultural landscape with its intensive dairy farming.
The hotspot 'shallow waters and peat meadow areas' encompassed the researchers from knowledge institutions (Utrecht University (UU), Wageningen University and Research centre (WUR), Institute for Environmental Studies of the Free University Amsterdam (IVM-VU) and Watercycle Research Institute (KWR)) and representatives of stakeholder organisations to build a good collaboration and knowledge transfer. The hotspot has focussed on three regions in the Netherlands, i.e. the peat areas with mostly meadows for dairy farming in the provinces of Noord- and Zuid-Holland, the peat areas in the province of Friesland and the crop fields on peat near Smilde in the province of Drenthe. Workshops have been organised in these areas for exchange of knowledge and opinions among stakeholders, authorities and scientists. These workshops were technically supervised by IVM-VU, specialised in decision support for environmental and spatial management. A mapping device (the touch table) was used to facilitate interactive land use planning. Spatial information on various landscape characteristics as well as models that relate environmental characteristics to agricultural yields are key components of this mapping device (Eikelboom and Janssen, 2013).

## 1.3 History of peatlands in the Netherlands

### 1.3.1 Peat formation

During the last glaciation (ending approximately 11,700 yr BP), a layer of sand with low permeability was gradually deposited on the land surface of the Netherlands. As drainage became more and more impeded, several depressions formed ideal sites for the formation of mires, i.e. wetland ecosystems with peat accumulation. Peat formation starts in wet depressions that fill up with the dead organic matter of submerged and floating vegetation as a result of the slow decay of dead plant remains (Figure 1.1). If the accumulation of organic material at the bottom reduces water depth to less than 2 m, a *Phragmites australis* stand will develop and give rise to a layer of *Phragmites* peat. Species-rich *Carex* spp. communities and *Carex* peat become establishd when the open water has totally filled in with vegetation and peat. In the next stage, forests and forest peat develop with *Betula* or *Alnus* spp. So far, the mire system has been affected by surface water or groundwater a stage commonly indicated as a fen. With ongoing succession, the mire system becomes more and more isolated from water flow other than rainfall and develops into a bog. *Sphagnum* spp. communities become progressively dominant and, given enough time, the bog can expand sideways, overgrowing the surrounding area (Zagwijn, 1986; Pons, 1992). The process of peat formation can be interrupted by sea or river water incursions. These events lead to the deposition of a layer of sand or clay on top of the peat, often followed by renewed peat formation. This has led to a pattern of peat layers reflecting the succession that has taken place, with local interruptions by layers of mineral overbank, flood basin or marine deposits. Eventually, a peat cover of up to several meters above sea level has covered 40% of the land surface in the Netherlands (TNO, 2007).

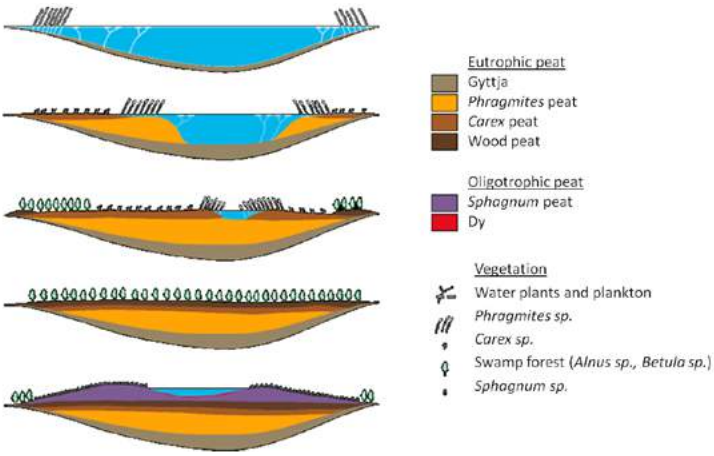


**Figure 1.1:** The process of peat formation (Berendsen, 2005).

### 1.3.2 Reclamation

After millennia of peat formation, the tide for the Dutch peatlands changed. First, natural threats like changes in river water discharge and seawater incursions caused a reduction in the peat surface area from 2500 years BP. From the fifth century onwards, peatlands were reclaimed for extensive grazing, followed by arable farming. In these reclaimed areas, peat accumulation stopped and soils started to subside as peat decomposition exceeded the accumulation of dead organic matter. The rise in the human population in the ninth century gave landlords the opportunity to increase their incomes through the reclamation of mires, so, peat cutting for fuel or salt extraction started to increase in scale. Drainage was improved by digging ditches, while the vegetation changed from typical bog communities dominated by *Sphagnum* spp. into stands with heather and moor grasses, which enabled more extensive sheep grazing. Burning was applied at local scale as a measure to remove undesirable vegetation and to release nutrients for agricultural production. Nutrients were also released from the mineralising peat soils and the drained peatlands enabled significant crop production of mainly rye, oats and barley. However, subsidence due to drainage and consequent wetter conditions hampered agricultural production after prolonged agricultural land use. From the fifteenth century onwards, drainage improved as the use of windmills became common practice. The windmills pumped the water from the polders upward into the outlet reservoir ('boezem'). This improved drainage once more boosted agricultural production. Consequently, subsidence accelerated distinctly (Borger, 1992; Pons, 1992). Figure 1.2 represents a time course for the subsiding soil surface in relation to drainage methods in the Dutch peatlands during the past millennium.

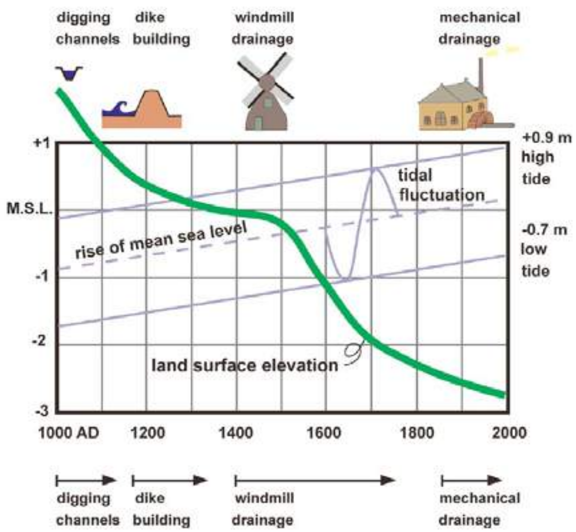


**Figure 1.2:** History of subsidence and sea level rise since 1000 AD (Van de Ven, 1993).

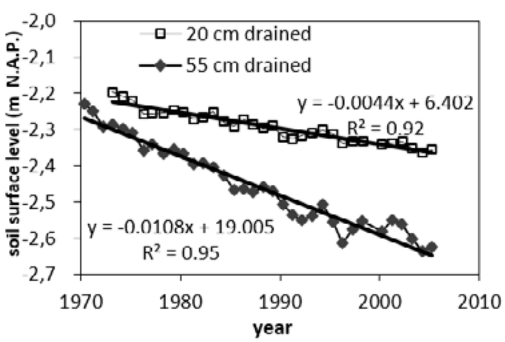


**Figure 1.3:** Soil surface level in relation to drainage depth, results of long-term monitoring in Zegveld (Van den Akker et al., 2007).

### 1.3.3 Subsidence

Aeration of peat causes soil subsidence, which is a combination of shrinkage, compression and oxidation. The shrinkage process is a reduction in volume caused by the withdrawal of water from the upper soil layer. The loss of the buoyant force of water in the upper layers also leads to the compression of deeper layers. The microbial oxidation of soil organic matter under oxic conditions leads to a major peat loss after drainage. In fact, up to 60-85% of subsidence can be attributed to decomposition (Schothorst, 1977). Figure 1.3 presents the soil surface level in two dairy meadows in Zegveld, the Netherlands. There is a highly significant correlation between drainage depth and subsidence rates. In Zegveld, average subsidence amounts to 10.8 $mm \cdot yr^{-1}$ and 4.4 $mm \cdot yr^{-1}$ in parcels with a ditch water level 55 cm and 20 cm below soil surface, respectively (Van den Akker et al., 2007).

### 1.3.4 Current situation

The surface area of peat soils, which originally amounted to 40% of the area of the Netherlands, has been reduced to less than 8% in 2007 (TNO, 2007). In the Netherlands, peat soils are defined as soils with more than 40 cm of material with ≥ 15% organic matter within the upper 80 cm (Stiboka, 1986). Most peatlands are still being drained to facilitate intensive agriculture. In the western provinces, the history of reclamation has created a characteristic landscape with long and narrow peat meadows intersected with ditches. Here, peat layers are still relatively thick, locally >10 m, while they are thinner than 2-3 m in the northern peat areas (Friesland and Drenthe) (Rienks et al., 2002). In the western and northern peat meadow areas, intensive dairy farming is the main land use, except for some relatively thin peat soils in Drenthe which are used for the production of sugar beets, maize and flower bulbs.

Most Dutch peat meadow areas are located in polders with controlled water levels, which are kept low in winter and high in summer to facilitate farming (Lamers et al., 2002). Most of these areas are currently located below sea level because of the combination of continuous subsidence and sea level rise; this complicates the removal of excessive water in the polder areas by ever more intensive pumping. Besides subsidence, there are associated problems like greenhouse gas emissions and poor surface water quality due to leaching of nutrients and dissolved organic carbon (Van Beek et al., 2007; Van den Akker et al., 2007; Vermaat and Hellmann, 2009; Querner et al., 2012). Apart from these management issues, the loss of this characteristic Dutch landscape, e.g. in the Groene Hart, a rural area in the Randstad surrounded by large cities as Amsterdam, Rotterdam and The Hague, is a main concern. It has been estimated that, under current conditions, virtually all peat will disappear from the Netherlands in the next 500 years, while the major part of the peat will already have disappeared after 200 years (Rienks et al., 2002).

## 1.4 Peat decomposition

The large carbon stock in peat soils provides a risk, because it can be converted to atmospheric carbon dioxide relatively quickly in a situation of drainage (Laiho, 2006). Estimations of the $CO_2$ production rates of drained peat meadows in temperate regions vary mostly between 2000-3000 kg $C \cdot ha^{-1} \cdot yr^{-1}$ (Best and Jacobs, 1997; Jacobs et al., 2007), and some estimates even exceed 5000 kg $C \cdot ha^{-1} \cdot yr^{-1}$ (Van den Akker et al., 2008; Joosten, 2010). To put this in perspective: in the Netherlands there are about 300,000 ha of peat soils, while a $CO_2$ emission of 3000 kg $C \cdot ha^{-1} \cdot yr^{-1}$ equals 1% of the $CO_2$ emission caused by road traffic in the Netherlands (Van Kekem, 2004; CBS et al., 2014).

Decomposition is the process of the transformation of organic material into inorganic compounds such as $CO_2$, $H_2O$, $NH_4^+$, $PO_4^{3-}$ and $SO_4^{2-}$. The rate of decomposition is controlled by biotic factors such as the quality of the organic material and the presence and composition of soil organisms (detritivores, bacteria, fungi, archaea) and by abiotic factors such as climate, pH, oxygen availability, hydrology and pore-water quality (Limpens et al., 2008).

### 1.4.1 Soil microbial enzymes

Decomposition of dead organic matter (DOM) often begins with physical fragmentation by detritivores. Thereupon, exo-enzymes, major drivers of decomposition and nutrient cycling in peat soils are excreted by soil heterotrophic microorganisms and start to hydrolyse and oxidise complex organic compounds (Sinsabaugh, 2010; Arnosti et al., 2014). These enzymes convert complex organic matter to simpler products such as glucose, amino acids and phosphate, and ultimately to carbon dioxide, water and mineral N species. Measuring potential exo-enzyme activities in soil samples is a way to study the functioning of soil microbes involved in the carbon, nitrogen and phosphorus cycling (Caldwell, 2005; Sinsabaugh and Follstad Shah, 2012; Arnosti et al., 2014).

Phenolic compounds are secondary metabolites produced by plants that consist of at least one aromatic ring bearing one (phenols) or more (polyphenols) hydroxyl substituents. Examples of phenolic compounds are lignin, tannin, cutin and humic substances (Sinsabaugh and Follstad Shah, 2012). Phenolic compounds enter the soil as leachates from above- and belowground plant parts and litter (Hättenschwiler and Vitousek, 2000). They have been indicated to inhibit enzyme activities, increase oxidative stress, act as antibiotics and be involved in mechanisms of plant defence against herbivores (Hättenschwiler and Vitousek, 2000; Sinsabaugh, 2010).

Phenolic compounds reduce the decomposition rates by inhibiting hydrolytic enzymes such as sulphatase, phosphatase and β-glucosidase; presumably, because these enzymes are inactivated when phenolic compounds bind to them (Wetzel, 1992; Verhoeven and Liefveld, 1997; Freeman et al., 2001; Sinsabaugh, 2010; Fenner and Freeman, 2011). Fungi and bacteria produce the enzyme phenol oxidase (POX) to reduce the toxicity of phenolic compounds and gain carbon and nutrients from its disintegration (Sinsabaugh, 2010). The 'enzymic latch theory' predicts that oxygen intrusion into peat soil, e.g. during drought, is followed by the degradation of phenolic compounds by POX, so that the phenolic inhibitors of hydrolytic enzymes are removed, which will enable peat decomposition to occur (Freeman et al., 2001; Fenner and Freeman, 2011). According to this enzymic latch theory, the enzyme phenol oxidase (POX) is considered an important regulator of peat decomposition as it can release a 'latch' on decomposition in oxic conditions (Freeman et al., 2001; Freeman et al., 2004; Sinsabaugh, 2010; Fenner and Freeman, 2011).

The dynamics of phenolic compounds and POX have not been completely unravelled yet. It is clear that this enzyme has a higher activity in oxic conditions, although anaerobic degradation of phenolic compounds and anaerobic POX activity has also been found[1] (Elder and Kelly, 1994; Freeman et al., 2001; Freeman et al., 2004; Fenner and Freeman, 2011). In literature, negative as well as positive correlations between POX activity and soluble phenol concentration were found (Pind et al., 1994; Williams et al., 2000; Toberman et al., 2010). The phenolic compounds (both condensed and soluble) form a pool of substrates for the enzyme. It is assumed that POX activity relates to the inward and outward fluxes from the soluble pool, rather than the size of this pool, as indicated by Sinsabaugh (2010). POX activity is generally higher at a higher pH (above 6) (Pind et al., 1994; Toberman et al., 2010), partly explaining the low POX activity generally observed in the acidic conditions in peat soils (Pind et al., 1994).

### 1.4.2 Terminal electron acceptor

The decomposition process ends with mineralisation of simple organic compounds to gases and nutrients via microbial respiration. This last step in the overall decomposition process requires the flow of electrons from organic matter (electron donor) to one of several terminal electron acceptors (TEAs). Oxygen functions as the TEA in oxic conditions. Oxygen diffuses 10,000 times faster through air than through water (Wilke and Chang, 1955), so the peat layers beneath the water table are mostly anoxic. Alternative TEAs become active under such suboxic or anoxic conditions. In the order of declining thermodynamic yields these alternative TEAs are: nitrate ($NO_3^-$), manganese ($Mn^{4+}$), ferric iron ($Fe^{3+}$), sulphate ($SO_4^{2-}$), and ultimately $CO_2$ which leads to $CH_4$ production (Rydin and Jeglum, 2006). Apart from these inorganic components, humic substances can function as TEA as well (Keller et al., 2009; Klüpfel et al., 2014). Adding electron acceptors such as nitrate or sulphate to anoxic peat soils can lead to a shift from methanogenesis, an anaerobic decomposition process with $CO_2$ as TEA, to denitrification or sulphate reduction, which are more energy-

1 The terms 'oxic' and 'anoxic' refer to conditions: with or without oxygen. 'Aerobic' and 'anaerobic' are used to describe a process, such as aerobic decomposition or respiration.

efficient decomposition processes than methanogenesis. An increased TEA availability can therefore result in an accelerated decomposition of peat soils under water-saturated conditions (Capone and Kiene, 1988; Canavan et al., 2006).

### 1.4.3 Peat origin

In this Ph.D. dissertation, the distinction is made between peat that originates from minerotrophic fen peatlands, consisting of the remains of *Carex* spp., *Phragmites* and/or wood (usually *Alnus* and/or *Betula* spp.), and peat formed in ombrotrophic bogs, with a large proportion of *Sphagnum*-derived material. These types of peat are two of the major classes that can currently still be identified in peat meadow soils in the Netherlands (Figure 1.4). The classification of peatlands into fens and bogs is based on their hydrology: minerotrophic fens receive base-rich water that has been in contact with mineral soils, whereas at least the upper layers of ombrotrophic bogs only receive water from precipitation (Table 1.1).

*Sphagnum* spp. are renowned for their adaptations that enable them to grow in nutrient-poor conditions, moreover, they create a habitat in which few vascular plant species can exist. Because of low nutrient availability, *Sphagnum* spp. produce plant material with a very low nitrogen content compared to other plants (Asada et al., 2005) and these mosses efficiently re-use the nutrients by reallocation from the senescent sphagnum remains to the capitulum, leaving poorly decomposed peat layers with high C/N ratios to build up peat. Moreover, *Sphagnum* spp. very effectively intercept nutrients from the atmosphere or the soil pore water (Schwintzer, 1983; Van Breemen, 1995; Jonasson and Shaver, 1999). The cell wall of *Sphagnum* spp. contain polymers of Sphagnum acid, which give it structural strength. These polymers make the (hemi)cellulose in dead peat mosses virtually inaccessible for microbial decomposition.

The differences in botanical composition and environmental conditions during peat formation have led to fen and bog peat substrates that strongly differ in their chemical quality. The specific chemical characteristics of *Sphagnum* spp. organic matter cause low decomposition rates. For example, the decomposition of *Sphagnum*-derived organic material is approximately 10 times slower than the decomposition of vascular-plant-derived organic material, such as *Carex* spp. litter (Scheffer et al., 2001). The low C:N ratio and phenolic compounds hamper the decomposition process of *Sphagnum* spp. organic material (Aerts and Ludwig, 1997). Additionally, poly-uronic acids in *Spahgnum* cell walls hamper microbial growth by lowering the pH, also the activity of phenol oxidase is limited at a low pH (Pind et al., 1994; Børsheim et al., 2001; Stalheim et al., 2009). It has been demonstrated that the presence of *Sphagnum* material, via the presence of the phenolic compounds, restricts the decomposition of other types of organic matter (Verhoeven and Toth, 1995; Verhoeven and Liefveld, 1997).

1

**Table 1.1:** Peat type classification, peat formation characteristics.

| Peat origin (thesis terminology) | Trophic status during peat formation | Hydrology, (origin of water) | Vegetation composition during peat formation |
|---|---|---|---|
| **Fen peat** | Eutrophic | Minerotrophic, (groundwater fed) | *Carex* spp., *Phragmites* spp., *Alnus* spp., *Betula* spp. |
| **Bog peat** | Oligotrophic | Ombrotrophic (rainwater fed) | *Sphagnum* spp., dwarf shrubs, grasses |

### 1.4.4 Land use

Natural peatlands with high water tables are a sink of carbon, as primary production is not exported and exceeds the decomposition of organic matter; whereas, current agricultural practices on peat soils cause a net release of carbon because decomposition exceeds the build up of DOM (Laiho, 2006). Agricultural land use strongly affects the cycling of carbon and nutrients in peatlands, because of drainage, fertilisation and the grazing or harvesting of aboveground plant biomass. Consequently, the soil surface level is subsiding. Oxygenation, which is a consequence of drainage, stimulates $CO_2$ production while $CH_4$ emission is slowed down (Van den Bos, 2003). Although $CH_4$ is a more powerful greenhouse gas than $CO_2$, which is reflected in a 28 times higher global warming potential at a 100-year timeframe, the drainage of peatlands enforces the $CO_2$ emission to such an extent that the net effect is a substantial contribution to global radiative forcing (IPCC, 2013).

*Roles of nutrients in peat decomposition*

Agricultural inputs and outputs rather than peat decomposition or water fluxes dominate the annual N and P budgets in agriculturally used peatlands in the Netherlands; the input of nitrogen and phosphorus by fertiliser application is a factor 2-4 larger than the nutrient release from the degrading peat. Modelling studies have revealed that the input of nitrogen equals the output in typical Dutch peat polders, whereas, there is a net surplus of phosphorus, so that 23% of the phosphorus input accumulates in Dutch peat soils (7 kg $P \cdot ha^{-1} \cdot yr^{-1}$) (Vermaat and Hellmann, 2009). In nature reserves, on the other hand, the input of nutrients is restricted to the atmospheric deposition and inlet water, while also the nutrient release from the soils is lower because of the higher water tables.

There are two hypotheses regarding the role of nutrient availability in organic matter decomposition. The first one is based on the stoichiometric theory of substrates and microbial demands for resources. According to this theory, decay rates are maximal when the C, N and P supplies match the demand of the microbes. Therefore, ecosystems will store less carbon when increasing atmospheric nitrogen availability reduces nitrogen limitation on organic matter degradation (Mack et al., 2004). To illustrate, in a 20-year fertilisation study in an Alaskan tundra system decomposition rates had increased substantially stronger than primary production, resulting in a distinct decline in the size of the SOC pool (Mack et al., 2004).

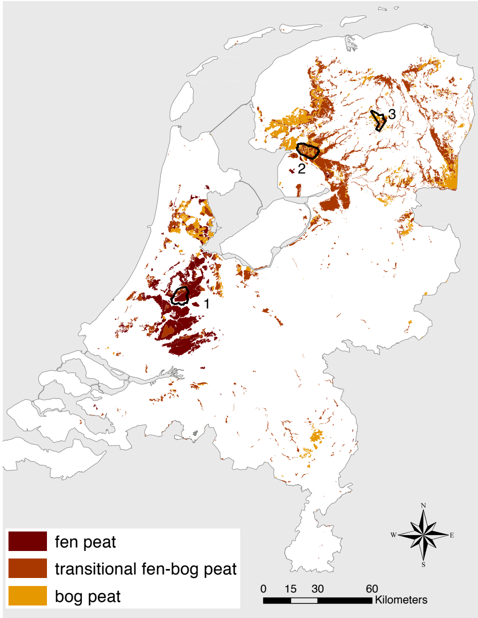


**Figure 1.4:** Peat types in the Netherlands (De Vries and Brouwer, 2005), with main research locations indicated. 1=fen peat agriculture and nature, 2 = bog peat agriculture, 3 = bog peat nature.

The second theory on the roles of nutrients in the regulation of organic matter decomposition rates, the so-called 'microbial nitrogen mining hypothesis' is directly opposed to the stoichiometric decomposition theory. According to the nitrogen mining hypothesis, microorganisms degrade recalcitrant compounds in order to acquire nitrogen. This process is often coupled to the degradation of labile carbon to gain energy. This hypothesis predicts that carbon storage increases with greater nitrogen availability as mining of recalcitrant organic matter for N is suppressed (Berg and McClaugherty, 2003; Moorhead and Sinsabaugh, 2006; Craine et al., 2007; Chen et al., 2014). In accordance with the microbial mining theory and the recalcitrant nature of peat material, cores of *Carex* spp. peat treated with nitrogen showed lower decomposition rates than unfertilised cores (Aerts and Toet, 1997). Summarising, the microbial nitrogen mining theory predicts declines in degradation of organic matter with N addition, while the basic stoichiometric decomposition theory predicts increases in degradation with N addition if the microbial demands for N had not been met before fertilisation.

Contrary to nitrogen, the role of phosphorus in organic matter decay is not linked to mining. Aerts and De Caluwe (1997) concluded that initial (3-20 months) *Carex* spp. litter decay in peatlands was controlled by P-related parameters. Hence, there is no evidence for phosphorus mining in the first phase of organic matter decomposition as P fertilisation increases decomposition. In soils, containing older organic matter than the litter experiment discussed above, phosphorus addition to soils with low P availability showed the greatest increases in respiration with P addition, so that there is no indication for phosphorus mining, in the initial phases as well as in later phases of decomposition (Craine et al., 2010).

*Biodiversity*

Plant biodiversity is generally much lower in agricultural meadows and fields than in nature reserves. These differences in species composition and biodiversity affect soil microorganisms. Each plant species has a unique contribution to the functioning of the belowground system because of the particular quality of its litter, the presence of microbial inhibitors and root exudates (Eisenhauer et al., 2010). Hence, plant biodiversity will directly and indirectly affect peat decomposition rates. Table 1.2 summarises the main differences between fen peat and bog peat in nature reserves and under agricultural use with respect to decomposition.

**Table 1.2:** Classification of peat soils in the Netherlands.

| | | **Characteristics** | **Peat origin** | |
|---|---|---|---|---|
| | | | **Fen peat** | **Bog peat** |
| **Land use** | **Nature** | GWT | I | I |
| | | Management | Low | None |
| | | Fertiliser | None | None |
| | | Nitrogen content | Low | Low |
| | | Phosphorus content | Low | Low |
| | | Phenolic compounds | Intermediate | high |
| | | Carbon input | Belowground DOM and part of the aboveground DOM (mainly grasses and *Phragmites* spp.).<br>Root exudes<br>Leachates | Belowground and aboveground DOM of mainly mosses, dwarf shrubs and grasses.<br>Root exudes<br>Leachates |
| | | pH | Low-Intermediate | Low |
| | **Agriculture** | GWT | II/ III | II/ III |
| | | Management | High | High |
| | | Fertiliser | Manure | Manure |
| | | Nitrogen content | High | High |
| | | Phosphorus content | High | High |
| | | Phenolic compounds | Low | Intermediate |
| | | Carbon input | Manure<br>Mainly belowground DOM, root exudates and leachates, primarily of grasses such as *Lolium perenne* | Manure<br>Mainly belowground DOM, root exudates and leachates, primarily of grasses such as *Lolium perenne* |
| | | pH | High | Low-intermediate |

# 1.5 Climate change

## 1.5.1 Climate change scenarios

The Royal Netherlands Meteorological Institute (KNMI) recently published their updated climate scenarios (KNMI, 2014). These scenarios predict higher temperatures, accelerating sea level rise, wetter winters, more intense showers and higher chances on drier summers in the near future compared to the past decades. The KNMI'14 scenarios are the four combinations of two possible values for the global temperature increase, 'Moderate' (G) and 'Warm' (W), and two possible changes in the air circulation pattern, 'Low value' and 'High value' (indicated with the letters $_L$ and $_H$ in subscription, Figure 1.5).
The scenarios predict an increase of the mean temperature between 1.3 °C ($G_L$ and $G_H$ scenarios) and 3.7 °C ($W_L$ and $W_H$ scenarios) in the year 2085 compared to the climate in the reference period 1981-2010. Especially the warmest days in summer and the coldest days in winter are predicted to show higher temperatures. With respect to precipitation, the Netherlands is situated between the patterns seen in northern and southern Europe. It is expected that the atmospheric circulation will continue to change in the 21$^{st}$ century, and that precipitation patterns in the Netherlands will become more similar to those in the south-western part of the continent. Consequently, precipitation quantity is expected to increase in winter and the frequency and length of summer droughts will increase as well. In the dry summers that are foreseen, peak precipitation events will increase while total precipitation amounts will decrease and evaporation will increase (Van Dorland and Jansen, 2007; KNMI, 2014).

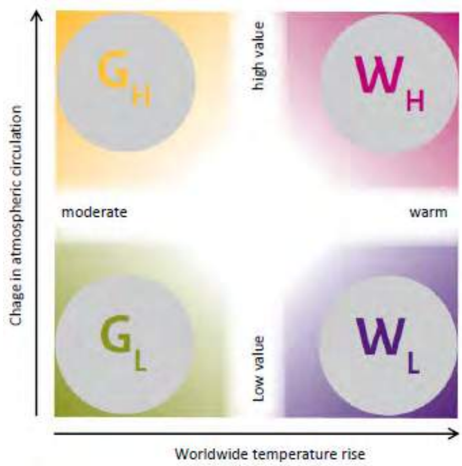


**Figure 1.5:** 2014 KNMI climate change scenarios (KNMI, 2014).

## 1.5.2 Possible effects of climate change on peat decomposition

*Temperature*

The predicted climate change could aggravate the environmental threats in Dutch peatlands (Witte et al., 2009). Higher temperatures will stimulate decomposition processes, as has been shown by laboratory incubations, model studies and current subsidence rates in peat areas throughout the world (Andriesse, 1988; Davidson and Janssens, 2006; Berglund et al., 2008; Dorrepaal et al., 2009). Figure 1.6 presents subsidence rates in relation to groundwater levels in different areas in the world.

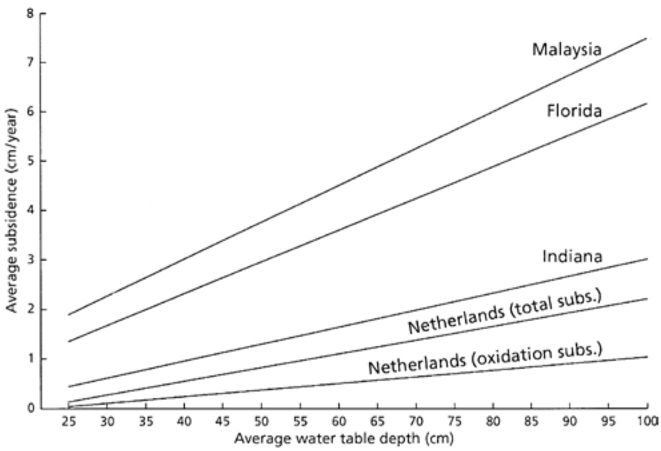


**Figure 1.6:** Subsidence rate versus groundwater level relationships for different areas in the world (Wösten et al., 1997).

The $Q_{10}$ represents the effect of a temperature change of 10 °C on the process rates, in this case the rate of degradation of organic matter. When looking separately at labile and more resistant organic matter (OM) it has been found that the $Q_{10}$ of labile OM is just around 2.2, whereas it is around 3.5 for organic matter that is more recalcitrant. Thus, temperature sensitivity increases with increasing molecular complexity of the substrate (Davidson and Janssens, 2006; Conant et al., 2008). Besides the effect of substrate quality, $Q_{10}$ varies with the temperature range. It has been found that $Q_{10}$ is higher in areas with a low temperature range (just above freezing point) than in the range between 13 and 25 °C (Berglund et al., 2008; Dorrepaal et al., 2009). These studies lead to the expectation that the higher temperatures associated with future climate change will accelerate decomposition of more recalcitrant organic matter especially in the Northern hemisphere, resulting in a distinctly faster subsidence of drained peat meadows.

*Summer drought*

Another predicted consequence of future climate change is an increase in frequency and severity of droughts at high latitudes, where much of the world's peat resides (IPCC, 2007a; KNMI, 2014). In the Netherlands, we expect that water tables in drained peat meadows will drop when evaporation exceeds precipitation, while the lateral flow of water from the ditches towards the meadow soil is too slow in peat soils to make up for these water losses due to the low hydraulic conductivity. Consequently, the water table will drop to the point that deeper permanently anoxic peat layers will become exposed to oxygen. The process of further decay of ancient peat that was stored below the water table after the onset of oxic conditions has been called secondary decomposition (Tipping, 1995). During such episodes of oxic conditions, phenolic compounds will start to decompose as a result of increased POX activity. After reestablishment of wet, anoxic conditions, the reduced content of phenolic compounds might result in higher anoxic decomposition than before the dry period (Fenner and Freeman, 2011).

*Salinisation*

During dry summers, surface water originating from rivers or lakes is supplied to peat areas to prevent drying out of the peat soils and limit enhanced decomposition due to more aerated conditions. However, during prolonged summer droughts, which are expected to occur more frequently with climate change, the river water has a poor quality and may become slightly brackish because of saltwater intrusion and evaporation (Satijn and Leenen, 2009). Supplying this water (inlet water) to peat areas to prevent peat desiccation will have the adverse effect of peat salinisation. Apart from surface water salinisation, groundwater is also prone to become more saline due to climate change. Due to water deficiencies in summer and other causes such as drainage, subsidence and sea level rise, the upward groundwater seepage pressure increases relative to the downward pressure of surface waters and superficial aquifers. Some peat areas are subject to brackish seepage as groundwater is in contact with marine sediments (De Louw et al., 2011).

Although peat areas had locally been influenced by brackish water during their formation (Bakker and Van Smeerdijk, 1982), questions arise about the effects of the recent summer salinisation on peat decomposition and mineralisation under anoxic conditions (Lamers et al., 1998; Smolders et al., 2006). When studying salinisation, the ionic salt composition is of primary importance. Chloride salts are expected to hamper peat degradation whereas the addition of sulphate might stimulate anaerobic decomposition as sulphate can be used as a TEA in anoxic conditions.

## 1.6 Outline of this thesis

### 1.6.1 Objectives

Peat meadows and associated shallow waters (e.g. lakes and ditches) encompass a major part of the Netherlands. Regional and local governments are under pressure to come up with adaptive management strategies to cope with the stresses posed by climate change on basic environmental requirements for agriculture, drinking water production and nature protection. Several major problems are foreseen in the peat meadow regions as a result of the expected changes in climate. More rapid soil subsidence due to accelerated peat oxidation associated with higher temperature, increased summer droughts and higher salinity are to be expected. Peat oxidation and high precipitation peaks might also lead to higher nutrient loading of surface waters. Aggravation of eutrophication problems with algal and cyanobacteria blooms is predicted with associated human health risks (Kosten, 2011). This study aims to understand the effects of different climate change drivers on decomposition of peat from different botanical origin and land use history in the Netherlands. Results from this study will contribute to the formation of new climate adaptation strategies.

### 1.6.2 Research questions and hypotheses

Based on the knowledge gaps identified in Chapter 1, the main research question of this thesis is:

**What are the effects of climate change on decomposition of peat in the Netherlands and what is the influence of peat origin and land use in the response to climate change?**

This main research question was subdivided into several more detailed questions:

- What are the effects of oxygenation periods of different duration on the decomposition and mineralisation of anoxic peat layers that are prone to be aerated during summer droughts?
- Do vertical profiles of phenolic compounds and potential phenol oxidase activity reflect long-term water levels?
- What is the effect of salinisation on anaerobic and aerobic decomposition of peat in the Netherlands?
- What are the effects of peat origin and land use on decomposition and microbial activity, respiration dynamics and exo-enzyme activities?
- What is the effect of climate change on the spatial distribution of predicted subsidence rates in Dutch peat areas?
- What are the effects of various adaptation measures on soil subsidence rates?

Overall, it is hypothesised here that the expected summer droughts result in a further amplification of carbon dioxide release from drained peatlands in the Netherlands, which will then function as a positive feedback to global warming. Furthermore, it is expected that salinisation will hamper aerobic decomposition, whilst the addition of sulphate salts might facilitate anaerobic decomposition. As discussed in §1.4.3 and §1.4.4, peat soils in the Netherlands are not uniform in their characteristics. It is expected that decomposition characteristics of peat soils are affected by peat origin (history of development) and the current land use. Bog peat is generally more resistant to degradation than fen peat, due to the presence of phenolic compounds and more acidic conditions. Drainage intensity related to land use affects the decomposition characteristics of peat in the Netherlands through the degree to which the peat is being oxygenated. It is expected that phenolic compound concentrations are lower in deeply drained peatlands. As the presence of phenolic compounds is linked to a latch on decomposition, it is expected that the aerobic breakdown of the phenolic compounds will release this latch and result in an amplified anaerobic peat decomposition rate. In addition, $Q_{10}$ values are higher for aerobic decomposition than for anaerobic decomposition (Szafranet-Nakonieczna and Stêpniewska, 2014), so that a combination of deeper drainage and rising temperatures will lead to even stronger increases in peat degradation rates. In short, it is hypothesised that peat origin and land use are strong determinants explaining the future fate of the Dutch peat landscape.

### 1.6.3 Outline

After the general introduction of the study in **Chapter 1**, **Chapter 2** focuses on the effect of summer droughts in peat areas. More specifically, with the experimental work presented, insight was gained on the oxidation of deep peat layers when they become exposed to air for the first time after many centuries of complete water saturation. This process has been named secondary decomposition (Tipping, 1995). The effects of oxygenation were studied in controlled conditions in an incubation study. The setup of the study took into account the effects of peat origin (fen peat vs. bog peat) and land use (agriculture vs. nature reserve). The peat samples were exposed to oxygen for 0-8 weeks. $CO_2$ and $CH_4$ production as well as soluble and condensed phenolic compounds and extractable nutrients were measured.

In **Chapter 3**, vertical profiles of peat soils under agricultural and natural land use are explored, again paying attention to the differences associated with peat origin and land use. In order to gain more insight into the dynamics of phenolic compounds and the enzyme phenol oxidase (POX), the concentrations of soluble and condensed phenolic compounds, together with nutrient concentrations were measured.

**Chapter 4** presents the results of a study on the effects of salinisation on decomposition and nutrient release. The effects of salinisation on decomposition and mineralisation of peat samples were studied in an incubation experiment. Samples from deep, anoxic peat layers were incubated in anoxic conditions and samples from superficial peat layers in oxic conditions. This set-up allowed differentiation between the effects of salinisation on deep and superficial peat samples.

The modifying effects of peat origin and land use were further explored by measuring soil respiration after nitrogen and/or glucose (energy) additions to peat samples in **Chapter 5**. Furthermore, total and glucose-responsive microbial biomass and exo-enzyme activities were determined to explore the effects on peat decomposition.

The results of the experimental work presented in this study, together with literature data, were used to model subsidence rates in several Dutch peat areas. **Chapter 6** presents the main results of stakeholder workshops that were organised in Frisian peat meadow areas within the hotspot 'shallow waters and peat meadow areas' of the research program 'Kennis voor Klimaat'. This chapter describes how expert knowledge on peat subsidence has been implemented in a spatially explicit decision support system, the 'touch table' (Eikelboom and Janssen, 2013), in the context of a new policy plan for the peat meadows developed by the province of Friesland. The effects of climate change on subsidence rates were explored as well as various adaptation measures. In collaboration with the provincial government and the water board of Friesland, the Netherlands, three study areas were selected in this province, together covering the range of variation in types of peat meadows (peat origin, thickness of peat layer, land use, ditch water level management). These study areas are examples of polders with a peat soil with clay cover, a thick peat layer (> 1.50 m) and a thin peat layer (< 1 m), respectively.

**Chapter 7** provides a general discussion of the results presented in this thesis from a scientific and a practice-orientated point of view. First, the effects of climate change on peat decomposition are discussed, followed by an evaluation of peat origin and land use effects on decomposition. This chapter ends with suggestions for further research, management recommendations and conclusions.

# Chapter 2

# Short period of oxygenation releases latch on peat decomposition

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*Science of the Total Environment 481 (2014) 61–68*

## Abstract

Extreme summer droughts are expected to occur more often in the future in NW Europe due to climate change. These droughts might accelerate the rate of peat oxidation in drained peat areas, with impacts on soil subsidence, GHG emission and water quality. This study aimed at providing more insight in the oxidation of deep peat layers that had not previously been exposed to air, the so-called secondary decomposition. We incubated four types of peat, i.e. fen peat and bog peat, sampled from permanently anoxic peat layers from nature reserves and agricultural peat meadows. Peat samples were incubated for thirteen weeks under anoxic conditions, but were exposed to air for one to eight weeks during that period. The production of $CO_2$ and $CH_4$ was quantified as a proxy for decomposition; concentrations of soluble nutrients and phenolic compounds were also measured. The results showed that oxygenation led to a steep increase in the rate of decomposition, indicated by higher carbon loss rates during and after oxygenation compared to non-oxygenated samples. Carbon loss rates increased both in fen peat (agricultural area: 352%, nature reserve: 182%) and in bog peat (83% and 159% respectively). Most peat samples investigated showed higher post-oxygenation $CO_2$ and/or $CH_4$ production compared to the anoxic pre-oxygenation period. This indicates that oxygenation stimulates decomposition, even after anoxic conditions have returned. Contrary to the enzymic latch theory, no effects of oxygenation on the concentrations of soluble or condensed phenolic compounds were detected. Soluble nutrient concentrations did not change due to oxygenation either. Noteworthy is the occurrence of pyrite mineralisation and associated acidification in fen peat. To conclude, low summer water levels, for example due to climate change, should be avoided in order to limit exceptionally high decomposition rates and associated problems such as increasing subsidence rates, greenhouse gas emission, sulphate release and acidification.

## 2.1 Introduction

Currently, 450-550 Pg of carbon is present in sequestered form in peat soils worldwide; whereas, the atmospheric carbon stock contains approximately 750 Pg of carbon (IPCC 2013). This large carbon stock provides a risk, because it can be converted into atmospheric carbon dioxide relatively quickly (Laiho, 2006). Aeration of peat soils, e.g. as a consequence of drainage for agricultural purposes, leads to increased peat decomposition rates, compression and physical shrinkage. Besides the soil subsidence due to drainage, peat has also been harvested as a source of fuel. These practices have lowered the soil surface level in the western part of the Netherlands substantially; the lowest point of the Netherlands is currently 6.76 m below sea level. In addition, greenhouse gas emission and surface water contamination are associated problems in peat areas reclaimed for agriculture (Schothorst, 1977; Van Beek et al., 2007; Van den Akker et al., 2008; Hellmann and Vermaat, 2012). These degraded peat areas continue to be used for agricultural practices, primarily dairy farming, removing aboveground biomass is by grazing and/or mowing. Furthermore, water levels are kept at a low level so that relatively rapid subsidence (1-2 $cm \cdot yr^{-1}$) occurs. Although Dutch agricultural peat areas are all characterised by drainage and fertilisation, parts of (near-)natural bog and fen ecosystems with high water levels still remain in both the northern and western peat areas.

Drought frequency and severity are predicted to increase at high latitudes, where much of the world's peat resides (IPCC, 2007b). Also in the Netherlands, the intensity of summer droughts is expected to increase in the near future as a result of climate change (Van den Hurk et al., 2006; KNMI, 2014). Water tables in peatlands will drop when evaporation exceeds precipitation and, consequently, deeper previously water-saturated peat layers will become exposed to oxygen, which stimulates decomposition (Laiho, 2006; Ellis et al., 2009; Reiche et al., 2009). The process of further decay of previously deposited and stored peat after the onset of oxic conditions has been called secondary decomposition (Tipping, 1995). The consequence of increased aeration is a stimulation of peat decomposition, moreover, Fenner and Freeman (2011) showed that the rewetting phase after drainage or drought was associated with even larger $CO_2$ emission rates than the drought phase. Thus, climate change and related higher frequency and intensity of dry summer periods could aggravate the problems in the drained Dutch peat areas as increased aeration stimulates decomposition and, hence, peat subsidence, both during and after a dry summer.

$CO_2$ production depends, among other factors, on the availability of terminal electron acceptors (TEAs). Oxygen is the primary TEA in oxic conditions, whereas, a range of alternative TEAs, such as nitrate, manganese, ferric iron and sulphate are used in anoxic conditions, resulting in thermodynamically less favourable processes. The supply of alternative electron acceptors as nitrate, ferric iron and sulphate is generally low in peatlands. Methanogens take over once these TEAs have been depleted. Dry periods can replenish the pool of alternative TEAs, which will enable anaerobic decomposition as soon as oxygen is depleted (Dodla et al., 2009). Besides, humic substances, which are ubiquitous in peat, can also be used as electron acceptors (Lovley et al., 1996; Keller et al., 2009).

Apart from the presence or absence of alternative TEAs, phenolic compounds in the peat substrate also play a crucial role in its resistance to decomposition. Hydroxyl groups that are directly attached to a benzene ring characterise these compounds. Phenolic compounds are present in plants as part of structural tissues and as secondary metabolites for defence against herbivory (Coley et al., 1985). Examples are sphagnum acid and coumaric and cinnamic acids in *Sphagnum* spp., whereas, lignin and tannin are present in eutrophic peat (Verhoeven and Liefveld, 1997). Phenolic compounds inhibit the release of hydrolytic enzymes. They also bind chemically to various hydrolytic enzymes, such as sulphatase, phosphatase and β-glucosidase, which are thereby inactivated (Wetzel, 1992; Verhoeven and Liefveld, 1997; Freeman et al., 2001; Fenner and Freeman, 2011).

The degradation of phenolic compounds is performed by the enzyme phenol oxidase (POX) that is excreted by soil microorganisms and its activity is primarily associated with oxic conditions (Freeman et al., 2001; Zibilske and Bradford, 2007). When phenolic concentrations decrease, the inhibiting effect on hydrolytic enzymes production is released, resulting in increased peat degradation rates, both under oxic and anoxic conditions. This mechanism is referred to as the 'enzymic latch theory' (Freeman et al., 2001; Freeman et al., 2004). Although the degradation of phenolic compounds is primarily associated with oxic conditions, these compounds can also be degraded in anoxic conditions by using alternative electron acceptors such as nitrate or sulphate (Bakker, 1977; Elder and Kelly, 1994; Levén et al., 2010). Although anaerobic phenol oxidase activity has been demonstrated, its rate is low compared to phenol oxidase activity in oxic conditions (Freeman et al., 2001).

Phenol oxidase activity has been shown to be very pH sensitive; the activity more than doubled when pH increases from 5 to 8 (Pind et al., 1994). Changes in the water table in peat areas have been associated with short and long-term changes in pH. For example, drainage leads to acidification (Toberman et al., 2010) and rewetting leads to a rise in pH (Toberman et al., 2008). These pH changes and the 'enzymic latch theory' bring us to the following hypothetical course of events when an intact peatland is drained: first, anaerobic decomposition of peat is slow due to the latch that phenolic compounds exert on decomposition and hydrolytic enzymes in particular; second, the expected rise in phenol oxidase activity due to increased aeration will be moderated due to a drop in pH; and third, pH rise following rewetting will stimulate decomposition to levels higher than in pre- or post-oxygenation periods (Fenner and Freeman, 2011).

There is an urgent need to obtain insight in the consequences of lower groundwater tables on decomposition rates of peat soils, especially regarding the effect of the duration of the oxygenation period in deep peat layers that have not yet been oxygenated. In this study, incubation experiments were carried out to assess the effects of oxygenation periods varying in duration on the decomposition and mineralisation of peat layers that have been anoxic for a very long time but are prone to be aerated during dry summers. More specifically, we used fen peat consisting of the remains of *Carex* spp., *Phragmites* spp. and/or woody species and bog peat with primarily *Sphagnum* spp. remains, the latter known for their higher concentration of phenolic compounds and more acidic conditions, which both result in higher resistance against decomposition (Verhoeven and Toth, 1995). In addition, the distinction was made between peat samples from nature reserves and from drained and fertilised agricultural meadows (Table 1.2). Responses were measured for $CO_2$ and $CH_4$ production, concentrations of soluble and condensed phenolic compounds, nutrients and pH. The following research questions were addressed: (1) How do oxygenation periods of different duration affect the decomposition of deep peat samples? (2) Does oxygenation release a latch on the decomposition of deep anoxic peat? This released latch would be recognised by higher post-oxygenation decomposition rates compared to decomposition rates of non-oxygenised peat samples. (3) Does oxygenation affect the concentrations of soluble and condensed phenolic compounds and nutrients? (4) If decomposition and/or mineralisation are affected by oxygenation, what are the effects of land use and peat origin?

## 2.2 Materials and methods

### 2.2.1 Study sites

Peat from two different origins, i.e. fen peat (consisting of plant remains from *Carex, Phragmites, Alnus, Salix* and *Betula* spp.) and bog peat (mainly *Sphagnum* spp.), were collected both from agriculturally used meadows and from nature reserves. This resulted in four peat types (Table 1.2). The peat meadows with fen peat are located in the Dutch western peat district; this area is characterised by soils with fen peat of up to 10 m thick. The agricultural meadow (N 52.138818, E 4.835930), near Zegveld, had been sown with *Lolium perenne*, with a ditch water level of 55 cm below soil surface. The site in the nature reserve Nieuwkoopse Plassen (N 52.140689, E 4.798340) had mesotrophic hay meadow vegetation belonging to the Calthion palustris alliance (Zuidhoff et al., 1996) and a ditch water level of 20 cm below soil surface. The agricultural meadow with bog peat was located in the province of Friesland, part of the northern peat area (N 52.874654, E 5.805269). It had a L. perenne monoculture and a water table approximately 30 cm belowground surface. The natural peat bog was located in the nature reserve Fochteloërveen (N 52.990897, E 6.394049). This site had a water table at the soil surface level and vegetation characterised by *Sphagnum* spp. and *Molinia caerulea*.

### 2.2.2 Soil collection

Peat samples were collected at the end of March 2011. All samples were taken from a depth of >120 cm below the mean water table, where year-round anoxic conditions can be assumed. Five replicate samples were collected at each location using an Edelman-Auger soil corer, over an area of approximately 400 $m^2$. The samples were directly transferred into gas-tight bags, in which oxygen was removed with an oxygen-binding reagent mixture (Anaerocult, A mini, Merck, the Netherlands) and transported in a cool box. The samples were stored in a fridge at 4 °C until the start of the experiment, which was within one week after sampling. Dry weight was determined by oven drying two samples from each replicate (70 °C, 48 h) and pH was measured by adding 100 mL of demineralised water to 10 g of fresh soil. After shaking for 2 h (rotary shaker, 100 rpm) pH was measured in the soil suspension (WTW Measurements Systems, Ft. Myers, FL, USA).

### 2.2.3 Incubation experiment

For the experiment, 10 g of fresh peat and 15 mL of demineralised water were incubated in 300 mL infusion flasks. Homogenising the samples, weighing them into the infusion flasks and closing the flasks with airtight stoppers was performed under a $N_2$ atmosphere in a glove box. In addition, the flasks were evacuated and backfilled with $N_2$ two times, for 6 min each time. For each peat sample, there were five treatments: 0, 1, 2, 4 and 8 weeks of oxic conditions during the thirteen-week experiment. Samples were put in a rotary shaker (100 rpm) to facilitate gas exchange, at 20 °C in dark conditions. At the start of the oxygenation period, the flasks were opened for 2 h and then closed. Oxygen availability might be rather high in incubation experiments compared to the slow diffusion rates in the field. Therefore, oxygenation was realised by flushing only once with air. Additional tests using a respiration monitor (Biometric Systems, Germany) equipped with $CO_2$ and $O_2$ sensors were used to ensure that $O_2$ concentrations in the course of the oxygenation periods in the incubation experiment did not drop below 16% and $CO_2$ levels stayed well below 2% (unpublished data). This is assumed to be adequate for aerobic decomposition studies (Parr and Reuszer, 1959). At the end of the oxygenation period flasks were evacuated and flushed with $N_2$ gas four times, for 6 min each time.

Gas samples were taken directly after setting up the experiment, after the first week of incubation, at the end of the variable oxic periods, four weeks after the oxic period and at the end of the experiment after 13 weeks. 15 mL of gas from the headspaces was sampled using a gas tight syringe with valve (SGE Analytical Science, Melbourne, Australia). The gas was stored in vacuum containers (Labco, Buckinghamshire, England) until analysis. $CH_4$ concentrations were assessed on a HP 5890A GC fitted with a Porapak N column and flame ionisation detector (FID) with external standards. The $CO_2$ concentrations were measured on an EGM-4 infrared gas analyser (PP Systems, Hertfordshire, UK). $CO_2$ dissolution and

bicarbonate equilibrium were taken into account when calculating the $CO_2$ production.
After thirteen weeks of incubation, water extractions were performed by adding 85 mL of demineralised water, shaking on a rotary shaker (1 h, 100 rpm) and filtering the samples (Whatmann GF/C, Dassel, Germany). pH was measured in the supernatant and the concentrations $NO_3^-$, $NH_4^+$, $PO_4^{3-}$ and DOC of the extracts were determined the day after extraction (Continuous Flow Analyser, Skalar, Breda, the Netherlands). The concentration of soluble phenolic compounds in terms of tannic acid equivalents in the extracts was determined two days after extraction (Box, 1983). Briefly, 250 µL of the Folin-Ciocalteau reagent was added to 4 mL of extract, after 8 min 750 µL $Na_2CO_3$ (10.6 g·100 mL$^{-1}$) was added and 40 min later adsorption was measured at 760 nm (Shimadzu UV-120-01 spectrophotometer, Shimadzu, Kyoto, Japan). In addition, a tannic acid calibration curve was made. After the extraction, the soil samples were frozen using liquid nitrogen and freeze-dried. Freeze-dried samples were ground using an MM200 mixer mill (Retsch GmbH, Haan, Germany) at 15 rps for 1 min. After grinding, organic matter content was determined by loss-on-ignition (550 °C, 5.5 h). The concentration of MeOH-soluble phenolic compounds was determined, which reflects the concentration of condensed phenolic compounds (Akowuah et al., 2005; Cicco and Lattanzio, 2011). Briefly, duplicate samples of 30 mg of dried and ground material were extracted in 5 mL 50% methanol. Samples were put in a water bath at 40 °C for 1 h, while shaking, and were centrifuged afterwards (10 min, 4000 rpm). The content of phenolic compounds in the supernatant was determined using a slightly modified protocol by Box (1983) and a standard curve was prepared in 50% methanol. We diluted 10 µL of the extract with 90 µL of MeOH solution to prevent precipitation of fine solids (Cicco and Lattanzio, 2011). After that, we added 30 µL of the Folin-Ciocalteau reagent, after 8 min 100 µL $Na_2CO_3$ (10.6 g·100mL$^{-1}$) was added and 40 min later adsorption was measured at 760 nm (BMG Spectrostar nano, BMG Labtech, Ortenberg, Germany).

### 2.2.4 Statistical analysis

Data were analysed by Repeated Measures ANOVA followed by Bonferroni post-hoc tests for the roles of peat origin and land use and treatment effects. In addition, differences between each combination of peat origin and land use were evaluated. Greenhouse–Geisser corrections were applied and, if necessary, data were logarithmically transformed to stabilise variances between groups. Repeated Measures ANOVA were also performed to evaluate the differences between no oxygenation and oxygenation in order to examine if aeration releases a latch on decomposition. All statistical analyses were done using IBM SPSS Statistics 20 (IBM Corporation, Armonk, New York, United States).

# 2.3 Results

## 2.3.1 Edaphic characteristics

The edaphic characteristics are presented in Table 2.1. The organic matter content and C:N ratio of the bog peat, especially the samples from the bog reserve Fochteloërveen, are clearly higher than in the other samples. pH did not differ significantly between the samples from different locations.

**Table 2.1:** Edaphic characteristics of the peat samples. Differing letters depict significant differences (RM ANOVA, $p<0.05$) between peat types. No significant differences in pH were found.

| Peat origin | Land use | Organic matter content (%) | C (%) | N (%) | C:N | pH |
|---|---|---|---|---|---|---|
| **Fen peat** | Agriculture | 79.04±0.7 [a] | 44.99±1.2 [a] | 2.72±0.18 [c] | 16.98±1.6 [a] | 5.33±0.21 |
| **Bog peat** | Agriculture | 90.84±0.5 [c] | 52.66±0.8 [b] | 1.84±0.05 [b] | 28.71±1.1 [b] | 5.66±0.05 |
| **Fen peat** | Nature reserve | 82.49±0.6 [b] | 46.26±1.9 [ab] | 2.57±0.07 [c] | 18.06±0.7 [a] | 5.60±0.18 |
| **Bog peat** | Nature reserve | 97.57±0.2 [d] | 51.80±1.2 [ab] | 1.26±0.04 [a] | 41.22±1.3 [c] | 5.08±0.04 |

## 2.3.2 $CO_2$ and $CH_4$ production

Oxygenation accelerated the average $CO_2$ production rates over the whole experimental period in incubations of fen peat, whereas $CO_2$ production of bog peat was not affected (Figure 2.1a, statistics: Appendix 2.A). Fen peat from a dairy meadow had the highest $CO_2$ production rates. The $CO_2$ production of fen peat was significantly higher during oxygenation than in the permanently anoxic samples (indicated by asterisks and dashed lines in Figure 2.1b) and increased with increasing length of the oxygenation period. These samples also showed higher post-oxygenation $CO_2$ production compared to permanently anoxic samples (Figure 2.1c). The fen peat from a nature reserve did show increasing post-oxygenation $CO_2$ production; however, this did not differ significantly from the non-oxygenated samples. The production rates of the other peat samples during the post-oxygenation period were apparently not affected by the duration of the treatments.

Short-term oxygenation enhanced $CH_4$ production rates (Figure 2.1d). Especially the samples exposed to oxygen for 1 week in the 13-week experimental period showed high overall $CH_4$ production rates (statistics: Appendix 2.A). In fen peat from an agricultural meadow and in bog peat from a nature reserve the $CH_4$ production rates increased over 7-fold as a result of one oxic week, compared to 2- to 3-fold in the other peat types. $CH_4$ production during the oxic period was overall low. The negative production rates found imply $CH_4$ oxidation (Figure 2.1e). Figure 2.1f presents the post-oxygenation $CH_4$ production rates, and for comparison, the production rates of the permanently anoxic samples are also shown (dashed lines). Fen and bog peat from agricultural meadows as well as bog peat from a nature reserve showed higher post-oxygenation $CH_4$ production rates compared to the non-oxygenated samples (asterisks). The fen peat samples from a nature reserve did not show significantly higher post-oxygenation production rates.

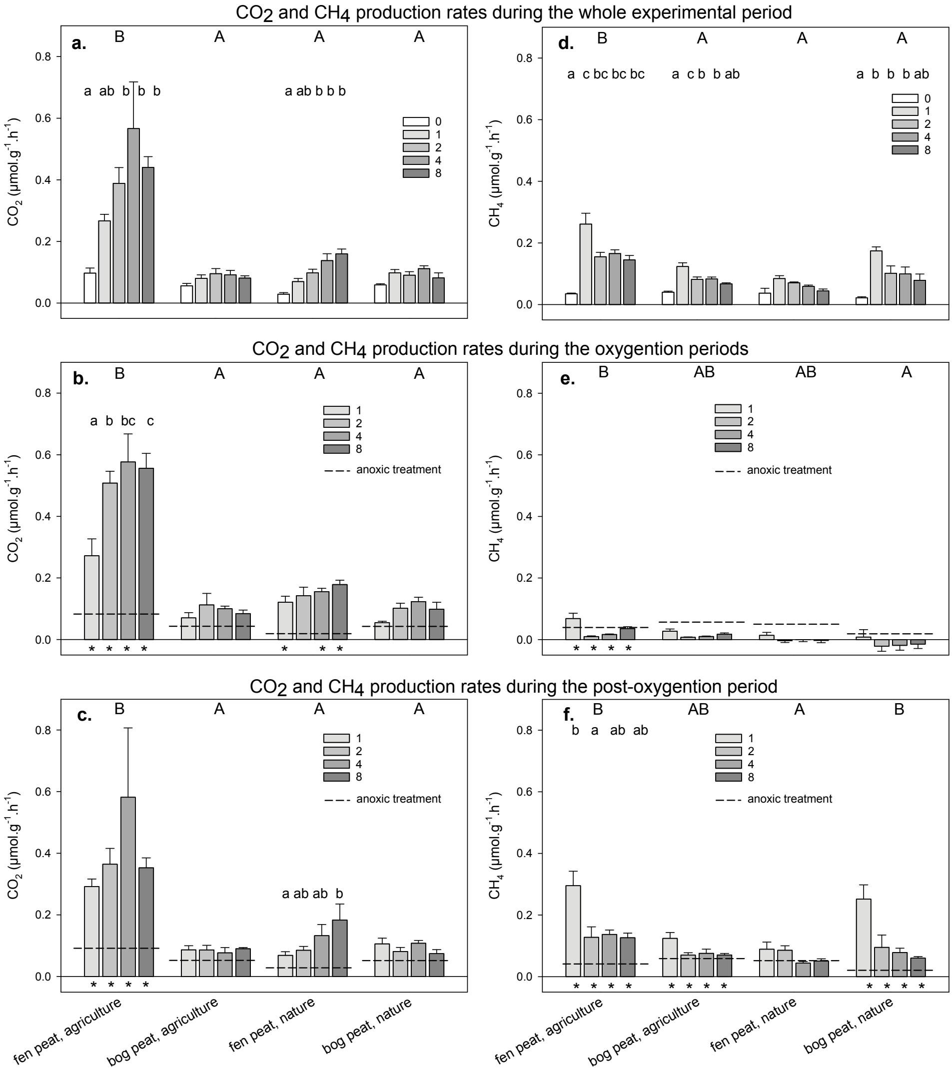


**Figure 2.1:** Average $CO_2$ (panels a, b, c) and $CH_4$ (panels d, e, f) production rates over the whole experimental period (a and d), during the oxygenation periods (b and e) and during the 4-week post-oxygenation period (c and f). Data are mean values of five replicates; error bars represent standard errors. Each sample type experienced 0, 1, 2, 4 or 8 weeks of oxygenation. Different capitals indicate significant differences between combinations of peat origins and land uses (ANOVA p<0.05); different small letters indicate treatment effects per combination of peat origin and land use (ANOVA p<0.05). The dashed lines in panels b, c, e and f represent the production rates without oxygenation; asterisks indicate significant differences between anoxic production rates and the production rates during and after oxygenation (one-way ANOVA, p<0.05).

In general, $CO_2$ production during and after the oxygenation period was affected by both land use and peat origin. Peat samples from agricultural areas showed higher $CO_2$ emissions than samples from nature reserves and fen peat produced more $CO_2$ than bog peat (Appendix 2.A). On the other hand, $CH_4$ production was only influenced by land use with samples from agricultural areas showing a higher production than samples from nature reserves.

### 2.3.3 Total carbon loss

Figure 2.2 depicts the total gaseous carbon loss during the experimental period expressed as units of carbon lost per unit of carbon present in the soil. The oxygenation periods have significantly increased the decomposition in three out of the four sites investigated. The bog peat from an agricultural meadow did not show a significant treatment effect. In general, carbon loss from the intensively used peat meadow on fen peat was higher than carbon loss from the other samples (statistics: Appendix 2.B), while this peat type had the lowest carbon content (Table 2.1).

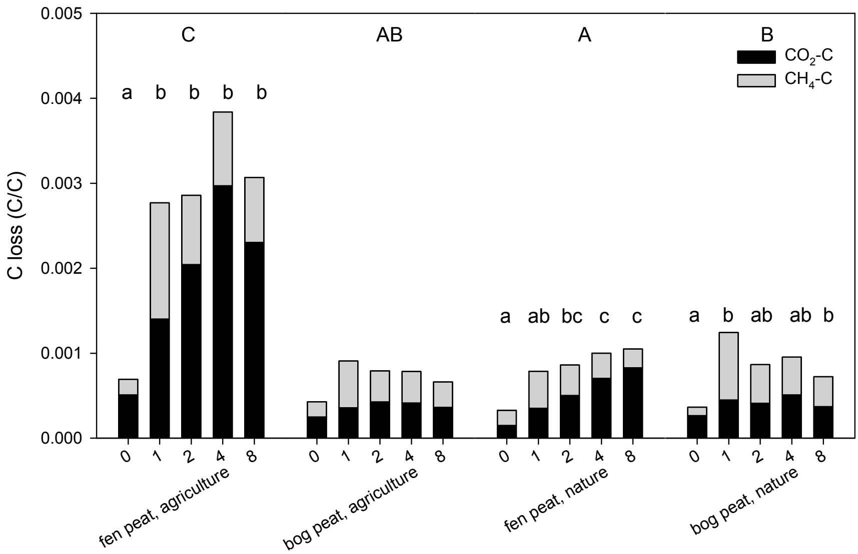


**Figure 2.2:** Total carbon loss per unit carbon during the experimental period (13 weeks), distinguishing between carbon loss as $CO_2$ and as $CH_4$. Different capitals indicate significant differences between total carbon loss between combinations of peat origins and land uses (ANOVA p<0.05); different small letters indicate treatment effects per combination of peat origin and land use (ANOVA p<0.05).

### 2.3.4 Phenolic compounds

The concentrations of soluble and condensed phenolic compounds at the end of the experimental period showed no significant differences between the oxygenation treatments. However, peat types significantly differed in phenolic compound concentrations (Table 2.2). Fen peat had lower concentrations of soluble phenolic compounds than oligotrophic peat, with a higher concentration in the agricultural meadow than in the nature reserve. For condensed phenolic compounds, bog peat samples contained higher concentrations than fen peat samples; land use did not affect the condensed phenolic compounds concentrations.



**Table 2.2:** Average concentrations ± standard error of water-soluble and MeOH-soluble phenolic compounds, $NO_3^-$, $NH_4^+$ and $PO_4^{3-}$. Differing letters indicate significant differences (ANOVA, $p<0.05$) between combinations of peat origin and land use.

| Peat origin | Land use | Soluble phenolic compounds ($g \cdot kg^{-1}$) | Condensed phenolic compounds ($g \cdot kg^{-1}$) | DOC ($gC \cdot kg^{-1}$) | $NO_3^-$ ($mgN \cdot kg^{-1}$) | $NH_4^+$ ($mgN \cdot kg^{-1}$) | $PO_4^{3-}$ ($mg \cdot kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| **Fen** | Agriculture | 1.25±0.30 $^{b}$ | 1.15±0.11 $^{a}$ | 4.20±0.2 $^{b}$ | 39.92±1.8 $^{c}$ | 116.52±18.7 $^{b}$ | 13.29±1.2 $^{b}$ |
| **Bog** | Agriculture | 4.23±0.66 $^{c}$ | 3.19±0.24 $^{b}$ | 6.15±0.7 $^{bc}$ | 26.74±2.8 $^{b}$ | 25.42±4.6 $^{a}$ | 95.04±12.5 $^{c}$ |
| **Fen** | Nature | 0.29±0.07 $^{a}$ | 1.43±0.13 $^{a}$ | 0.85±0.1 $^{a}$ | 15.63±0.9 $^{a}$ | 32.96±2.4 $^{a}$ | 3.51±0.2 $^{a}$ |
| **Bog** | Nature | 3.22±0.26 $^{c}$ | 3.48±0.17 $^{b}$ | 6.43±0.5 $^{c}$ | 30.78±1.5 $^{b}$ | 141.32±7.6 $^{c}$ | 13.69±0.8 $^{b}$ |

### 2.3.5 Water quality

Dissolved Organic Carbon (DOC) concentrations showed no treatment effect and highest amounts were found in bog peat (Table 2.2). The dissolved nutrient concentrations $NO_3^-$, $NH_4^+$ and $PO_4^{3-}$ in water extracts did not show any significant treatment effect either. Sulphate, on the other hand, showed significant treatment effects as sulphate concentrations tended to increase with the length of the oxygenation period (Figure 2.3, statistics: Appendix 2.C). These increasing $SO_4^{2-}$ concentrations were in particular seen in the fen peat samples. In addition, acidification took place due to oxygenation in this fen peat. The combination of increasing sulphate concentrations and pH suggests pyrite oxidation.

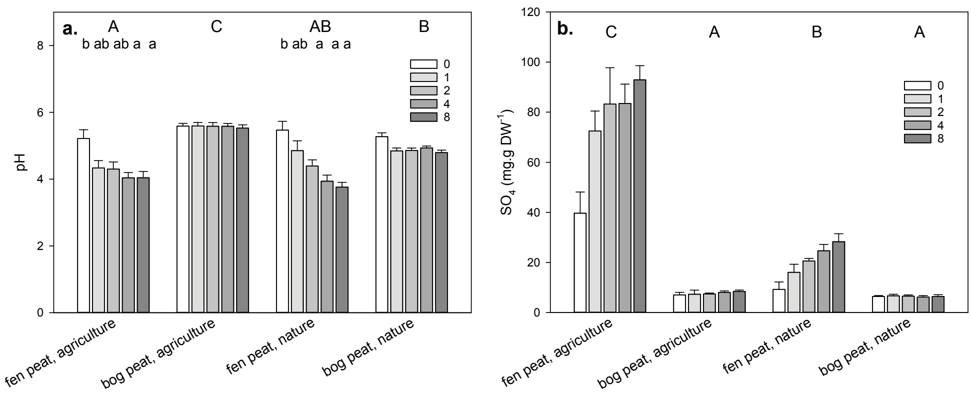


**Figure 2.3:** Average pH (a) and $SO_4^{2-}$ concentrations (b) at the end of the experimental period. Data are mean values of 5 replicates; error bars represent standard errors. Each peat type experienced 0, 1, 2, 4 or 8 weeks of oxic conditions. Different capitals indicate significant differences between the peat types (ANOVA $p<0.05$); different small letters indicate, per combination of peat origin and land use, differences between the treatments (ANOVA $p<0.05$).

# 2.4 Discussion

## 2.4.1 Short-term oxygenation releases latch on decomposition

Our experiment showed that even one week of oxygenation released a latch on the decomposition of normally anoxic peat samples, because after restoring anoxic conditions, $CO_2$ production remained high and $CH_4$ production kicked off. The largest increase in total carbon loss was already achieved after one week of oxygenation and did not increase much after longer oxygenation periods. These results were found in four different types of samples, viz fen and bog peat under both agricultural and natural land use. So, in addition to confirming that the effect of oxygenation is also measurable in the post-oxygenation period (Freeman et al., 2001; Fenner and Freeman, 2011), we showed that even one week of oxygenation sufficed to ease the latch on organic matter breakdown, although oxygen supply in our setup was probably higher than in actual vertical peat profiles under field conditions.

In this experiment, the so-called secondary decomposition was studied; this is the aerobic decomposition of peat that already has been stored for a long time in the anoxic subsurface (Tipping, 1995; Borgmark and Schoning, 2006). Despite the acidification of the fen peat, which might have prevented an even larger decomposition response (Toberman et al., 2010), fen peat decomposition rates increased directly within the first week of oxygenation, while there was not such stimulation in bog peat samples. The higher concentration of phenolic compounds in the bog peat, which hamper decomposition (Freeman et al., 2001), might explain the lack of a direct response to oxygenation on peat decomposition in this peat type.

In our experiment, the observation of higher $CO_2$ production rates during oxygenation periods of 2 to 8 weeks, compared to one week, suggests that a period of adaptation or growth of specific microbial organisms is needed. Nevertheless, the stimulating effect of oxygenation on bog peat became apparent in the post-oxygenation period, as $CH_4$ production of the bog peat was significantly higher than $CH_4$ production in the permanently anoxic samples. This indicates that crucial changes occurred during oxygenation that facilitated subsequent anaerobic decomposition, which is in line with the enzymic latch theory (Freeman et al., 2001; Fenner and Freeman, 2011). Another explanation for the high $CH_4$ production after one week of oxygenation compared to 2-8 weeks of oxygenation could be a recovery of the methane consumers after the oxygen treatment.



It must be noted that the transition from oxygenation to post-oxygenation did not lead to a substantial drop in $CO_2$ production rates, although oxygen was not directly available anymore. This is partly in line with the results presented by Fenner and Freeman (2011). They found that a water table drop caused an increase in $CO_2$ fluxes that remained, and increased even further, when anoxic conditions returned. The continuation of high $CO_2$ production in the post-oxygenation period compared to anoxic conditions could be caused by crucial changes in the peat material, e.g. a change in the phenolic compounds, which might have been qualitative rather than quantitative as the concentrations of phenolic compounds did not change. Furthermore, oxidised chemical species could have accumulated during oxygenation and used as TEAs in the post-oxygenation period, like $NO_3^-$, $SO_4^{2-}$ or humic substances (Lovley et al., 1996; Keller et al., 2009; Deppe et al., 2010; Yavitt, 2013). The increase in $CO_2$ production (Fenner and Freeman, 2011) was not found in our incubation study, but the anoxic conditions prevented oxidation of $CH_4$ to $CO_2$, which is normally a common process in the superficial peat layers in field conditions (Heipieper and De Bont, 1997). Furthermore, it was remarkable that all peat types showed high $CH_4$ production rates in the period directly after the oxygenation, especially after only one week of oxygenation. Perhaps, $CH_4$ consumers need time to get going again after the oxygenation. This contrasts the results of Yavitt (2013) who found a lag time before the onset of methanogenesis once anoxic conditions returned. This was explained by the accumulation of oxidised components, recovery of methanogens after drought and/or recovery of fermenting bacteria. In our experiment, oxidised components had indeed accumulated; see for example the increased sulphate concentrations. However, our peat samples did not suffer from drought as the samples were turned into slurries. In our opinion, this resembles best the conditions of deep peat layers that experience a dry summer, as oxygen intrusion does not necessarily imply drought stress in deep soil layers.

Both $CO_2$ and $CH_4$ were produced in the post-oxygenation period. Methanogenic pathways generally produce equimolar amounts of carbon dioxide and methane, but $CO_2$ production is often reported to be higher than $CH_4$ production in both field and laboratory incubation studies of peat decomposition (Conrad, 1999; Corbett et al., 2013). The $CO_2$:$CH_4$ ratio has been used previously to gain a better understanding of anaerobic organic matter degradation (Kane et al., 2013). In our experiment, $CO_2$:$CH_4$ ratios were generally between 1 and 3 in the permanently anoxic samples, which is quite low and suggests a low electron acceptor capacity (Kane et al., 2013). Two factors should be considered while evaluating $CO_2$ and $CH_4$ production rates. Firstly, in field conditions part of the produced $CH_4$ would be oxidised travelling up in the soil profile when $CH_4$ passes oxic soil layers (Bodelier, 2011). Secondly, anaerobic $CH_4$ oxidation can occur, although there is no conclusive evidence yet for the electron acceptor(s) involved in this process (Bodelier, 2011; Smemo and Yavitt, 2011). Therefore, it is likely that $CH_4$ release in our lab incubation experiment is higher compared to the field situation; therefore, we did not calculate global warming potentials based on the results of this experiment. Furthermore, the removal of $CO_2$ by flushing the samples could ease the end-product-related inhibition on decomposition.

### 2.4.2 Phenolic compounds

We did not find any effect of oxygenation on the concentrations of soluble or condensed phenolic compounds. This was despite the fact that it has previously been shown that summer droughts cause a decrease in the concentration of phenolic compounds, which is then generally followed by higher decomposition rates (Freeman et al., 2001; Fenner and Freeman, 2011). It could be that subtle changes occurred in the concentrations of phenolic compounds, which were not detected. The method we used for measuring soluble phenolic compounds works with the Folin-Ciocalteau reagent, which is most commonly applied in ecological studies (Freeman et al., 2004; Bragazza and Freeman, 2007; Fenner and Freeman, 2011). We also measured concentrations of condensed phenolic compounds, but they did not show a treatment effect either. Nonetheless, the expected differences between peat origins were confirmed: bog peat samples had a substantially higher concentration of condensed phenolic compounds than fen peat samples. The concentrations of phenolic compounds in our peat samples fit well within the range found by others (Bragazza and Freeman, 2007; Rimmer and Abbott, 2011). Furthermore, we can state that even without changes in the concentration of phenolic compounds, oxygenation releases a latch on decomposition.

Carbon loss was especially raised in the fen peat samples. Bog peat, which is more recalcitrant to decomposition, as indicated by the high C:N ratio and phenolic compound concentrations, reacted less strongly on oxygenation than fen peat. However, we do not see an indication for a clear correlation between the phenolic compound content or C:N ratio and decomposition rates and their reaction to oxygenation. Within one peat origin, e.g. the fen peat, both the highest and lowest carbon loss rates are found, while the content of phenolic compounds is low. Moreover, the bog peat samples, with the highest content of phenolic compounds, do not necessarily show the lowest carbon loss rates.

### 2.4.3 Relevance for Dutch peat areas

During dry summers, which are expected to occur more often due to climate change, water levels become extremely low, so anoxic peat layers are being more and more oxygenated, the deepest ones often for the first time. In this experiment, we found that even only one week of oxygenation of this deep peat already increased decomposition rates during and after oxygenation. Decomposition rates of fen peat and bog peat from nature reserves and dairy meadows increased with a factor 2-4 due to one week of oxygenation in a thirteen-week period. No clear peat origin or land use effects were detected.



Although carbon loss in the whole experimental period was substantially higher in the oxygenated samples, aerobic and anaerobic decomposition rates of bog peat increased less than the decomposition rates of fen peat. The presence of oxygen or alternative TEAs is apparently not limiting the decomposition of this peat that much. More information on the comparison of aerobic and anaerobic decomposition rates can be found in Chapter 4 and Chapter 7 of this thesis.

Subsidence, the result of higher decomposition rates than accumulation rates of organic matter, in addition to shrinkage and compression is one of the main problems in Dutch peat meadow areas. The absolute values of gas exchange presented here cannot be used to derive field gas emission or subsidence rates because of differences between the small-scale lab incubations and field conditions. Nevertheless, we proved that low groundwater levels, which are expected to occur more often in the Netherlands due to climate change, can significantly increase decomposition rates and associated problems, even after the water levels have gone up again during subsequent rain periods. If peatland managers aim at reducing subsidence rates, this study would suggest investing in preventing very low summer water levels in these areas.

The oxygenation of deep peat layers in extremely dry summer periods will not only lead to faster soil subsidence, but also can affect ground and surface water quality, even though no direct effects on nutrient concentrations were found during our incubations. $NO_3^-$ concentrations were not affected by the treatments; however, denitrification could have taken place, resulting in nitrogen loss as $N_2$ or $N_2O$ gas, the latter is a GHG with a large global warming potential.

We found indications that pyrite oxidation took place in the fen peat as pH decreased and sulphate concentrations increased; no indications for pyrite oxidation were detected in the ombrotrophic bog peat. The peat in the western part of the Netherlands, mainly minerotrophic peat, contains considerable amounts of pyrite, whereas the ombrotrophic peat in the northern peat areas is much lower in pyrite (Lowe and Bustin, 1985; Van Gaans et al., 2007). In addition to the release of sulphate into the peat soil, also the acidification associated with pyrite oxidation might have significant consequences for ecosystem functioning. Firstly, one of the supposed key regulators of peat decomposition, i.e. the activity of phenol oxidase (Freeman et al., 2004) is strongly regulated by pH and has a pH optimum at around 8 (Pind et al., 1994; Williams et al., 2000). Despite acidification, oxygenation still enhanced decomposition. Secondly, pyrite oxidation is often related to an increase in phosphate concentrations, because $SO_4^{2-}$ and $PO_4^{3-}$ compete for the same anion adsorption sites (as reviewed by Smolders et al. (2006)). However, we did not observe such phenomena here as phosphate concentrations were not affected by oxygenation. Thirdly, acidification might cause desorption of metals from the soil complex and form sulfide which can leach to ground and surface water (Lucassen et al., 2005).

## 2.5 Conclusion

The goal of this paper was to gain insight into the consequences of lower summer groundwater levels for peat decomposition and mineralisation of deeper anoxic peat layers. Because of climate change, it is expected that dry summers will occur more often. We showed that oxygenation of deep peat layers that had not previously been exposed to air led to acceleration of decomposition. Even one week of oxygenation led to a released latch on decomposition, indicated by higher post-oxygenation carbon loss rates compared to non-oxygenated samples. Carbon loss was highest in fen peat currently used for dairy farming. Contrary to the enzymic latch theory, no effect of oxygenation on the concentrations of soluble and condensed phenolic compounds was detected. Nutrient concentrations did not change due to oxygenation either. Noteworthy is the occurrence of pyrite mineralisation and associated acidification and $SO_4^{2-}$ release to the pore water and surface water. So, low summer water levels, for example due to climate change, should be avoided in order to limit exceptionally high decomposition rates and associated problems such as increasing subsidence rates, greenhouse gas emission, sulphate release and acidification.

### Acknowledgements

This study was supported by the research program Knowledge for Climate (Kennis voor Klimaat), hotspot 'shallow waters and peat meadow areas' project code HSOV1a. We are grateful to Natuurmonumenten for facilitating peat sampling in the nature reserves Nieuwkoopse Plassen and Fochteloërveen. In addition, farmer Gert Diever and the Veenweide Innovatiecentrum (peat meadow innovation centre) are greatly thanked for allowing us to take soil samples at their sites. We would like to acknowledge Gerrit Rouwenhorst (Utrecht University) and Richard van Logtestijn (VU University, Amsterdam) for their help with chemical and gas analyses.

# Appendix

**Appendix 2.A:** Results of Repeated Measures ANOVAs and Bonferroni post-hoc tests on total $CO_2$ (upper table) and $CH_4$ (lower table) production rates and production rates during and after the oxygenation period.

| | **Log tot $CO_2$** | | | **Log $CO_2$ oxygenation period** | | | **Log $CO_2$ post-oxygenation period (µmol·g dw$^{-1}$·h$^{-1}$)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | F | p | df | F | P | df | F | p |
| **Treatment** | 3.32 | 36.925 | 0.000 | 2.34 | 7.452 | 0.001 | 2.73 | 1.606 | 0.205 |
| | 0(a), 1(b), 2(b), 4(b), 8(b) | | | 1(a), 2(b), 4(b), 8(b) | | | | | |
| **Land use** | 1 | 54.690 | 0.000 | 1 | 15.546 | 0.001 | 1 | 37.093 | 0.000 |
| | Agriculture > nature | | | Agriculture > nature | | | Agriculture > nature | | |
| **Peat origin** | 1 | 68.206 | 0.000 | 1 | 59.359 | 0.000 | 1 | 73.140 | 0.000 |
| | Eutrophic > Oligotrophic | | | Eutrophic > Oligotrophic | | | Fen peat > bog peat | | |
| **Treatment * land use** | 3.32 | 0.157 | 0.958 | 2.34 | 0.279 | 0.791 | 2.73 | 0.491 | 0.673 |
| **Treatment * peat origin** | 3.32 | 13.270 | 0.000 | 2.34 | 1.087 | 0.356 | 2.73 | 2.669 | 0.064 |
| **Treatment * land use * peat origin** | 3.32 | 1.005 | 0.404 | 2.34 | 1.081 | 0.357 | 2.73 | 2.600 | 0.069 |

| | **Log tot $CH_4$** | | | **Log $CH_4$ oxygenation period** | | | **Log $CH_4$ post-oxygenation period (µmol·g dw-1·h-1)** | | |
|---|---|---|---|---|---|---|---|---|---|
| | df | F | p | df | F | P | df | F | p |
| **Treatment** | 2.65 | 106.46 | 0.000 | 1.26 | 25.321 | 0.000 | 1.95 | 19.963 | 0.000 |
| | 0(a), 1(d), 2(c), 4(c), 8(b) | | | 1(d), 2(a), 4(b), 8(c) | | | 1(b), 2(a), 4(a), 8(a) | | |
| **Land use** | 1 | 24.295 | 0.000 | 1 | 8.664 | 0.010 | 1 | 7.442 | 0.015 |
| | Agriculture > nature | | | Agriculture > nature | | | Agriculture > nature | | |
| **Peat origin** | 1 | 1.387 | 0.256 | 1 | 2.417 | 0.140 | 1 | 1.705 | 0.210 |
| **Treatment * land use** | 2.65 | 0.695 | 0.543 | 1.26 | 2.188 | 0.151 | 1.95 | 0.532 | 0.588 |
| **Treatment * peat origin** | 2.65 | 0.378 | 0.745 | 1.26 | 1.005 | 0.348 | 1.95 | 0.155 | 0.852 |
| **Treatment * land use * peat origin** | 2.65 | 11.240 | 0.000 | 1.26 | 4.422 | 0.041 | 1.95 | 7.443 | 0.002 |

**Appendix 2.B:** Results of Repeated Measures ANOVAs and Bonferroni post-hoc tests on total carbon loss per unit carbon present in the peat.

| | df | F | p |
|---|---|---|---|
| **Treatment** | 3.1 | 45.399 | 0.000 |
| | 0(a), 1(b), 2(b), 4(b), 8(b) | | |
| **Land use** | 1 | 59.826 | 0.000 |
| | Agriculture > nature | | |
| **Peat origin** | 1 | 24.424 | 0.000 |
| | Fen peat > bog peat | | |
| **Treatment * land use** | 3.1 | 1.194 | 0.322 |
| **Treatment * peat origin** | 3.1 | 15.607 | 0.000 |
| **Treatment * land use*peat origin** | 3.1 | 0.969 | 0.417 |

**Appendix 2.C:** Results of Repeated Measures ANOVAs and Bonferroni post-hoc tests on pH and $SO_4^{2-}$ concentrations at the end of the experimental period.

| | **pH** | | | **Log $SO_4$ ($g.g^{-1}$)** | | |
|---|---|---|---|---|---|---|
| | df | F | p | df | F | p |
| **Treatment** | 1.84 | 30.050 | 0.000 | 1.95 | 18.661 | 0.000 |
| | 0(d), 1(c), 2(bc), 4(ab), 8(a) | | | 0(a), 1(ab), 2(bc), 4(bc), 8(c) | | |
| **Land use** | 1 | 4.704 | 0.046 | 1 | 71.838 | 0.000 |
| | Agriculture > nature | | | Agriculture > nature | | |
| **Peat origin** | 1 | 43.940 | 0.000 | 1 | 320.680 | 0.000 |
| | Fen peat < bog peat | | | Fen peat > bog peat | | |
| **Treatment * land use** | 1.84 | 2.529 | 0.101 | 1.95 | 0.063 | 0.936 |
| **Treatment * peat origin** | 1.84 | 16.334 | 0.000 | 1.95 | 13.756 | 0.000 |
| **Treatment * land use * peat origin** | 1.84 | 1.812 | 0.183 | 1.95 | 1.653 | 0.208 |

# Chapter 3

# Phenolic compounds, phenol oxidase and nutrients in drained peat areas

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Photo by Steven Verbeek, Waterschap Noorderzijlvest

## Abstract

Peatlands store vast amounts of carbon. Peat formation takes place when primary productivity of the vegetation exceeds decomposition of organic matter. The layered variation over the peat profile reflects the age of the organic material, botanical composition and degree of decomposition and humification. This implies that the chemical composition of the peat, including the presence of phenolic compounds, varies with depth. The concentrations of phenolic compounds, which are considered to be regulating factors in peat decomposition also vary with depth due to differences in the activity of the enzyme phenol oxidase (POX) which is primarily active in oxic conditions. In this study it was hypothesised that there would be a transition from low concentrations of phenolic compounds in the aerated part of peat profiles towards higher concentrations of phenolic compounds in the deeper, permanently anoxic parts because of higher potential POX activity in oxic conditions. However, no such relation was found. Agricultural land use (drainage and fertilisation), did not affect the concentrations of soluble and condensed phenolic compounds and potential POX activity either. Furthermore, a higher potential POX activity and soluble phenolic compound concentration with increasing depth was found in profiles of some of the peat soils studied. Statistical analysis only explained a small proportion of the variance in potential POX activity. Surprisingly, potential POX activity correlated positively with sulphate concentrations. Furthermore, the presence of phenol oxidase activity deeper in the profile could indicate that anaerobic degradation of phenolic compounds does occur with alternative electron acceptors. Nevertheless, this study does not give any evidence of the actual occurrence of such anaerobic decay of phenolic compounds as the potential, rather than the actual POX activity was measured. Summarising, the relation between the concentration of phenolic compounds and oxygen availability is not as straightforward as previously thought. Also, the presence of potential POX activity in deeper, anoxic, soil layers was surprising because of the presumed inactivity of this enzyme in anoxic conditions. The results indicate that quality, rather than quantity, of phenolic compounds is worth further investigation.

## 3.1 Introduction

Peat formation takes place when primary productivity of the vegetation exceeds decomposition of organic matter. Consequently, dead organic matter accumulates and a peat layer is formed. As a result of limited bioturbation in peatlands, vertical peat profiles represent the succession that has taken place over time with older organic material situated deeper in the profile than recently produced organic material. In general, the remains of aquatic plants are encountered deepest in a peat profile, followed by typical fen species such *Phragmites*, *Carex*, *Betula* and *Alnus* species. An oligotrophic *Sphagnum* layer often overlies these minerotrophic layers (Figure 1.1) (Zagwijn, 1986; Pons, 1992).

The layered variation over the peat profile reflects the age of the organic material, the botanical composition and the succession during peat accumulation and the degree of decomposition and humification. This implies that the botanical composition and chemical quality of the peat substrate is expected to vary with depth, which will critically influence peat decomposition rates. Variation in phenolic compound concentrations across the profile could play an important role here. Phenolic compounds are plant secondary metabolites, involved in herbivory defence and regulation of nutrient availability. Phenolic compounds enter the soil as leachates from above- and belowground plant parts and in condensed form via above and belowground litter (Hättenschwiler and Vitousek, 2000). Examples of phenolic compounds are lignin, tannin and sphagnum acid (Figure 3.1). The 'enzymic latch theory' has put forward a crucial role for soluble phenolic compounds in the decomposition of peat (Freeman et al., 2001). Their presence supposedly hampers the activity of hydrolytic enzymes such as sulphatase, phosphatase and β-glucosidase. Microorganisms can degrade phenolic compounds through excretion of the enzyme phenol oxidase (POX). The behaviour of this group of phenol degrading enzymes has not been fully understood to date.

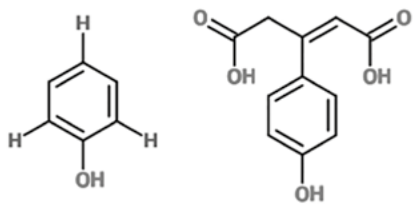



**Figure 3.1:** Examples of phenolic compounds, left: phenol, right: sphagnum acid.

Factors known to influence POX activity in soils include oxygen availability, soil pH, the concentration of soluble phenolic compounds and nitrogen availability (Sinsabaugh, 2010). Firstly, it has been hypothesised that POX degrades phenolic compounds primarily in oxic conditions (Pind et al., 1994; Freeman et al., 2001). However, field measurements do not consistently show higher potential POX activities in superficial peat layers compared to

deeper layers (Williams et al., 2000) and laboratory incubations showed that POX activity were only halved when 0.5% instead of 21% oxygen was present (Zibilske and Bradford, 2007). Pind et al. (1994) found higher concentrations of phenolic compounds in superficial peat layers than in deeper peat layers. However, this could also be related to the fact that the sources of these compounds are living and dead organic matter, which is produced aboveground and in the root-zone of the soil. In addition to oxygen, other electron acceptors might affect the decay of phenolic compounds in anoxic conditions. In 1934, it was proven that the aromatic nucleus of common phenolic compounds was completely decomposed when incubated without oxygen with sewage sludge (Tarvin and Buswell, 1934). Later, it has been found that that anoxic decay processes such as sulphate reduction, denitrification, iron$^{3+}$ reduction, fermentation and methanogenesis play a role in the degradation of phenolic compounds (Elder and Kelly, 1994; Heider and Fuchs, 1997).
Secondly, various studies indicate that pH is another important factor with a possibly stronger controlling effect on POX rates than oxygen availability. The optimal pH for the enzyme POX is around 8 (Pind et al., 1994; Toberman et al., 2008; Sinsabaugh, 2010; Toberman et al., 2010; Fenner and Freeman, 2011). It was shown in a two-month experiment that impeded drainage resulted in less acidic conditions due to processes like denitrification and sulphate reduction, which led to higher POX activity in peat cores from a riparian gully, although oxygen availability was most likely low (Toberman et al., 2008). A long-term experiment comparing drained and undrained forest sites in Finland, revealed that POX activities were lower and soluble compound concentrations were higher in the drained site. This effect was also attributed to the lower pH caused by drainage due to processes like nitrification and pyrite oxidation (Toberman et al., 2010). Fenner and Freeman (2011) suggested that their observation of increased post-drought decomposition rates is related to an increase in pH. Other factors potentially affecting POX activity are the concentrations of phenolic compounds and of mineral nitrogen. Phenolic compounds are a primary substrate for the enzyme POX, so that a higher POX activity is expected at higher concentrations of phenolics. On the other hand, high POX activity might reduce the size of the pool of phenolic compounds. In a dry summer in *Sphagnum*- and *Carex*-dominated peatlands in New York State, low soluble phenolic compound concentrations tended to induce POX, whereas high concentrations tended to inhibit POX activity (Williams et al., 2000). In general, POX activity relates to the fluxes of phenolic compounds rather than the absolute amounts at any one time. Furthermore, the pool of soluble phenolic compounds can be replenished continuously by the degradation of condensed phenolic compounds.
The discovery that POX activity is not only associated with depolymerisation of phenolics in soil decomposition processes, but also with polymerisation in plants to synthesise lignin and other secondary compounds, further complicates the interpretation of POX activities (Sinsabaugh, 2010). Studies on effects of N addition on POX activity have generated

conflicting results, but, in general, N addition reduced POX activity in recalcitrant organic matter (Sinsabaugh, 2010).

Most studies conducted on phenolic compounds and POX in peat soils have been carried out in natural peat areas. The present study examines vertical profiles of peat soils under agricultural and natural land use. Dairy farming is currently the most common land use on drained peat soils, whereas small proportions are used for crop production or protected as a nature reserve with nearly natural conditions. Hence, the conditions in Dutch peatlands now strongly differ from natural peat areas because of differences in vegetation type, groundwater levels and fertilisation intensity. In the Netherlands, most natural peatlands have been drained for agriculture many centuries ago, which facilitates deeper aeration (Iiyama and Hasegawa, 2009) and decomposition, this contrasts with natural peatlands where oxygen intrusion is limited to the upper centimetres or one to two decimetres. Agricultural practices as grazing and mowing reduce the proportion of the plant primary production that could contribute to peat formation. Consequently, it is hypothesised that agricultural practice minimises the input of phenolic compounds because of the removal of aboveground biomass.

This process of further decay of previously deposited and stored peat after the onset of oxic conditions has been called secondary decomposition (Tipping, 1995). Hence, in drained peatlands the concentration of soluble phenolic compounds is expected to be lower in the superficial peat layers and to increase with depth, in contrast to natural peat areas where phenolic compounds are still being formed (Pind et al., 1994; Hättenschwiler and Vitousek, 2000). More specifically, in our agricultural peatlands a transition zone is expected to occur at the level of the groundwater table, beneath which the concentration of phenolic compounds suddenly increases with depth.

Apart from drainage, the agricultural land use strongly affects belowground cycling of carbon and nutrients through the inputs of chemical fertilisers and manure. Although nitrogen addition stimulates the decomposition of easily degradable organic matter, it has been found to hamper the decay of recalcitrant compounds (Mack et al., 2004; Knorr et al., 2005a; Craine et al., 2007). Nitrogen addition to recalcitrant organic matter leads to a lower phenol oxidase activity, whilst the effects on easily degradable litter are smaller and either positive or negative (Sinsabaugh, 2010). Particularly in peat areas, the response of POX activity to nitrogen addition has often been reported to be negative (Matocha et al., 2004; Dell et al., 2012). Therefore, lower POX activities are expected in agricultural sites as nitrogen availability is supposed to be higher in agricultural sites than in nature reserves.

Furthermore, we made the distinction between fen peat and bog peat, the latter known for its higher concentration of phenolic compounds and more acidic conditions that both result in higher resistance against decomposition (Verhoeven and Toth, 1995), supposedly because of lower POX activities (Freeman et al., 2001; Freeman et al., 2004).

The main research questions addressed in this study are: Is there a transition zone with lower concentrations of phenolic compounds above the groundwater level and higher concentration below? Do agricultural land use and peat origin (fen or bog) affect the concentrations of soluble and condensed phenolic compounds and potential POX activity? Which factors explain POX activity most? What is the potential POX activity in deep anoxic peat layers compared to well-aerated superficial soil layers?

## 3.2 Materials and methods

### 3.2.1 Sample areas

Peat profile samples were taken in three regions in the Netherlands (Figure 3.2, Appendix 3.A), between July and November 2011 and between August and November 2012. The Zegveld/Nieuwkoopse Plassen region is located in the western peat district. The landscape is characteristic with the appearance of grassland parcels intersected by drainage ditches on a thick layer of fen peat (with the remains of *Alnus*, *Betula*, *Carex* and/or *Phragmites* spp.). In this region, samples were taken at two parcels used for dairy farming in Zegveld, in addition, three meadows in the nearby nature reserve Nieuwkoopse Plassen were sampled.


**Figure 3.2:** Location of the sample areas. 1: Zegveld and Nieuwkoopse Plassen. 2. Tjeukemeer area. 3. Fochteloërveen area

This nature reserve is a mosaic of semi-natural grasslands and reed swamps interwoven with ditches located on fen peat, the groundwater level is within decimeters of the soil surface (Van Den Pol-Van Dasselaar et al., 1997). The Tjeukemeer region is located in the province of Friesland. The peat in this area is generally less nutrient-rich than in the western peat district and both bog peat and fen peat are commonly encountered. In this study, peat profiles up to 3.0 m depth from meadows, agricultural fields and a nature reserve (Rottige Meente) were sampled. The third study area involves the bog reserve Fochteloërveen and surroundings in the province of Drenthe. The bog reserve Fochteloërveen is characterised by ombrotrophic *Sphagnum* peat. This area has been restored between 1986 and 1990, after partial excavations in the past. The core of the area, where sampling took place, is now actively producing *Sphagnum* peat (Schouwenaars, 2002). Furthermore, three agricultural fields near the Fochteloërveen were sampled. A wide range of crops is produced on these fields (sugar beet, potato, corn) but also more capital-intensive cultivations such as cut flowers. To recapitulate, the peat samples that were included in the study, include fen peat and bog peat, from agricultural sites and nature reserves, spread over three regions in the Neherlands.



### 3.2.2 Sampling

Peat samples were collected using an Edelmann soil corer or a peat corer (Eijkelkamp, Breda, the Netherlands). Samples were collected at 19 locations, varying in peat origin and land use intensity. Firstly, at each location four vertical profiles were inspected visually to determine spatial variability. A fifth vertical profile was made, and if samples from this profile were representative for the average pattern of the first four vertical profiles, they were collected. Sampling depths were 20, 40, 60, 80, 100, 120, 140, 160, 180 and 300 cm below soil surface, occasionally samples were collected at 10 cm intervals, e.g. around the groundwater table, or when visual changes were observed. An anaerocult® A mini bag was added to samples from below the water table to ensure anoxic conditions during transport and storage (Anaerocult a mini, Merck, Darmstadt, Germany). Samples were stored at 4 °C until further processing.

### 3.2.3 Measuring potential phenol oxidase (POX) activity

Within 24 h after sampling potential POX activity was determined following the protocol of Pind et al. (1994) and the modifications of Williams et al. (2000). For each peat sample, four subsamples of 2 g of peat were put in separate centrifuge tubes. 10 mL of demineralised water was added and samples were put on a rotary shaker (20 min, 120 rpm) to create soil homogenates. 2 mL of demineralised water was added to two homogenates, to the other two homogenates 2 mL of 3,4-Dihydroxy-L-phenylalanine (L-DOPA) solution (10 mM, Sigma-Aldrich, St. Louis, Missouri, United States) was added. Samples were incubated on a rotary shaker for 20 min (120 rpm). During incubation, part of the L-DOPA is oxidised and the red coloured compound 2,3-dihydroindole-5,6-quinone-2-carboxolate (dicq) is formed. Samples were centrifuged (10 min, 4000 g) to stop the reaction and absorption of the supernatant was measured at 460 nm (in 2011: Shimadzu UV-120-01 spectrophotometer, Shimadzu, Kyoto, Japan; in 2012: Spectrostar, BMG Labtech, Ortenberg, Germany). Average activity of the duplicate samples was calculated using Beer's Law, using the difference in absorbance between samples with and without L-DOPA added and a molar extinction coefficient for diqc. This coefficient of diqc was determined by using a commercial POX preparation (Sigma T7755) to completely oxidise a known amount of L-DOPA and then measuring the absorbance of the reaction product. The activity was expressed in terms of nmol diqc·g $DW^{-1}$·$min^{-1}$). Dry weight was determined by oven drying a subsample of each sample (70 °C, 48 h).

### 3.2.4 Extractions

Within 48 h after sampling, peat samples were extracted with demineralised water. 100 mL of demineralised water was added to 8 g of fresh peat and shaken on a rotary shaker (60 min, 120 rpm). Afterwards, samples were centrifuged (10 min, 4000 g) and filtrated with glass fibre filters (Whatman GF/C, Whatman, Dassel, Germany). The concentration of soluble phenolic compounds was determined using the Folin-Ciocalteau reagent (Box, 1983). 30 µL Folin-Ciocalteau reagent was added to 100 µL of the extract. After eight minutes, 100 µL $Na_2CO_3$ (10.6 g/100 mL) was added. After 40 minutes, absorbance at 760 nm was measured (anno 2011: Shimadzu UV-120-01 spectrophotometer, Shimadzu, Kyoto, Japan, anno 2012: Spectrostar, BMG Labtech, Ortenberg, Germany). In addition, pH of the extracts was measured and $NO_3^-$, $NH_4^+$, $PO_4^{3-}$, $SO_4^{2-}$ and DOC were determined within 2 weeks on a Continuous Flow Analyser (Skalar Analytical, Breda, the Netherlands), after storing the samples at -20 °C.

### 3.2.5 C:N analysis, condensed phenolic compounds and organic matter content

Within 24 h after sampling, peat samples were frozen using liquid nitrogen. Afterwards they were freeze-dried and ground using a ball mill for 1 minute at 15 rps (Retsch MM 200 ball mill, (Retsch GmbH, Haan, Germany) for later analysis of condensed phenolic compounds, C:N ratio and organic matter content. The concentration of total phenolic compounds was determined by a methanol extraction. 30 mg of dried and ground material were extracted in duplicate in 5 mL 50% methanol. Samples were put on a shaker in a water bath at 40 °C for 1 hour and were centrifuged afterwards (10 min, 4000 rpm). The content of phenolic compounds in the supernatant was determined using a slightly modified protocol of Box (1983) for which a standard curve was prepared in 50% methanol. 10 µL of the extract was diluted with 90 µL of MeOH solution to prevent precipitation of fine solids

(Cicco and Lattanzio, 2011), after that, the procedure described above was followed. The concentration of condensed phenolic compounds was calculated as the difference between the total amount of phenolic compounds and the amount of soluble phenolic compounds. C:N ratios were determined using an EA/110 CHNS-O analyser (Interscience BV, Breda, the Netherlands). Organic matter content was determined by loss on ignition (5.5 h, 550 °C).

### 3.2.6 Statistical analysis

Univariate ANOVAs were conducted on the data for the peat profiles. Depth, peat origin and land use were included as covariates. Per study area it was examined whether depth, land use and peat origin affected the concentrations of soluble and condensed phenolic compounds as well as the POX activity. If necessary, data were log-transformed to produce comparable variances. Data were analysed with a principal component analysis to derive the most important relations. Missing values were replaced by the mean. One of the aims of the study was finding more clarity concerning the dynamics of POX activity; therefore, this variable was not included in the factor analysis. However, the component scores were related to the POX activity via a stepwise regression. In addition, regression analyses were done, both per area and per land use type in this area. All statistical analyses were performed with IBM SPSS 20 (IBM software, Armonk, New York, United States). Only samples with an organic matter content higher than or equal to 25% were included in the analysis, samples with a lower organic matter content were not regarded 'peat' samples, but originated from Pleistocenic sand, sandy or clayey layers in the peat profile or sand amendment through agricultural parcels.



## 3.3 Results

### 3.3.1 Profile descriptions

The vertical profiles in Zegveld and Nieuwkoopse Plassen consist fully of fen peat, except for some samples with very low organic matter contents (Figure 3.3a). The profiles in Zegveld in use for dairy farming are drained deeper than those in the nature reserve Nieuwkoopse Plassen. In the Tjeukemeer area both fen and bog peat occurs in the profiles (Figure 3.3b). Here, drainage is considerably deeper, with water tables down to 120 cm below soil surface during summer, except for the meadow in Echtenerbrug, where the water table is generally just a few decimetres below soil surface. In Smilde, there was one field with fen peat and the other locations were characterised by bog peat (Figure 3.3c). Although the agricultural area Smilde is characterised by deep drainage, the Smilde field 1 was relatively wet due to a confined layer of boulder clay at 110 cm, which caused a perched water table. There, relatively pristine peat at a depth of 60 cm and deeper was found, which was not the case in the other Smilde samples. The nature reserve Fochteloërveen had bog peat down to ca 2 m depth.

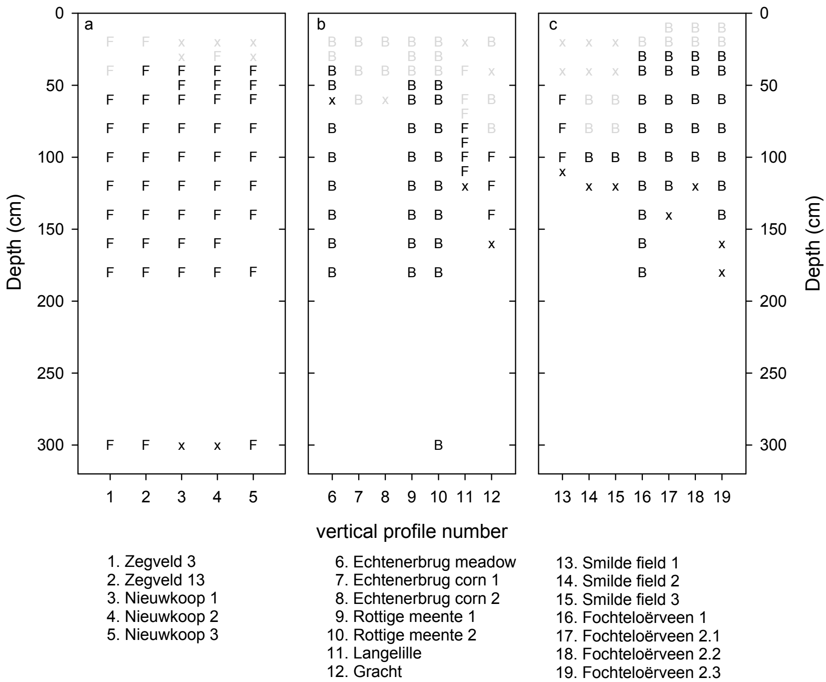



**Figure 3.3:** Description of the vertical profiles of the three case study areas. a=Zegveld and Nieuwkoopse Plassen, b=Tjeukemeer area, c=Smilde and Fochteloërveen area. Samples from above the groundwater at time of sampling are indicated with grey letters, below groundwater at time of sampling is indicated with black letters. F= fen peat, B=bog peat, x=organic matter content <25%, these samples are omitted from further analyses.

## 3.3.2 Soluble phenolic compounds

The fen peat in the Zegveld/Nieuwkoopse Plassen region had generally the lowest concentrations of soluble phenolic compounds, while concentrations were highest in the bog peat region Smilde/Fochteloërveen, see Figure 3.4 (Univariate ANOVA, covariates: depth, peat origin, land use, df=1, F=45.076, $p$<0.001). The concentration of soluble phenolic compounds was higher in the dairy meadow profiles in Zegveld compared to the semi-natural grassland profiles of the Nieuwkoopse Plassen area (df=1, F=47.772, $p$<0.001) (Figure 3.4a, Appendix 3.B). In both areas, concentrations of soluble phenolic compounds increased with depth (F=5.950, $p$<0.001). There was no significant interaction between land use and depth. Similarly, in the Tjeukemeer region, higher concentrations of soluble phenolic compounds were found in the agricultural peat meadows than in the nature reserve Rottige Meente (Figure 3.4b, df=1, F=11.926, $p$<0.001), with concentrations significantly increasing with depth. On the contrary, the concentration of soluble phenolic compounds did not increase significantly with depth in the Smilde/Fochteloërveen region (Figure 3.4c). The nature reserve Fochteloërveen had higher concentrations of soluble phenolic compounds compared to the agricultural fields nearby. In this bog peat, the concentration did increase with depth, while this was not the case in the nearby agricultural fields.

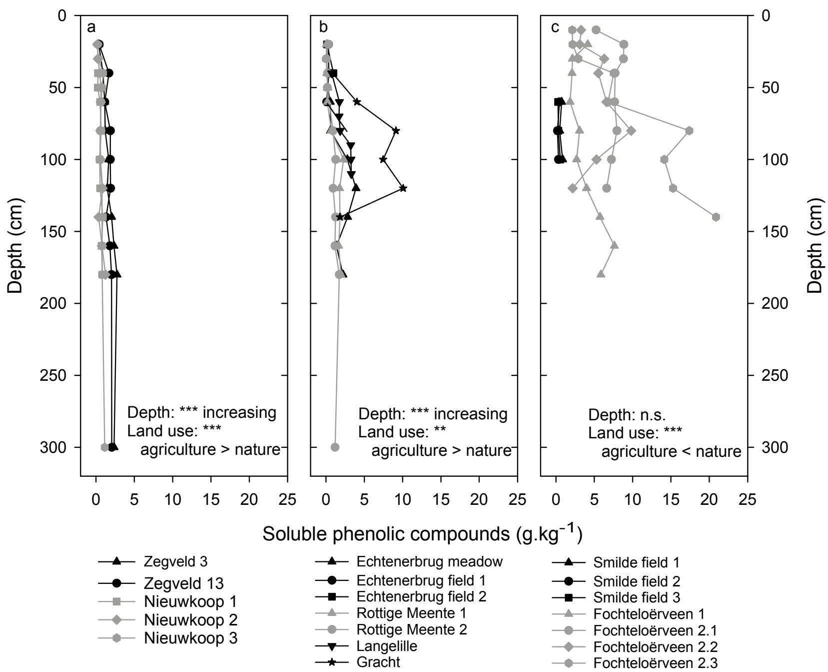



**Figure 3.4:** Concentration of soluble phenolic compounds in vertical profiles in 3 study areas (a=Zegveld and Nieuwkoopse Plassen, b=Tjeukemeer area, c=Smilde and Fochteloërveen area). Agricultural areas are indicated in black, nature reserves are indicated in grey. Only soil samples with OM ≥ 25% are shown. Main results of Univariate ANOVA are shown (*** $p<0.001$, ** $p<0.01$, * $p<0.05$).

### 3.3.3 Condensed phenolic compounds

The concentrations of condensed phenolic compounds were in the same order of magnitude as the soluble phenolic compound concentrations (paired samples t-test $p=0.547$). Highest concentrations were found in the Smilde/Fochteloerveen region (Univariate ANOVA, covariates: depth, peat origin, land use, df=1, F=3.417, $p<0.05$), while the differences between the Zegveld/Nieuwkoopse Plassen and the Tjeukemeer area were non-significant. Generally there were few significant effects of land use and depth on the concentrations of condensed phenolic compounds (Figure 3.5a,b,c). Only in the Tjeukemeer region, depth partly explained the concentrations of condensed phenolic compounds (Appendix 3.B), while the concentration was higher in the nature reserve than in the agricultural fields in the Smilde/Fochteloerveen region.

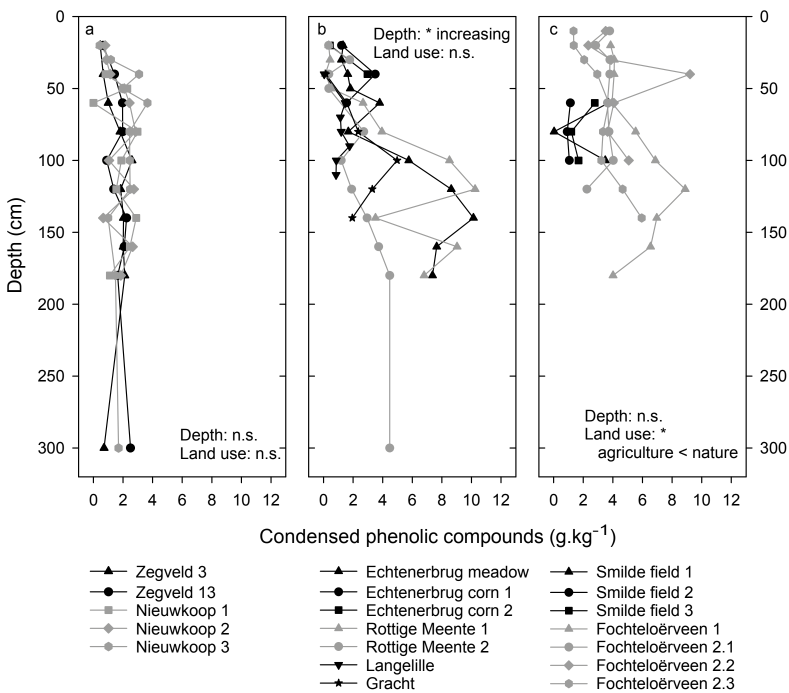



**Figure 3.5:** Concentration of condensed phenolic compounds in vertical profiles in 3 study areas. (a=Zegveld and Nieuwkoopse Plassen, b=Tjeukemeer area, c=Fochteloërveen area). Agricultural areas are indicated in black, nature reserves are indicated in grey. Only soil samples with OM ≥ 25% are shown. Main results of Univariate ANOVA are shown (*** $p$<0.001, ** $p$<0.01, * $p$<0.05).

### 3.3.4 Potential phenol oxidase (POX) activity

Overall, the POX activity was highest in the fen peat than in the bog peat (df=1, F=6.047, $p$<0.05) and higher in the nature reserves than in the agricultural sites (df=1, F=31.065, $p$<0.001). The potential POX activity was higher in the nature reserve Nieuwkoop than in the agricultural area Zegveld ($p$<0.001, Figure 3.6a, Appendix 3.B). ANOVA analyses did not reveal any other significant relations between potential POX activity and land use or depth. Regression analyses, however, showed that in Zegveld, the Tjeukemeer area and Fochteloërveen the potential POX activity significantly increased with depth.

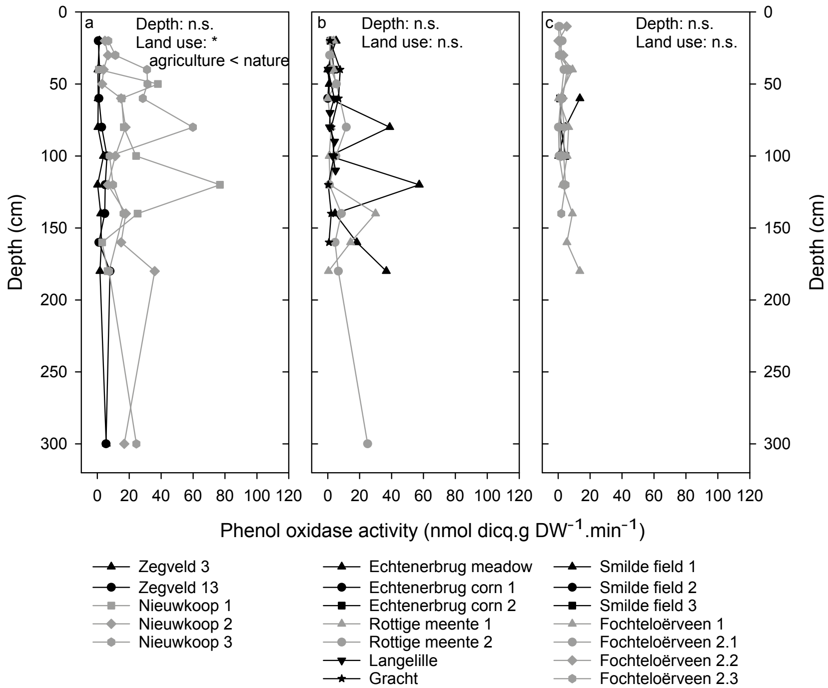



**Figure 3.6:** The potential POX activity in vertical profiles in 3 study areas. (a=Zegveld and Nieuwkoopse Plassen, b=Tjeukemeer area, c=Fochteloërveen area). Agricultural areas are indicated in black, nature reserves are indicated in grey. Only soil samples with OM ≥ 25% are shown. Main results of Univariate ANOVA are shown (*** $p$<0.001, ** $p$<0.01, * $p$<0.05).

## 3.3.5 Nutrients and alternative electron acceptors: Nitrate and sulphate

Nitrate and sulphate are not only nutrients, because in anoxic conditions nitrate and sulphate can function as alternative terminal electron acceptors (TEAs), thereby facilitating anaerobic decomposition. Overall, land use did not affect water-extractable nitrate concentrations (df=1, F=0.474, $p$=0.493), whereas we had expected higher $NO_3$ concentration in the fertilised areas. Concerning peat origin, nitrate concentrations were higher in the fen peat samples than in the bog peat samples (df=1, F=8.149, $p$<0.01). Nitrate concentrations did not show differences across the peat profiles. If the data are analysed for each region separately, there are significantly lower nitrate concentrations in the peat meadows in Zegveld than in the nature reserve Nieuwkoop (Figure 3.7a,d).

Water-extractable sulphate concentrations were generally higher in the fen peat than in the bog peat (df=1, F=8.583, $p$<0.01), as well as higher in nature reserves than in agricultural areas (df=1, F=6.660, $p$<0.05). If analysed for each region separately, sulphate concentrations increased with depth in the Zegveld/Nieuwkoop area and Tjeukemeer area.

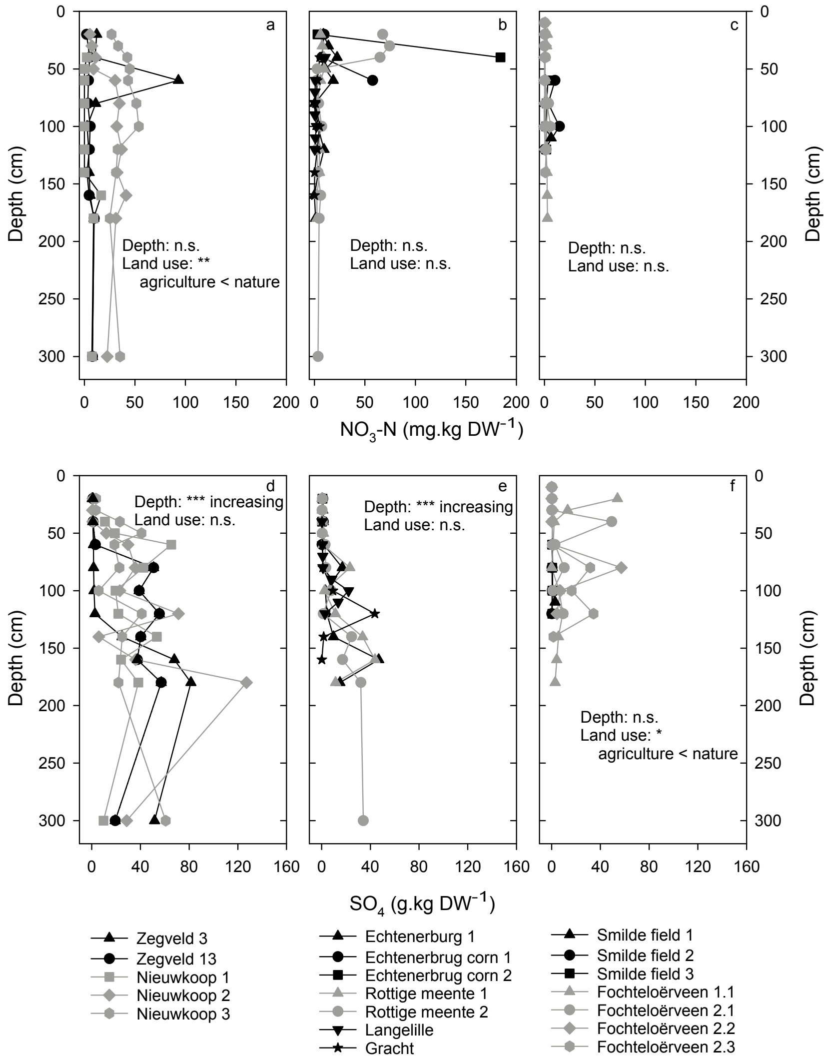



**Figure 3.7:** Water-extractable nitrate and sulphate concentrations in vertical profiles in 3 study areas. (a=Zegveld and Nieuwkoopse Plassen, b=Tjeukemeer area, c=Fochteloërveen area). Agricultural areas are indicated in black, nature reserves are indicated in grey. Only soil samples with OM ≥ 25% are shown. Main results of Univariate ANOVA are shown (*** $p$<0.001, ** $p$<0.01, * $p$<0.05).

### 3.3.6 Nutrients: ammonium and phosphate

Water-extractable ammonium concentrations did not differ between agricultural areas and nature reserves (df=1, F=3.233, $p$=0.075). Whereas, extractable phosphate concentrations were higher in the nature reserves than in the agricultural areas (df=1, F=8.277, $p$<0.01). Ammonium and phosphate concentrations were not influenced by peat origin and both generally increased with depth (df=15, F=2.410, $p$<0.01 and df=15, F=4.521, $p$<0.01).

If the data are considered for each region separately, there are significantly higher ammonium concentrations in the peat meadows in Zegveld than in the nature reserve Nieuwkoop (Figure 3.8a). There is a significant interaction between land use and depth; the ammonium concentrations in Zegveld increase more with depth than in the Nieuwkoopse Plassen. Ammonium concentrations in the peat samples from the Tjeukemeer region and Smilde/Fochteloërveen are generally low except for one of the profiles from the nature reserve Rottige Meente.

3

Water-extractable phosphate concentrations generally increased with depth. In Zegveld, two parcels were sampled with long-term differences in drainage depths. The profile from parcel 13 with superficial drainage (30 cm) showed a sudden increase in phosphate concentrations from a depth of 60 cm downward (below detection limit at 40 cm to 23.90 $mg \cdot kg^{-1}$ at 60 cm), while the deeper drained parcel 3 (55 cm) showed this increase from 80 cm downward (4.36 $mg \cdot kg^{-1}$ at 60 cm to 80 cm 23.93 $mg \cdot kg^{-1}$). From Figure 3.8d it is apparent that the phosphate concentrations in the nature reserve Nieuwkoopse Plassen are higher than in the agricultural area Zegveld, with the highest concentrations closer to the soil surface. The high phosphate concentrations in the nature reserve Fochteloërveen (Figure 3.8f), and to a lesser extent in the Nieuwkoopse Plassen (Figure 3.8e), underline the overall significant effect of land use on phosphate concentrations.

### 3.3.7 Factors explaining potential POX activity

A principle component analysis was performed that grouped the variables in the dataset into two components with an eigenvalue larger than 1, which together explained 44.8% of total variance (Table 3.1 and Figure 3.9a). The first component explained 27.5% of the variance and was correlated to the soil variables: water content, organic matter content, concentration of condensed phenolic compounds, C:N ratio, concentration of soluble phenolic compounds and DOC concentration. The second component explained 17.4% of the variance and was characterised by positive correlations with depth and the concentrations of water-extractable $SO_4^{2-}$, DOC, $PO_4^{3-}$ and $NH_4^+$. When the component scores were correlated to the potential POX activity, the second component correlated significantly with POX activity although the proportion of total variances explained is low ($R^2$= 0.096, $p$<0.001).

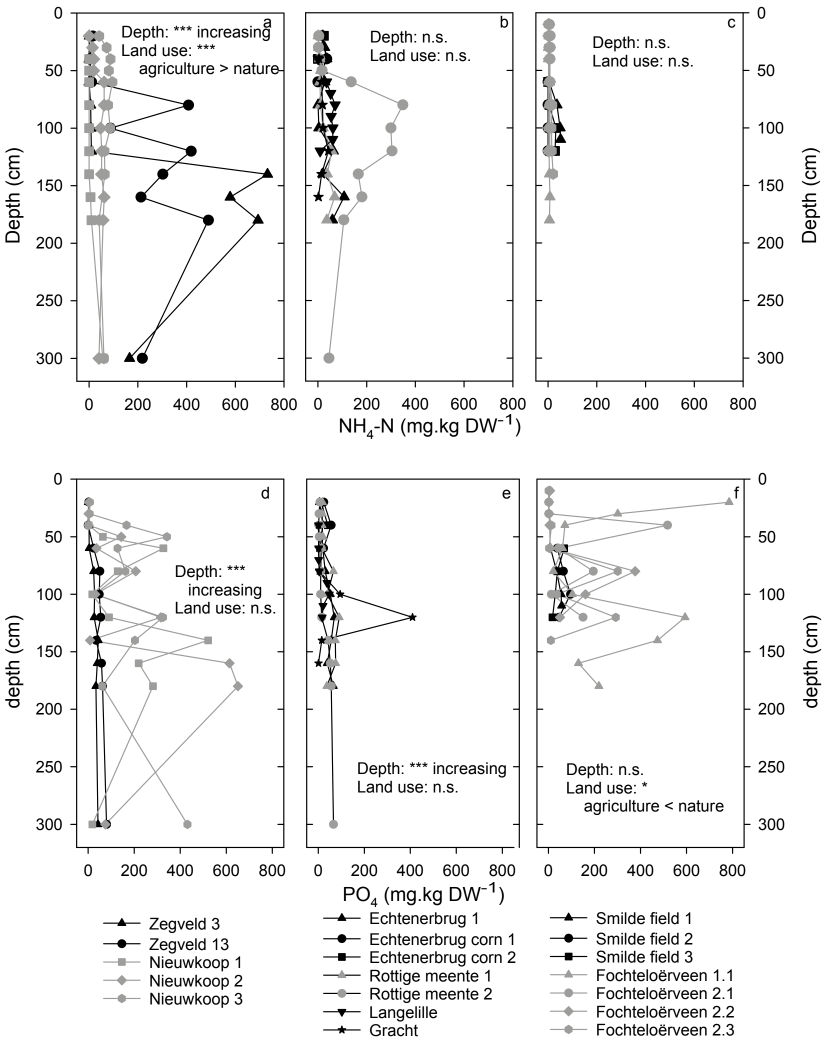



**Figure 3.8:** Water-extractable ammonium and phosphate concentrations in vertical profiles in 3 study areas. (a=Zegveld and Nieuwkoopse Plassen, b=Tjeukemeer area, c=Fochteloërveen area). Agricultural areas are indicated in black, nature reserves are indicated in grey. Only soil samples with OM ≥ 25% are shown. Main results of Univariate ANOVA are shown (*** $p$<0.001, ** $p$<0.01, * $p$<0.05).

Cluster centroids (average scores on each component, with standard errors) for the combinations of land use and peat origin are presented in Figure 3.9b. The cluster centroid of peat samples from the Fochteloërveen and Rottige Meente (bog peat from nature reserves) is positioned in the right part of the figure, close to the horizontal, indicating a strong correlation with the first component and little correlation with the second component. As we saw in the vertical profiles, the samples from the Fochteloërveen, and to a lesser extent those from the Rottige Meente, are characterised by high concentrations of soluble and condensed phenolic compounds. Furthermore, the Fochteloërveen and Rottige Meente showed significantly higher organic matter contents (Fochteloërveen: 92 ± 1%, Rottige Meente: 70 ± 5%) and C:N ratio (Fochteloërveen: 41.5 ± 2, Rottige Meente: 22 ± 2.8) compared to the other peat profiles. The bog peat from the intensive grassland is positioned further to the left on this first axis. Therefore, it is associated with lower concentrations of phenolic compounds and lower C:N (26.5 ± 2). In addition, it is associated with lower nutrient concentrations. The fen peat samples from both land use types seem to be more related to each other than the bog peat samples. This fen peat is associated with higher concentrations of nutrients and $SO_4^{2-}$ and lower organic matter content and C:N.

As the PCA analysis did not lead to a clear explanation of variation in POX activity, individual correlations of POX activity and soil variables were also explored (Table 3.2). Potential POX activity correlated significantly and positively with depth, pH, $PO_4$ and $SO_4$ ($p$<0.05); whilst, potential POX activity tended to be negatively correlated with the concentration of soluble phenolic compounds ($p$<0.1). In general, the variance that was explained was rather low, the highest $R^2$ was found for the correlation between $SO_4^{2-}$ and POX ($R^2$=0.094). The coefficient of determination for this correlation is hardly lower than that for the second factor of the factor analysis ($R^2$=0.096).



**Table 3.1:** Results of factor analysis and regression of component scores with POX. Coefficients smaller than 0.4 are presented in grey, n=145.

| | **Factor 1** | **Factor 2** |
|---|---|---|
| **Water content (g water/g soil)** | 0.961 | -0.170 |
| **Organic matter content (%)** | 0.781 | -0.011 |
| **Condensed phenolic compounds** | 0.669 | 0.213 |
| **C:N ratio** | 0.771 | -0.221 |
| **Soluble phenolic compounds** | 0.677 | -0.013 |
| **$SO_4^{2-}$** | -0.173 | 0.929 |
| **Depth** | -0.011 | 0.649 |
| **DOC** | 0.464 | 0.694 |
| **$PO_4^{3-}$** | 0.137 | 0.596 |
| **$NH_4^+$** | -0.147 | 0.437 |
| **$NO_3^-$** | -0.295 | -0.023 |
| **pH** | -0.330 | 0.159 |
| **Variance explained (%)** | **27.5** | **17.4** |
| **Cumulative variance explained (%)** | **27.5** | **44.8** |
| **Correlation with POX activity** | ***p*=0.366** | ***p*<0.001 $R^2$=0.096** |

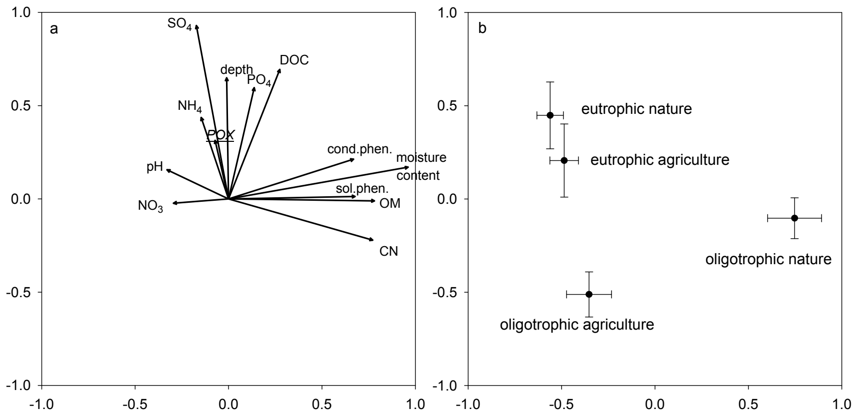



**Figure 3.9:** a. Correlation bi-plot from the principle component analysis on soil variables. Components and component scores are given in Table 3.2. Correlations of the soil variables and POX activity with the main axes/components are given by arrows. b. Correlation bi-plot from the principle component analysis with cluster centroids for the land use and peat origin combinations. n=145.

**Table 3.2:** Correlation coefficients of potential POX activity with the other variables. n=145, slope of the regression line, coefficient of determination and P value (uncorrected for number of comparisons) are reflected. P values <0.05 are black, other values are grey.

| | **Beta** | **$R^2$** | **P value** |
|---|---|---|---|
| **Depth (cm)** | 0.048 | 0.051 | 0.004 |
| **Water content (g water·g dry peat$^{-1}$)** | -0.001 | 0.000 | 0.998 |
| **pH** | 3.689 | 0.038 | 0.018 |
| **OM** | -0.028 | 0.002 | 0.569 |
| **CN** | -0.095 | 0.012 | 0.200 |
| **Condensed phenolic compounds** | 0.558 | 0.010 | 0.225 |
| **Soluble phenolic compounds** | -0.552 | 0.025 | 0.057 |
| **DOC** | 0.068 | 0.013 | 0.179 |
| **$NO_3^-$** | 0.056 | 0.011 | 0.214 |
| **$NH_4^+$** | -0.002 | 0.001 | 0.761 |
| **$PO_4^{3-}$** | 0.017 | 0.045 | 0.010 |
| **$SO_4^{2-}$** | 0.172 | 0.094 | 0.000 |

## 3.4 Discussion

This study was aimed at evaluating the concentrations of phenolic compounds and POX activities in the context of their role in peat decomposition. It was hypothesised that there would be a gradient across the peat profile with low concentrations of phenolic compounds in the aerated zone of peat profiles towards higher concentrations in deeper, anoxic zones, as a result of higher POX activity in oxic conditions (Freeman et al., 2001; Sinsabaugh, 2010). In contrast to these expectations, either a positive effect of depth on potential POX activity or no effect of depth was found, implying that the enzyme POX is present even at great depths in normally anoxic peat conditions. Contrasting information on the correlation between potential POX activity and oxygen availability or depth has been reported in literature as well (Pind et al., 1994; Williams et al., 2000; Zibilske and Bradford, 2007). Most of the locations that have been studied are characterised by downward seepage. Potentially, this could transport the enzyme to deeper peat layers. However, some of the sites we studied (Rottige Meente, Langelille and Smilde) have upward seepage or neutral water flow (pers. comm. P.C. Jansen, Wageningen University and Research centre), so that water flow cannot be exclusively responsible for the presence of POX in deeper soil layers as depth only explained a small part of the variance in potential POX activities. To gain more understanding in the dynamics of POX, we tried to link other variables to POX activity, such as pH. In accordance with a body of literature (Pind et al., 1994; Toberman et al., 2008; Sinsabaugh, 2010; Toberman et al., 2010; Fenner and Freeman, 2011), potential POX activity correlated with pH in our study as well, although only a small part of the variance was explained. The relation between nitrate and sulphate concentrations and POX were also explored as the anaerobic processes sulphate reduction and denitrification have been found to be correlated to the degradation of phenolic compounds (Elder and Kelly, 1994; Heider and Fuchs, 1997). However, POX was not correlated with nitrate concentrations.

The increasing sulphate concentrations with depth were unexpected. Based on the results of Chapter 2, where oxygenation led to almost a doubling in soluble sulphate, higher sulphate concentrations in the upper part of the soil profiles were expected. Other studies on peat soils either did not find a clear pattern of sulphate concentrations in soil profiles (Kravchenko and Sirin, 2007) or found that sulphate rapidly decreased in the upper 10 cm and remained unchanged deeper down the peat profiles studied (Blodau et al., 2007). The individual regression analyses in our study showed that pH, $SO_4^{2-}$ and $PO_4^{3-}$ correlated with potential POX activity. Possibly, sulphate is used as a terminal electron acceptor in the oxidation of phenolic compounds (Elder and Kelly, 1994). Another suggestion is that in conditions where pyrite oxidation takes place, also POX activity can be found. However, pyrite oxidation releases $H^+$ into the environment and POX activity is low in acidic conditions. Hence, this biogeochemical part of the factors involved in POX activity remains unclear.

Nitrate and ammonium concentrations did not affect POX activity, although we had expected negative effects of nitrogen availability on POX activity based on Sinsabaugh (2010). Moreover, nitrate and ammonium concentrations did not differ between natural areas and agricultural peatlands, while the latter are fertilised. It is plausible that the added nutrients are efficiently taken up by the vegetation or microorganisms and do not accumulate in the pore water (see also Chapter 5). A positive correlation between POX and $PO_4^{3-}$ concentrations was found, which could indicate a phosphate limitation of decomposition. Phosphate limitation of decomposition processes has been reported repeatedly in wetlands, although mostly in fresh litter (Qualls and Richardson, 2000; Craine et al., 2007).
Perhaps the most intriguing result of this study of vertical soil profiles is the presence and high potential activity of POX in deeper, anoxic peat layers. In addition, POX was correlated with abiotic factors such as sulphate concentrations, depth, phosphate concentrations and pH, whereas, variables related to the organic matter quality such as C:N, concentrations of soluble and condensed phenolic compounds and organic matter content did not affect potential POX activity. Correlation bi-plots show that fen peat is associated with higher potential POX activity than bog peat. This is also found in Chapter 5. Our results indicate that there are (unmeasured) peat origin related factors that have a major influence on potential POX activity. It was shown that various phenolic compounds have a different role in decay resistance (Hájek et al., 2010). So, the quality, rather than the quantity, of phenolic compounds could be a master factor affecting the decomposition process.

## 3.5 Conclusion

Vertical soil profiles were studied in fen and bog peat soils under natural and agricultural land use. Phosphate and sulphate concentrations were higher in nature reserves than in agricultural sites, whereas, water-extractable nitrate and ammonium concentrations were not affected by land use. Sulphate concentrations where higher in fen peat than in bog peat, which is attributed to the pyrite that is present in minerotrophic peat. Although POX activity is generally higher in oxic conditions, we found a higher potential POX activity with increasing depth. Although potential activities do not necessarily correspond to actual activities, the presence of a presumed oxidative enzyme at 3 m depth was remarkable. Contradictory to our hypotheses, the phenolic compound concentrations were not evidently related to drainage depth or other land use related factors. The examined soil properties only explained a small proportion of the variance in POX activity. Surprisingly, POX correlated positively with abiotic factors such as sulphate and phosphate concentrations, depth and pH. The correlation with sulphate could indicate a role of sulphate in anaerobic POX activity, or a correlation with pyrite oxidation. However, the positive correlation with pH is contradictory with a correlation with pyrite oxidation. Variables related to organic matter quality such as C:N, phenolic compound concentration and organic matter content did not explain potential POX activity. In general, fen peat was associated with higher potential POX activities than bog peat. Perhaps, the quality rather than the quantity of phenolic compounds or other organic matter affect potential enzyme activities.

## Acknowledgements

This study was supported by the research program Knowledge for Climate (Kennis voor Klimaat), hotspot 'shallow waters and peat meadow areas' project code HSOV1a. We are grateful to Natuurmonumenten Nieuwkoopse Plassen and Fochteloërveen, the Veenweide Innovatiecentrum (peat meadow innovation centre) and several farmers for allowing us to take soil samples at their properties.

# Appendix

**Appendix 3.A:** Characteristics of the sample locations (BIS Nederland, 2013).

| Location | Coordinates | Land use | Vegetation | Description | Watertable (highest-lowest, cm below soil surface) |
|---|---|---|---|---|---|
| **Zegveld and Nieuwkoopse Plassen** | | | | | |
| Zegveld 3 | N 52.138818, E 4.835930 | Dairy meadow | Lolium perenne | Earthified top soil, wood/Carex peat, ca 5 m peat | 55-70 |
| Zegveld 13 | N 52.136975, E 4.837528 | Dairy meadow | Lolium perenne | Earthified top soil, wood/Carex peat, ca 5 m peat | 30-50 |
| Nieuwkoop 1 | N 52.138398, E 4.794520 | Nature reserve | Calthion palustris-alliance | Carex peat | <25-<50 |
| Nieuwkoop 2 | N 52.141045, E 4.800936 | Nature reserve | Calthion palustris-alliance | Carex peat | <25-<50 |
| Nieuwkoop 3 | N 52.140689, E 4.798340 | Nature reserve | Calthion palustris-alliance | Carex peat | <25-<50 |
| **Tjeukemeer area** | | | | | |
| Echtenerbrug meadow | N 52.878385, E 5.806065 | Dairy meadow | Lolium perenne | Organic clay cover on clayey Sphagnum peat | <25-120 |
| Echtenerbrug field 1 | N 52.862636, E 5.807438 | | corn | Sphagnum peat | <25-120 |
| Echtenerbrug field 2 | N 52.851019, E 5.809481 | | corn | Sphagnum peat | <25-120 |
| Rottige meente 1 | N 52.827851, E 5.918675 | Nature reserve | Calthion palustris-alliance | Clayey Sphagnum peat | <25-80 |
| Rottige meente 2 | N 52.827637, E 5.885827 | Nature reserve | Calthion palustris-alliance | Clayey Sphagnum peat | <25-80 |
| Langelille | N 52.840141, E 5.835934 | Dairy meadow | Lolium perenne | Carex peat | <25-120 |
| Gracht | N 52.817997, E 5.859451 | Dairy meadow | Lolium perenne | Barely earthified Sphagnum peat on Carex, Phragmites or wood peat | <25-120 |

**Appendix 3.A:** Continued

| Location | Coordinates | Land use | Vegetation | Description | Watertable (highest-lowest, cm below soil surface) |
|---|---|---|---|---|---|
| **Fochteloërveen area** | | | | | |
| Smilde field 1 | N 52.954118, E 6.413564 | | this year: mustard | Topsoil consisting of sand, sometimes organic or humic. From 60 cm: wood/ Phragmites peat | 40-120 |
| Smilde field 2 | N 52.986572, E 6.458576 | | this year: sugar beet | Topsoil consisting of sand, sometimes organic or humic. From 60 cm: Sphagnum peat | 40-120 |
| Smilde field 3 | N 52.985720, E 6.460980 | | this year: potato | Topsoil consisting of sand, sometimes organic or humic. From 60 cm: Sphagnum peat | 40-120 |
| Fochteloërveen 1 | N 52.990897, E 6.394049 | Nature reserve | Sphagnum spp., Molinia spp. | Sphagnum peat | 0-25 |
| Fochteloërveen 2.1 | N 52.995198, E 6.391571 | Nature reserve | Sphagnum spp., Molinia spp. | Sphagnum peat | 0-25 |
| Fochteloërveen 2.2 | N 52.992976, E 6.393995 | Nature reserve | Sphagnum spp., Molinia spp. | Sphagnum peat | 0-25 |
| Fochteloërveen 2.3 | N 52.990768, E 6.395819 | Nature reserve | Sphagnum spp., Molinia spp. | Sphagnum peat | 0-25 |

**Appendix 3.B:** Results of statistical analyses of soluble phenolic compounds, condensed phenolic compounds and POX activity in vertical peat profiles

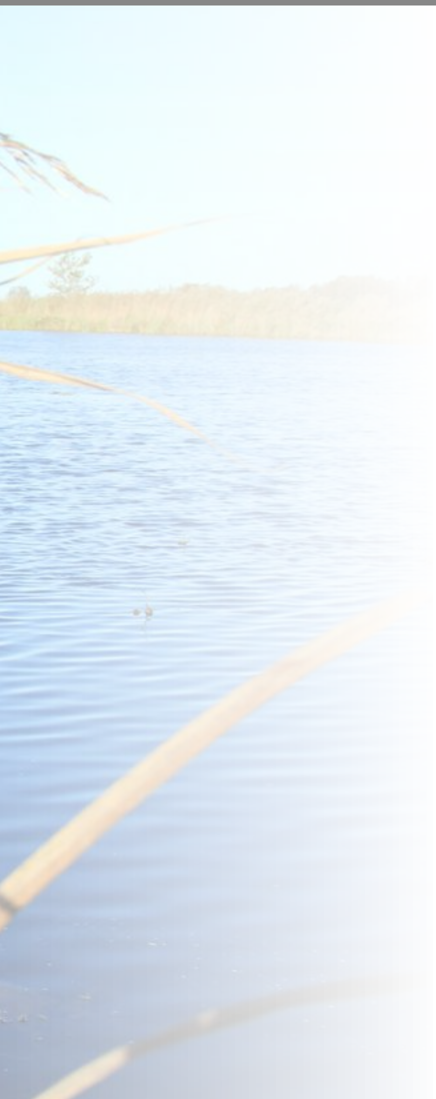
| | | **Soluble phenolic compounds (log)** | | **Condensed phenolic compounds (log)** | | **POX activity (log)** | |
|---|---|---|---|---|---|---|---|
| | df | F | p | F | p | F | P |
| **Zegveld & nieuwkoop** | | | | | | | |
| Land use | 1 | 47.772 | 0.000 | 1.254 | 0.272 | 4.253 | 0.049 |
| | | Agriculture > nature | | | | Agriculture < nature | |
| Depth | 11 | 8.950 | 0.000 | 1.891 | 0.085 | 1.007 | 0.465 |
| | | increase with depth | | | | | |
| Depth * land use | 9 | 1.091 | 0.400 | 0.380 | 0.935 | 0.634 | 0.758 |
| Parcel | 1 | 2.863 | 0.102 | 2.680 | 0.113 | 1.458 | 0.237 |
| **Regression with depth** | | | | | | | |
| All land uses | | $R^2$=0.330<br>*p*=0.000<br>*f*=0.441+0.005*depth | | n.s. | | n.s. | |
| Agriculture | | $R^2$=0.550<br>*p*=0.000<br>*f*=0.921+0.006 *depth | | $R^2$=0.469<br>*p*=0.000<br>*f*=-0.288 +0.041*depth | | $R^2$=0.358<br>*p*=0.005<br>*f*=0.625+0.17*depth | |
| Nature | | $R^2$=0.492<br>*p*=0.000<br>*f*=0.336+0.003 *depth | | n.s. | | n.s. | |
| **Tjeukemeer area** | | | | | | | |
| Land use | 1 | 11.926 | 0.002 | 1.309 | 0.264 | 0.420 | 0.523 |
| | | Agriculture > nature | | | | | |
| Depth | 14 | 5.209 | 0.000 | 3.351 | 0.005 | 1.412 | 0.222 |
| | | increase with depth | | increase with depth | | | |
| Depth * land use | 10 | 0.645 | 0.762 | 0.185 | 0.996 | 1.237 | 0.318 |
| Parcel | 1 | 14.624 | 0.001 | 7.405 | 0.012 | 1.813 | 0.191 |
| **Regression with depth** | | | | | | | |
| All land uses | | $R^2$=0.091<br>*p*=0.031<br>*f*=0.697+0.011*depth | | $R^2$=0.343<br>*p*=0.000<br>*f* =0.432+0.029*depth | | $R^2$=0.181<br>*p*=0.002<br>*f* =-0.582 +0.085*depth | |
| Agriculture | | $R^2$=0.203<br>*p*=0.014<br>*f*=0.198+0.027*depth | | n.s. | | $R_2$= 0.207<br>*p*=0.013<br>*f*=-3.529+0.140*depth | |
| Nature | | $R^2$=0.501<br>*p*=0.000<br>*f*=0.160 +0.008*depth | | $R^2$= 0.287<br>*p*=0.010<br>*f*=0.665+0.024*depth | | $R^2$= 0.321<br>*p*=0.006<br>*f*=-0.160+0.064 *depth | |

**Appendix 3.B:** Continued

| | | Soluble phenolic compounds (log) | | Condensed phenolic compounds (log) | | POX activity (log) | |
|---|---|---|---|---|---|---|---|
| | df | F | p | F | p | F | P |
| **Fochteloërveen area** | | | | | | | |
| Land use | 1 | 12.118 | 0.002 | 29.378 | 0.000 | 0.166 | 0.687 |
| | | Agriculture < nature | | Agriculture < nature | | | |
| Depth | 10 | 2.150 | 0.054 | 1.825 | 0.102 | 1.533 | 0.180 |
| Depth * land use | 2 | 0.792 | 0.463 | 2.629 | 0.090 | 0.804 | 0.458 |
| Parcel | 1 | 17.302 | 0.000 | 5.987 | 0.021 | 1.389 | 0.248 |
| **Regression with depth** | | | | | | | |
| All land uses | | n.s. | | $R^2$=0.097<br>*p*=0.042<br>*f* =2.669 +0.014*depth | | n.s. | |
| Agriculture | | n.s. | | n.s. | | n.s. | |
| Nature | | $R^2$=0.164<br>*p*=0.017<br>*f*=4.065 +0.039 *depth | | $R^2$=0.223<br>*p*=0.005<br>*f*=2.953+0.018*depth | | n.s. | |

# Chapter 4

# The effects of salinisation on aerobic and anaerobic decomposition and mineralisation in peat meadows: the roles of peat origin and land use



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*Journal of Environmental Management 143 (2014) 44-53*

## Abstract

Peat soils can be commonly found in the western and northern Netherlands. Drainage for agriculture has caused increased soil aeration which has stimulated decomposition and, hence, soil subsidence, currently amounting to 1-2 $cm \cdot yr^{-1}$. River water is supplied to part of these peat areas in summer to prevent drying out of the soils. Saltwater intrusion and evaporation make this surface water slightly brackish during drought periods. In addition, brackish seepage can surface more easily during such dry periods. Incubation experiments were carried out to study the effects of salinisation on aerobic decomposition and mineralisation of superficial peat samples and anaerobic decomposition and mineralisation of deep peat samples. Peat samples originated from sites with two different land uses: agricultural peat meadows and nature reserves, and two different peat substrates within each of these: fen peat and bog peat. In general, salinisation reduced the aerobic decomposition by 50%, whereas the anaerobic decomposition rates remained unchanged. Remarkably, the response of decomposition to salinisation did not depend on peat origin and land use. Regarding mineralisation, however, ammonium concentrations increased in samples from nature reserves, probably as a result of reduced nitrification. Whereas, in samples from agricultural sites, nitrate accumulated. Phosphate concentrations increased, possibly caused by changes in desorption and adsorption processes due to higher sulphate concentrations. DOC concentrations decreased in the salinified samples due to precipitation of particulates. Furthermore, the fen peat samples showed increasing sulphate concentrations, which was attributed to pyrite oxidation. Independently of salinisation, nitrification rates were higher in the agricultural, fertilised, peat soils. In conclusion, while salinisation might reduce subsidence rates, it will have adverse effects on water quality.

## 4.1 Introduction

Peat meadows and associated shallow waters comprise a major part of the land area of the Netherlands. These peat areas have been formed in the course of the Holocene, when wet conditions prevailed and decomposition of organic material was impeded. Large parts of the Dutch peatlands have been in contact with seawater during their formation regularly. The geological events of transgression, periods in which the sea found its way onto the land surface due to relatively fast sea level rise, and regression, periods with a retreat of the sea, caused layered patterns of clay and peat, especially seen near the river mouths. In these landscapes, eutrophic fen peat was formed near rivers and in groundwater seepage areas, while oligotrophic bog peat developed on higher grounds.

Since their reclamation for agriculture in the Middle Ages, drainage for agricultural use has resulted in enhanced peat decomposition rates. Among the consequences of the drainage are soil subsidence (Schothorst, 1977), greenhouse gas emissions (Best and Jacobs, 1997; Blodau and Moore, 2003; Limpens et al., 2008; Van den Akker et al., 2008; Berglund and Berglund, 2011) and surface water pollution (MNP & RIVM, 2002; RIVM, 2009). In the past 50 years, water levels in the Dutch peat meadow areas have been drawn down even more to facilitate intensive dairy farming, resulting in land subsidence rates up to 1-2 $cm \cdot yr^{-1}$ (Schothorst, 1977; Janssen, 1986; Querner et al., 2012).

Dry summers are a concern to water boards, think about the dyke breach in Wilnis, the Netherlands, in 2003, but also the increased aeration of the peat stimulating its decomposition and drought damage for farmers are amongst the main reason to keep water levels in summer artificially high. In summer, additional water originating from rivers or lakes is supplied to the peat areas. However, during prolonged summer droughts, which are expected to become more frequent with climate change, the river water has a poor quality and may become slightly brackish because of saltwater intrusion and evaporation (Satijn and Leenen, 2009). Supplying this water to peat areas causes salinisation. Apart from surface water salinisation, groundwater is also prone to become more saline due to climate change. Due to water deficiencies in summer and other causes such as drainage, subsidence and sea level rise, the upward groundwater seepage pressure increases relative to the downward pressure of surface waters and superficial aquifers. In some peat areas, this seepage is brackish because the groundwater is in contact with marine sediments (De Louw et al., 2011).

Although peat areas had locally been influenced by brackish water during their formation (Bakker and Van Smeerdijk, 1982), questions have been arising about the effects of the recent summer salinisation on peat decomposition and mineralisation (Lamers et al., 1998; Smolders et al., 2006). Generally, a sudden increase in salinity, usually expressed as the concentration of chloride ions, will impede decomposition as the increase in osmotic value can pose a stress to microorganisms (Laura, 1974; Pathak and Rao, 1998; Setia et al., 2010).

However, there are salinity-resistant archaea, bacteria and eucaryota which can accumulate salts or osmolytes to adjust their osmotic potential; nonetheless, such mechanisms demand energy (Oren, 1999) and carbon mineralisation efficiency becomes lower (Setia et al., 2011). Hence, a higher salinity is expected to hamper both aerobic and anaerobic decomposition rates.

Next to chloride salts, sulphate salts are an important component of brackish river water and groundwater, as the chemical composition reflects sea salt. In contrast to the expected reduction in decomposition rates because of chloride salts, sulphate salts, the other hand, stimulate anaerobic decomposition because sulphate is one of the alternative terminal electron acceptors (TEAs). When studying the effect of salinisation on decomposition rates, understanding the role of terminal electron acceptors (TEAs) is crucial. In oxic conditions, oxygen functions as the main TEA. Alternative TEAs under anoxic conditions, in the order of declining thermodynamic yields, are nitrate ($NO_3^-$), manganese ($Mn^{4+}$), ferric iron ($Fe^{3+}$), sulphate ($SO_4^{2-}$), and ultimately $CO_2$ which leads to $CH_4$ production (Rydin and Jeglum, 2006). Adding electron acceptors such as sulphate to anoxic peat soils can therefore lead to the shift from methanogenesis, an anaerobic slow process, to sulphate reduction, which is a faster process (Capone and Kiene, 1988; Canavan et al., 2006). In inundated salt-affected soils, sulphate reduction is known to be one of the most important decomposition pathways (Canavan et al., 2006; Jørgensen et al., 2009). Hence, the addition of sulphate might stimulate anaerobic peat decomposition.

Conflicting results have been reported in the literature on the effects of salinity on nitrogen mineralisation in organic matter in general; literature on the effects of salinity on mineralisation of peat soils in particular is scarce. The results of these studies were often dependent on the methodology, in addition to differences between soil types and salt composition and concentration. However, it has been found that ammonification and nitrification can be affected by salinisation. Laura (1974) found that nitrogen mineralisation increased after salinisation as total nitrogen content of the organic matter decreased substantially with higher salt concentrations. Experimental additions of artificial seawater to inundated peat cores resulted in lower nitrification rates (Portnoy and Giblin, 1997). McCLung and Frankenberger Jr. (1987) compared nitrification and ammonification rates in silt loam soils after chloride and sulphate additions and noticed that, while NaCl treatments almost completely stopped nitrification, the same concentration of $Na_2SO_4$ resulted in only 27% reduction in nitrification. Ammonification rates also decreased due to salinisation, and also more due to NaCl addition than due to $Na_2SO_4$ (McClung and Frankenberger Jr., 1987). In a study in which also fertilisation was included, which is highly relevant in the framework of our study as most of the Dutch peatlands have been intensively fertilised, lower ammonification rates in salt-amended samples were found (Irshad et al., 2005). The concentrations of ammonium and nitrate declined more strongly with increasing salt concentration in samples treated with urea and manure than in the samples without fertilisers. In general, both ion adsorption and mineralisation rates are likely to be affected by salinisation, with consequences for nutrient release and water quality. Increased ionic strength due to salinisation could increase ion release from the peat complex as a result of higher cation exchange. On a longer time span, nitrification might be hampered, which could lead to increased ammonium concentrations; however, when also ammonification is hampered, both ammonium and nitrate concentrations are expected to decrease.

Phosphate and DOC release from the soil complex can be affected by salinisation as well. Firstly, sulphate, chloride and phosphate compete for the same anion adsorption sites (Beltman et al., 2000). In addition, sulphide, which is produced when sulphate is reduced, interferes with the iron-phosphorus cycle by reducing iron$^{3+}$(hydr)oxides and iron$^{3+}$-phosphates. The insoluble $FeS_x$ that is formed reduces the availability of iron to bind phosphate, thereby increasing phosphate mobility, as reviewed by Smolders et al. (2006a), although no clear relations between soluble sulphate and phosphate were found in our studies (Chapter 2 and Chapter 3). Secondly, the dynamics of Dissolved Organic Carbon (DOC) might also change due to salinisation. It has been hypothesised that DOC concentrations in the surface water decrease with salinisation because of DOC precipitation (Monteith et al., 2007; Hruska et al., 2009).

In this study, experiments were performed in order to explore the effects of groundwater and surface water salinisation on anaerobic and aerobic decomposition and net N and P mineralisation rates of peat in peat meadow areas in the Netherlands. More specifically, the effect of salinisation on the aerobic and anaerobic decomposition and mineralisation rates were compared in peat samples differing in origin and land use history in a full factorial comparison. We used fen peat as well as bog peat samples, both peat origins were sampled in nature reserves as well as in dairy meadows (Table 1.2). The fen peat samples consisted of the remains of a *Carex* spp. and *Phragmites* spp. dominated vegetation and the bog peat samples are formed by the remains of a *Sphagnum* spp. dominated vegetation. *Sphagnum* peat is known for its higher concentration of phenolic compounds and more acidic conditions, which both result in higher resistance against decomposition (Verhoeven and Toth, 1995). Next to water level manipulations, agricultural land use affects belowground cycling of carbon and nutrients through inputs of chemical fertilisers and manure, which has led to the degradation of the pristine peat to amorphous peat and a change in the microbial community (Jaatinen et al., 2008). Although nitrogen addition stimulates the decomposition of easily degradable organic matter, it has been found to hamper the decay of recalcitrant compounds (Mack et al., 2004; Knorr et al., 2005b; Craine et al., 2007). This study aims at clarifying the effects of land use and peat origin on decomposition and mineralisation characteristics as well as their response to salinisation.

# 4.2 Material and methods

## 4.2.1 Study sites

Soil samples for the experiment were collected at four sites contrasting in peat origin (fen peat vs. bog peat) and land use (agriculture vs. nature) (Table 1.2). Two sample sites were located in agricultural peat meadows used for dairy farming with a monoculture of *Lolium perenne*, i.e. in the polder Zegveld, characterised by a thick layer of fen peat, and in the Veenpolder van Echten in Friesland, characterised by bog peat. In addition, samples were collected in two Dutch nature reserves, i.e. the Fochteloërveen and the Nieuwkoopse Plassen. The Fochteloërveen is a restored ombrotrophic bog in Friesland and is characterised by bog peat, whereas the Nieuwkoopse Plassen is a nature reserve with species-rich grasslands underlain by fen peat (Table 4.1).

**Table 4.1:** Sample locations and peat characteristics. *) highest-lowest, cm below soil surface, source (BIS Nederland, 2013). Differing letters behind mean ± standard error of pH, OM and C/N indicate significant differences according to ANOVA and Bonferroni-corrected post-hoc tests.

| **Location characteristics** | | | | **Sample characteristics** | | | | |
|---|---|---|---|---|---|---|---|---|
| **Peat origin** | **Land use** | **Location** | **Water Table*** | **Oxygen status** | **Sampling depth (m)** | **pH** | **OM (%)** | **C:N** |
| **Fen peat** | Agriculture | N 52.138818, E 4.835930 | 35-80 | oxic | 0.5 | 5.9±0.04 b | 62±0.7 a | 9.6±0.2 a |
| | | | | anoxic | 2.0 | 5.9±0.02 b | 77±0.7 b | 13±0.3 b |
| **Bog peat** | Agriculture | N 52.874654, E 5.805269 | 10-75 | oxic | 0.3 | 6.4±0.01 c | 58±1.0 a | 12±0.2 b |
| | | | | anoxic | 1.5 | 6.4±0.11 c | 94±0.1 c | 31±1.1 e |
| **Fen peat** | Nature | N 52.140689, E 4.798340 | 5-40 | oxic | 0.2 | 6.1±0.08 b | 61±1.7 a | 12±0.4 ab |
| | | | | anoxic | 1.5 | 6.3±0.02 c | 74±1.0 b | 16±0.7 c |
| **Bog peat** | Nature | N 52.990897, E 6.394049 | 0-25 | oxic | 0.1 | 5.9±0.12 b | 98±0.2 d | 25.1±0.78 d |
| | | | | anoxic | 0.7 | 4.5±0.05 a | 97±0.1 cd | 32±1.40 e |

## 4.2.2 Experiment 1: incubation experiment

At each field site, five samples were collected from both oxic and anoxic layers, using an Edelman corer (see table 4.1 for sampling depths). The samples from anoxic layers were transferred immediately into bags containing an Anaerocult A Mini incubation bag (Merck, Darmstadt, Germany) to prevent oxidation and stored at 4 °C for four days. After storage, the samples were mixed and coarse material was removed. Subsamples were taken to determine dry weight (70 °C, 48 h) and the organic matter content (550 °C, 5.5 h). Sample bags with samples from the anoxic layers were flushed with liquid nitrogen (5 min) to restore anaerobic conditions. Pore water was sampled from the soil samples that were still in the sample bags using rhizons (Eijkelkamp, the Netherlands). Part of the sampled water was mixed with artificial seawater to increase the pore water salt concentration to 4‰ (for comparison: seawater contains 35‰ of salts). The obtained brackish pore water was mixed with the peat samples to create samples with a brackish treatment. The control samples were mixed with pore water diluted with demineralised water to obtain the same dilution factor as the brackish pore water. Maximum water-holding capacity was obtained for the anoxic samples and WHC was 60% in the oxic samples. The seawater was prepared according to the Woods Hole recipe (Cavanaugh, 1956). The weight based $SO_4^{2-}$:$Cl^-$ ratio in this mixture is approximately 1:7, the mole based ratio is approximately 1:200.

After mixing the pore water through the samples, 10 g of each sample was weighed into a 300 mL infusion flask for each time step (0, 4, 8, 16 weeks) and five replicates were used, amounting to 320 flasks (2 salinity treatments*2 land uses*2 peat origins*2 oxygen conditions*5 replicates*4 time steps). Flasks were closed with airtight stoppers. The flasks for the anoxic treatments were degassed and flushed with $N_2$ gas three times and then further flushed with $N_2$ gas for 10 min. Samples were incubated in dark conditions at 20 °C. Headspaces were flushed every four weeks with $N_2$ or fresh air for the anoxic and oxic treatments respectively, to prevent too high levels of $CO_2$ and $CH_4$ or too low $O_2$ levels in the oxic treatments.

The flasks that were incubated for the whole experimental period of 16 weeks were repetitively sampled for gas analysis at 12 times during the incubation. At each of these times, 15 mL of gas was sampled and, directly afterward 15 mL of $N_2$ gas or air, depending on the treatment, was added to compensate for lost pressure. $CH_4$ concentrations were measured on a HP 5890A gas chromatograph fitted with a Porapak N column and flame ionisation detector (FID) with external standards. The $CO_2$ concentrations were measured on an EGM-4 infrared gas analyser (PP Systems, Hertfordshire, UK).

After 0, 4, 8 and 16 weeks, 80 flasks were taken for analyses of soil and water chemistry. 100 mL demineralised water was added to each flask, samples were shaken (3 h, 100 rpm) and filtered (Whatman 595½, pore size 4-7 μm) at 4 °C. For soil extractions of the anoxic samples, the demineralised water was first purged with $N_2$ gas to remove oxygen. Subsequently it was supplied to the soil samples via a syringe that was pierced through the airtight stopper, while simultaneously air was released via a needle. Dry weight was determined by drying and weighing the filters containing peat (70 °C, 48 h) and deposits in the infusion flask. pH of extracts was measured. Extracts were analysed on a continuous flow analyser (SA-40, Skalar Analytical, Breda, the Netherlands) for $NO_3^-$, $NH_4^+$, $PO_4^{3-}$, $SO_4^{2-}$ and DOC concentrations. C:N ratio of peat samples from incubation times t0 and t16 was measured using a CHNS analyser (Interscience Instruments, Breda, the Netherlands).

### 4.2.3 Experiment 2: potential nitrification

The effect of salinisation on potential nitrification rates of bog peat samples from the nature reserve and the agricultural peat meadow was evaluated, since the results of experiment 1 indicated that nitrification might be hampered due to salinisation in the oxic bog peat from the nature reserve Fochteloërveen. Therefore, in six-fold replication, slurries of 10 g of freshly sampled peat and 150 mL solution were created. Solutions consisted of 2 mM $KH_2PO_4$ to buffer pH changes and 1 mM $(NH_4)_2SO_4$ as an ammonium source (Smits et al., 2010). In addition, artificial seawater was added to part of the solutions to create a brackish treatment with an $SO_4^{2-}$ concentration of 0.28 $g \cdot L^{-1}$ (Cavanaugh, 1956). Samples were put on a rotary shaker at 25 °C in dark conditions. 5 mL subsamples of the slurries were taken after 6, 12, 24, 48, 96, 168, 240 and 360 h, centrifuged (6 min, 10,000 rpm), decanted and stored at -20 °C until analysis on a continuous flow analyser for $NO_3^-$ and $NH_4^+$ concentrations. At each sampling time, the pH of the incubation medium was checked and restored to its original value with 0.1 M NaOH or 0.1 M HCl, if necessary.

### 4.2.4 Experiment 3: salt effects on DOC concentrations

Extracts of fen and bog peat samples from the nature reserves Nieuwkoopse Plassen and Fochteloërveen were measured in a time series to evaluate the effects of salt on DOC concentrations in soil. 5 g of fresh peat from aerobic peat layers were extracted with 100 mL demineralised water. Two 5 g subsamples of each replicate soil sample were weighted and placed in a 300 mL incubation flask. One sample was amended with 100 mL demineralised water, whereas the other sample was amended with 100 mL 0.2 M $Na_2SO_4$. These samples were shaken for three hours. Afterward, samples were filtered (Whatman GF/C 1.2 µm) and 80 mL was stored at 4 °C and analysed colourimetrically on a continuous flow analyser for DOC concentration (SA-40, Skalar Analytical, Breda, the Netherlands) after 0, 1, 2, and 3 weeks.

### 4.2.5 Statistical analysis

Data analysis was carried out using a mixed design ANOVA (Repeated Measures, IBM SPSS 20). Treatment (control and salt) was used as a within subjects variable when analysing average gas production rates; land use and peat origin were the between-subjects factors. The within subjects variable time was added in case of measurements in time, e.g. for nutrient levels, DOC or $SO_4^{2-}$ concentrations. If necessary, data were logarithmically transformed to stabilise variances between groups. Log transformations were done after adding a constant to the data in order to obtain values larger than 1. Greenhouse-Geisser corrections were applied. Treatment effects within combinations of peat origin and land use were analysed with a bootstrapped ANOVA (1000 iterations).

## 4.3 Results

### 4.3.1 Experiment 1: incubation experiment

*$CO_2$ and $CH_4$ production*

Figure 4.1 presents the main results of the $CO_2$ and $CH_4$ production rates during the experimental period. In oxic conditions mainly $CO_2$ was produced while in anoxic conditions both $CO_2$ and $CH_4$ production took place. The average $CO_2$ production rates over the whole experimental period of the samples under oxic conditions revealed that salinisation slowed down the $CO_2$ production in all peat origins and land uses (df 1,16; F=43.368; $p$<0.001, Figure 4.1a).

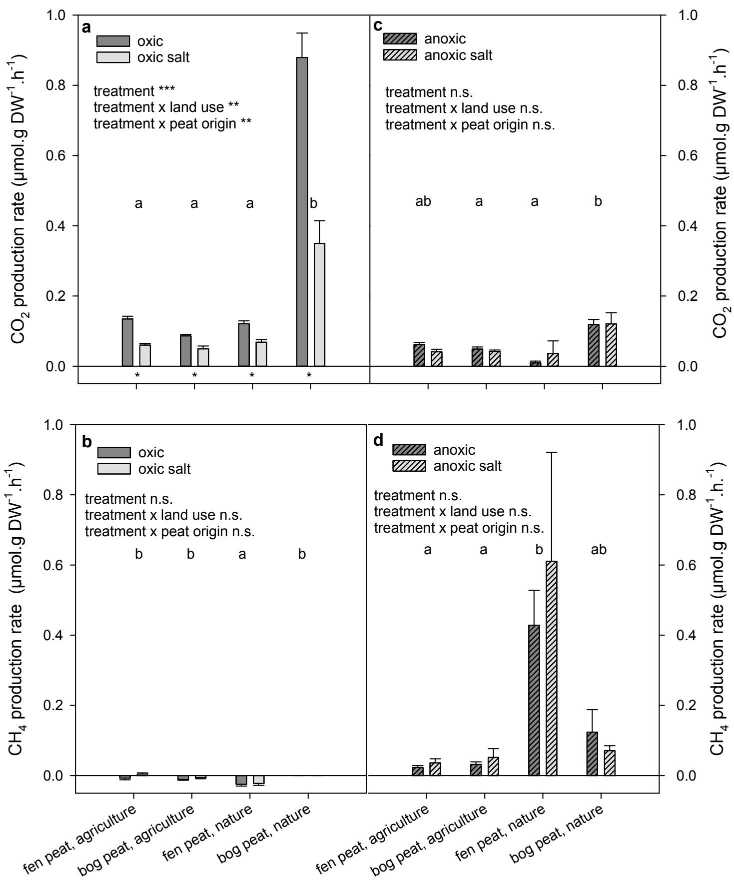


**Figure 4.1:** Average $CO_2$ (panels a and c) and $CH_4$ (b and d) production rates in the oxic (left) and anoxic (right) parts of the experiment. Error bars indicate standard errors. Main results of RM ANOVA are shown. (***$p$<0.001, **$p$<0.01, *$p$<0.05), as well as results from bootstrapped ANOVA for individual treatment effects (* below the bar means $p$<0.05). Differences between peat origins are indicated with differing letters above the bars ($p$<0.05). Note: the $CH_4$ production in the oxic samples from a nature reserve on bog peat was below detection limits.

A reduction of 40-60% in $CO_2$ production was observed in all types of peat samples due to salinisation. The $CO_2$ production in the bog peat from a nature reserve in oxic conditions was high (Figure 4.1a), as well as the $CH_4$ production from the fen peat from a nature reserve in anoxic conditions Figure 4.1d). $CH_4$ production rates were nil during aerobic incubations (Figure 4.1b). In contrast to the oxic part of the experiment, $CO_2$ and $CH_4$ production rates of the anoxic samples did not show any effect of salinisation (Figure 4.1c,d). $CO_2$ production was slightly lower in anoxic incubations than in oxic incubations, whereas $CH_4$ production was substantially higher.

*Total carbon loss*

Figure 4.2 depicts the average total gaseous carbon ($CO_2$ + $CH_4$) loss rates during the experiment, as a measure for total decomposition. In the oxic conditions, salinisation significantly reduced the carbon loss (df 1,15; F=51.395; $p<0.001$) whereas, in anoxic conditions no effect of salinisation was detected. Highest carbon loss was found in the bog peat samples from a nature reserve in oxic conditions, while in anoxic conditions the carbon loss in fen peat from a nature reserve was amongst the highest values.

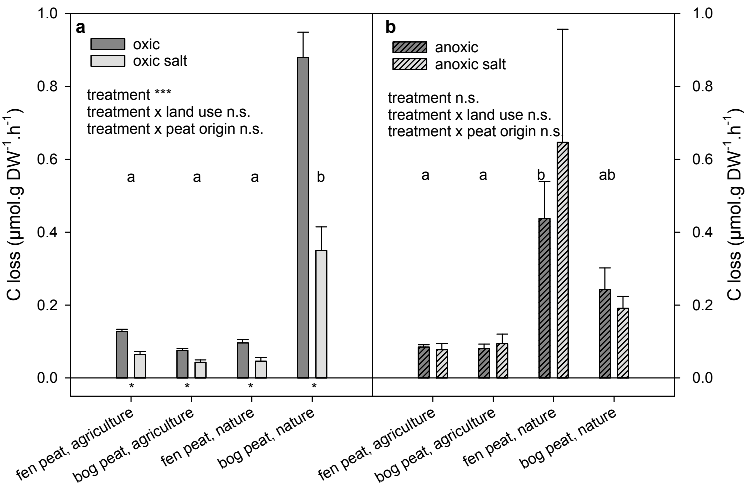


**Figure 4.2:** Total gaseous C loss rates during the oxic (a) and anoxic (b) part of the experiment. Error bars indicate standard errors. Main results of RM ANOVA are shown. (***$p<0.001$, **$p<0.01$, *$p<0.05$), as well as results from bootstrapped ANOVA for individual treatment effects (*$p<0.05$). Differences between peat origins are indicated with differing letters above the bars ($p<0.05$).

*Chemical soil parameters*

The concentrations of $NH_4^+$, $NO_3^-$, $PO_4^{3-}$ and DOC were measured after 0, 4, 8 and 16 weeks (Figure 4.3). Remarkable are the increasing $NH_4^+$ concentrations in the oxic bog peat from a nature reserve treated with salt. However, the control samples showed also increasing $NH_4^+$ concentrations, as well as the fen peat samples from a nature reserve. Furthermore, salt amendment in oxic and anoxic conditions led to lower $NO_3^-$ concentrations compared to control samples. All oxic agricultural samples showed increasing $NO_3^-$ concentrations with time, in contrast to the samples from nature reserves. $PO_4^{3-}$ tended to increase in the oxic samples and decrease in anoxic samples. In oxic conditions, salt addition caused a higher $PO_4^{3-}$ concentration, while there was no significant effect in the anoxic conditions. In general, salt addition lowered the DOC concentration, both in oxic as anoxic conditions. Bog peat had the highest DOC concentrations and samples from nature reserves contained more DOC than samples from agricultural meadows.



$SO_4^{2-}$ concentrations changed during the course of the oxic and anoxic incubations (Figure 4.4). In oxic conditions, the $SO_4^{2-}$ concentrations in fen peat increased with time, both in control and in salt-amended samples. This is in contrast to the bog peat samples, where no increase of sulphate concentrations was found. Remarkable is that the increase in sulphate concentration due to the salinisation treatment did not always coincide with changes in sulphate concentrations in the samples. For example, in bog peat from a nature reserve, the instant increase of sulphate concentrations was substantially higher than can be explained by $SO_4^{2-}$ addition alone.

The $SO_4^{2-}$ concentrations in the anoxic incubations were generally higher than in the oxic incubations. Fen peat from an agricultural meadow had sulphate concentrations amongst the lowest values found in oxic conditions, but amongst the highest values found in anoxic conditions. Sulphate concentrations in the anoxic fen peat samples tended to increase during the course of the experiment; whereas, the opposite was the case in the bog peat samples. pH was generally higher in the salt-amended samples than in the control samples (data not shown. Oxic: df=1, F=64.237, $p$<0.001, anoxic: df=1, F=100.902, $p$<0.001). Although, interaction effects of peat origin, land use and time were mostly significant.

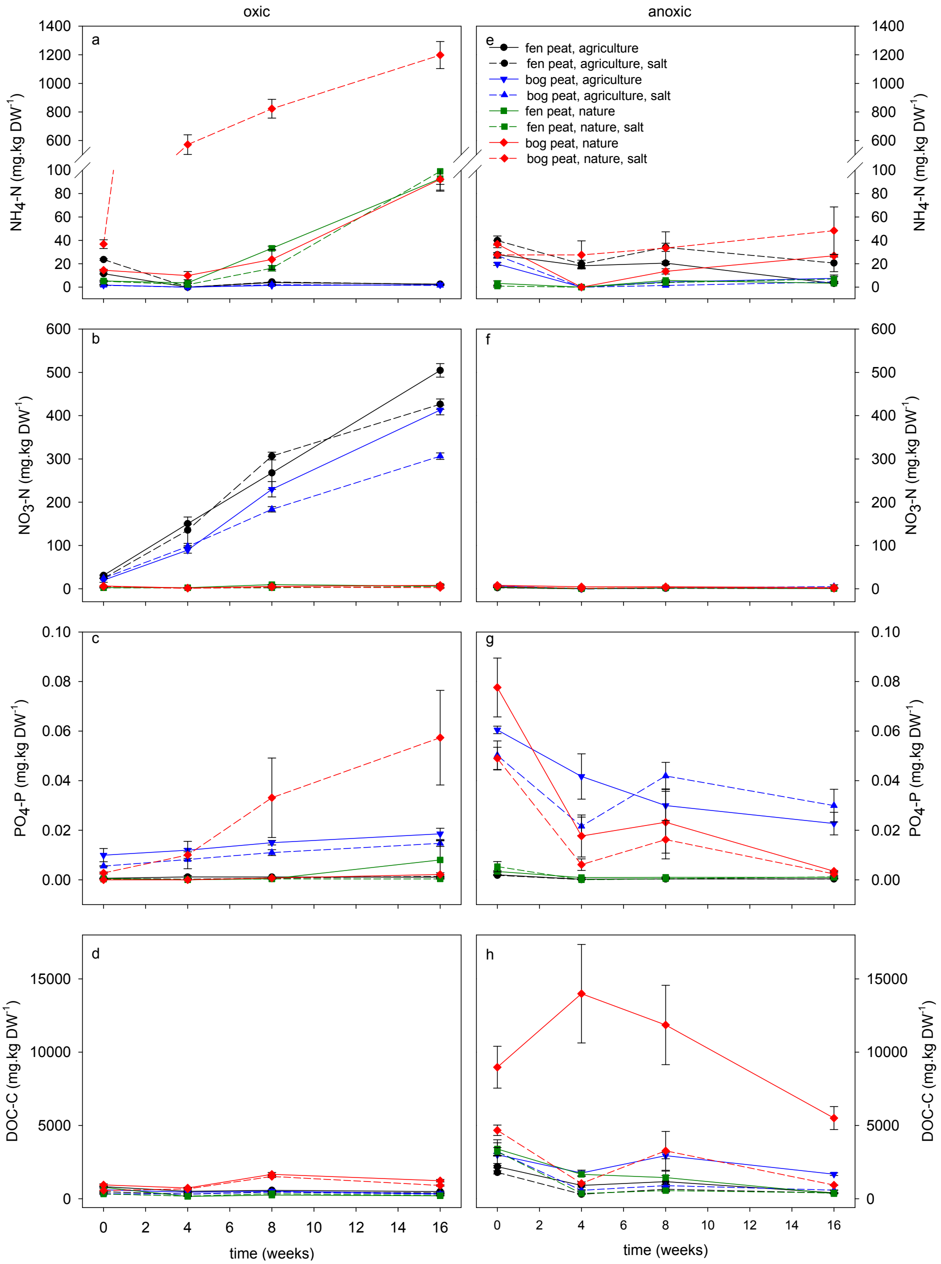


**Figure 4.3:** Soluble $NH_4^+$, $NO_3^-$, $PO_4^{3-}$ and DOC after 0, 4, 8 and 16 weeks in the oxic part of the experiment (left hand panels) and anoxic part of the experiment (right hand panels). Error bars represent standard errors.

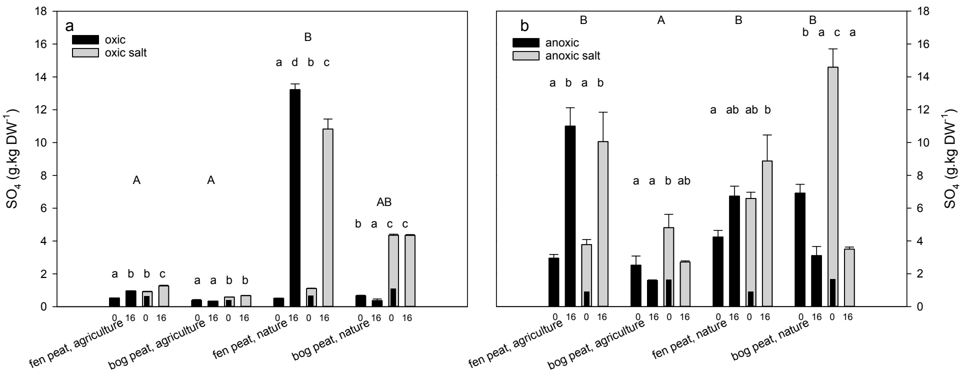


**Figure 4.4:** $SO_4^{2-}$ concentrations after 0 and 16 weeks in oxic (a) and anoxic incubations (b). Error bars indicate standard errors. Differences between peat origins are indicated with differing capitals above the bars ($p$<0.05). Differing small letters indicate significant differences per peat origin. A vertical bar indicates the amount of added $SO_4^{2-}$.

## 4.3.2 Experiment 2: potential nitrification

Potential nitrification in bog peat samples from the nature reserve Fochteloërveen was affected by salinisation. In brackish samples, significantly less ammonium was oxidised to nitrite or nitrate than in control samples (RM ANOVA, df=1,10, F=33.544, $p$<0.001). Furthermore, ammonium concentrations increased with time. This is in contrast to the ammonium concentrations in the bog peat samples from a dairy meadow. In these samples, ammonium concentrations decreased in time, irrespective of the presence of salt (RM ANOVA, df=1,10, F=0.436, $p$=0.524) (Figure 4.5).

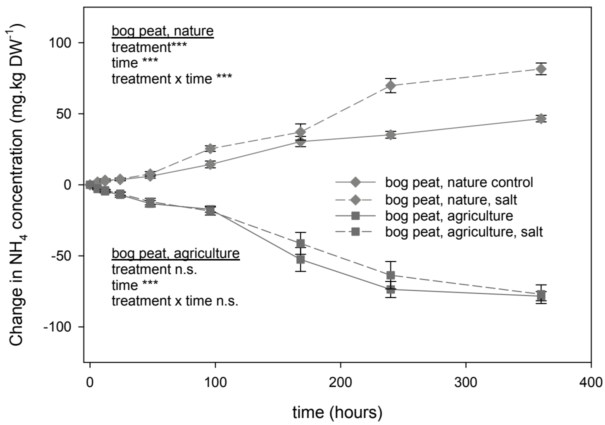


**Figure 4.5:** Change in concentration after the start of the potential nitrification experiment. Main results of RM ANOVA are indicated (***$p$<0.001, **$p$<0.01, *$p$<0.05).

### 4.3.3 Experiment 3: salt effects on DOC

The degradation of DOC in the presence and absence of salt was tested in fen peat and bog peat samples from nature reserves. As visible in Figure 4.6, the extracts of both peat origins contained lower concentrations of DOC in the brackish samples compared to the control samples (RM ANOVA, df=1, F=24.762, $p$<0.01), but the decrease of DOC concentrations with time was independent of salt addition (RM ANOVA, df=1.880, F=0.900, $p$=0.418).

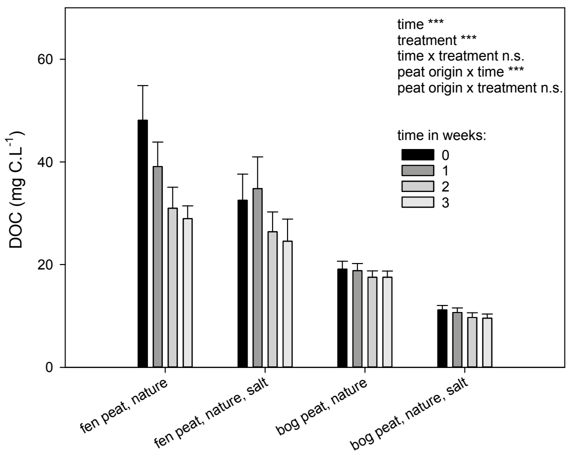


**Figure 4.6:** Effect of salt on DOC concentrations in peat extracts from fen peat and bog peat from nature reserves. DOC concentrations were measured after 0, 1, 2 and 3 weeks of storage. Main results of RM ANOVA are indicated. (***$p$<0.001, **$p$<0.01, *$p$<0.05).

## 4.4 Discussion

### 4.4.1 Salinity effects on decomposition

*Aerobic decomposition*

In our experiment, salinisation with a natural salt mixture reduced aerobic decomposition rates of peat samples from the unsaturated zone by approximately 50% in all our peat origin-land use combinations. The effect of salinisation was consistent despite large differences in the decomposition rates between peat origins and land uses. The lower aerobic decomposition rates after salinisation were due to a decline in $CO_2$ production as there was no net $CH_4$ production inoxic incubations. A decline of decomposition rates in brackish conditions has been found in earlier studies (Pathak and Rao, 1998; Setia et al., 2010; Mavi et al., 2012). This is explained by a detrimental effect of a sudden salinisation event on soil microorganisms as the high osmotic potential outside the microbes relative to the within the microbes can cause their cells to lose water (Oren, 1999; Wichern et al., 2006).

The carbon loss in oxic conditions in the bog peat samples from a natural peat bog was remarkably high compared to the other peat samples, both in brackish and in control conditions, although it fits well within the range of other peat incubation studies (Aerts and Toet, 1997; Moore and Dalva, 1997; Basiliko et al., 2007; Kechavarzi et al., 2010), see also Chapter 7. The comparatively high decomposition rates might have been due to the relatively young age of the organic matter in the superficial, unsaturated peat layer in the bog where the samples had been taken, in contrast to the material from the other locations which has had a history of aerobic decay. This fibric organic material in the bog is considered to contain more easily degradable carbon compounds than the hemic fen peat samples. The $CO_2$ production of such weakly decomposed material is high and usually decreases with time and increasing recalcitrance of organic matter (Berg and Meentemeyer, 2002; Glatzel et al., 2004). Furthermore, oxygen availability in the lab experiment might have been higher compared to the field situation as the disturbed lab samples lost part of their structure and water holding potential by capillary rise. To recapitulate, the higher oxygen intrusion in the lab samples might have caused higher decomposition rates in the bog samples from the nature reserve Fochteloërveen.



*Anaerobic decomposition*

In anoxic incubations of peat samples from the saturated zone, contrary to our expectations, no effect of salinisation on decomposition was found. Neither $CO_2$ production nor $CH_4$ production were affected by salinisation. Comparable to the anoxic conditions, the osmotic potential of the surrounding of the microorganisms could have counteracted the stimulating effect of $SO_4^{2-}$. We had hypothesised that the production of $CO_2$ would increase after salinisation because of the addition of $SO_4^{2-}$ as an alternative TEA. $SO_4^{2-}$ does not directly inhibit methanogenesis, only $NO_3^-$ and $O_2$ do so, but sulphate-reducing bacteria are more efficient competitors for labile carbon and hydrogen than methanogens (Lovley and Klug, 1983). Increased sulphate concentrations in peat soils have been found to result in higher anaerobic decomposition rates (Portnoy and Giblin, 1997; Smolders et al., 2006). Indeed, in anoxic conditions sulphate concentrations decreased, which could be an indication of the use of sulphate as a TEA, on the other hand, sulphate could have been reduced to pyrite. $CH_4$ production is often decreased as the activity of sulphate-reducing bacteria has a distinct negative effect on methane fluxes (Bartlett et al., 1987). $CH_4$ emissions were found to decrease due to sulphate addition in fen peatlands (Dise and Verry, 2001; Knorr et al., 2009). However, increases in both methanogenesis and $CO_2$ emission have been found after salinisation as well (Weston et al., 2011).

The lack of an effect of salinisation on both anaerobic $CO_2$ production and methanogenesis might have been due to the sulphate concentrations remaining below a threshold for sulphate reducers to become more competitive than methanogens. Such a threshold has not been precisely quantified for peatlands, but has been suggested to be at approximately 40 µM (Nedwell, 1995). In our experiment, the addition of the artificial seawater solution increased the salt concentration of the pore water with 4‰ point; this corresponded with a concentration of approximately 3 mM of $SO_4^{2-}$ in pore water. This value is far beyond the minimum concentration for sulphate reduction to take place. Another relevant factor might be the availability of small organic compounds like acetate, which often control the processes of sulphate reduction and methanogenesis, the final steps in the degradation of organic matter (Rydin and Jeglum, 2006). These terminal metabolic processes therefore depend on the generation of low molecular weight DOC substrates by hydrolysis and fermentation. In our experiment, we saw a decline in DOC concentration, as the dissolved organic molecules precipitate out at higher salt concentrations (Evans et al., 2006; Hruska et al., 2009). The unchanged $CO_2$ and $CH_4$ production rates with sulphate addition compared to the control treatments can be attributed to this lower substrate availability.

*Comparison aerobic and anaerobic decomposition*

$O_2$ is the energetically most favourable electron acceptor in decomposition processes and increased aeration stimulates peat decomposition (Domisch et al., 2006; Ellis et al., 2009; Reiche et al., 2009). Therefore, it would be logical to find higher aerobic than anaerobic decomposition rates; however, comparable amounts of carbon were lost per unit of dry soil in oxic and anoxic incubations. Here, one must be aware that we used peat material from superficial peat layers from the unsaturated zone for oxic incubations and peat samples from deep, permanently anoxic peat layers for anoxic incubations in order to obtain practically orientated information on the effect of salinisation on peat areas. One of the differences between these peat samples is the organic matter content. The samples from superficial layers are further decomposed because of a long history of drainage and therefore contain a larger mineral fraction; except for the bog peat samples from the peat forming nature reserve Fochteloërveen. Moreover, the advanced decomposition stage of the samples from the unsaturated peat layers most likely resulted in a higher fraction of recalcitrant material, which often shows lower decomposition rates (Berg and Meentemeyer, 2002; Glatzel et al., 2004). Nevertheless, expressed per unit organic matter the samples incubated with oxygen decomposed faster than those incubated without oxygen.

The superficial and deep peat layers were formed in wet, anoxic conditions but due to current low water levels most of these superficial peat layers in the Netherlands are currently undergoing aerobic decomposition, the so-called secondary decomposition (Tipping, 1995). It has been suggested that these peat layers, which have been exposed to oxygen through drainage, currently have different chemical properties than the deep peat layers that have not been in contact with oxygen for centuries. It has been found that anoxic conditions lead to a preferential loss of C compared to N, leading to lower C:N ratios (Tipping, 1995;

Mauquoy et al., 2002; Laiho, 2006). The latter was not found in our experiment, possibly due to the combination of a longer history of oxygenation, a younger age of the organic matter and differences in botanical composition between superficial and deep peat layers. Besides differences in organic matter content and recalcitrance of the organic matter, the microbial communities of superficial and deep peat layers probably differ. The input of root exudates and also manure in the agricultural areas are likely to affect the microbial communities that facilitate organic matter decomposition (Chapter 5; Wlash et al., 2012). It should be noted that samples used in this study were disturbed because of the sampling procedure and transfer to the incubations at the lab scale. They also were incubated over a short period at a higher temperature than the soils in the field. Therefore, results represent qualitative differences in potential rates of gas production in relation to salinisation and aerobicity that cannot be directly extrapolated in a quantitative sense to the field situation.

### 4.4.2 Nutrient concentrations

Water quality effects upon salinisation may be caused by three processes (1) the addition of ions that are present in the brackish water; (2) the processes of desorption and adsorption and (3) biogeochemical oxidation or reduction reactions, e.g. nitrification or pyrite oxidation. Firstly, salinisation causes higher ion concentrations, thus directly changing water quality. In our experiment, mostly sodium, chloride, sulphate, potassium, calcium and magnesium were present in the salt mixture.

Secondly, changes in desorption and adsorption processes due to higher salt concentrations also affected concentrations of several nutrients, e.g. phosphate concentrations became substantially higher after salinisation. In addition, sulphate concentrations in the samples from the Fochteloërveen (bog peat, nature) increased more than can be explained purely by the addition of sulphate-salt. Not only phosphate and sulphate, but also chloride and DOC compete for the same anion adsorption sites (Beltman et al., 2000; Kalbitz et al., 2000; Lucassen et al., 2004; Smolders et al., 2006). The added salt, e.g. chloride, might have replaced some of the sulphate and phosphate that was bound to the soil complexes. An additional relevant process is the interaction between sulphide with the iron-phosphorus cycle, leading to insoluble iron-sulphide minerals and phosphate mobilisation (Smolders et al., 2009).

Thirdly, regarding oxidation and reduction reactions, nitrate concentrations were lower in the salt-amended samples than in control samples in the oxic conditions. In combination with the higher ammonium concentrations in the brackish samples, this suggests that salinisation hampered nitrification. Nitrification is a process that primarily takes place in oxic conditions but has also been reported to occur in anoxic conditions (Hu, 2011). Our additional potential nitrification experiment also indicated inhibition of nitrification by salinisation for the bog peat samples from the Fochteloërveen. This negative effect of salinisation is a well-known phenomenon (Laura, 1974; Inubushi et al., 1999). The accumulation of nitrate in the samples from drained agricultural soils, whereas in the samples from nature reserves ammonium accumulated, indicates that the microbial nitrifier community is quite active in agricultural sites due to decades of fertilisation. This is a common phenomenon (Lu et al., 2011) and an immediate relation of nitrifier activity with nitrogen fertiliser use was also observed in two Dutch peat meadows (Best and Jacobs, 2001).

The consistently increasing $SO_4^{2-}$ concentrations in the fen peat samples during our incubations in oxic and anoxic circumstances are most likely due to pyrite ($FeS_2$) oxidation. In oxic conditions, oxygen serves as the electron acceptor in the oxidation of $FeS_2$ to $Fe^{2+}$ and $SO_4^{2-}$, while in anaerobic conditions pyrite oxidation can be coupled to $NO_3^-$ or $Fe^{3+}$ reduction (Jørgensen et al., 2009). Van Gaans et al. (2007) indicated that approximately 8% of the fen peat in the western part of the Netherlands consists of pyrite. These pyrite enrichments in the fen peat samples are explained by microbial sulphate reduction under brackish conditions with seawater as the dominant source of sulphate and Fe supply from freshwater sources (Dellwig et al., 2002). No pyrite oxidation was detected in the bog peat samples originating from the northern part of the Netherlands, where the peat is generally less influenced by (saline) groundwater and therefore lower in pyrite content (Lowe and Bustin, 1985).

The lower dissolved organic carbon concentrations in salt-amended samples compared to control samples, both in oxic and anoxic conditions of the main salinisation experiment is most likely due to the transfer of carbon compounds from dissolved into particulate state. Large-scale surveys of lake and stream water in the United Kingdom and surface water in both North America and Europe indicated that DOC solubility is suppressed under high sulphur or salt concentrations (Clark et al., 2005; Evans et al., 2005; Monteith et al., 2007; Hruska et al., 2009).

So, besides the hampering effects of salinisation on aerobic decomposition rates, water quality is expected to be influenced as adsorption-desorption processes might cause higher phosphate concentrations. Besides, higher ammonium concentrations might occur due to a hampering of nitrification. On the other hand, DOC concentrations are expected to decrease due to precipitation.

### 4.4.3 Management implications

The important message for peatland managers is that the episodic supply of brackish water via groundwater flows or inlet water will not increase but rather decrease subsidence rates. However, the changes in salt and nutrient concentrations will potentially affect agricultural practices and nature values. It was previously shown that dry episodes enhance the decomposition of deep pristine peat layers substantially, not only during but also after the dry event during which oxygenation took place (Brouns et al., 2014c, Chapter 2 of this thesis). Brief periods of supply of slightly brackish water do not affect grass production and are therefore not harming the interests of dairy farming in the region; arable farming is generally more sensitive to salinisation. On the other hand, in areas with low water quality, e.g. high nutrient concentrations, salinisation would result in an increase in phosphate and ammonium concentrations that could aggravate eutrophication problems. Adaptive water management in peat meadow areas should take into account such site-specific differences.

## 4.5 Conclusion

The experiments presented here aimed at exploring the effects of groundwater and surface water salinisation on anaerobic and aerobic decomposition and effects on nutrient concentrations in peat meadow areas in the Netherlands in the context of climate change effects. Salinisation is expected to occur mainly during dry summers, in short episodic pulses rather than over a longer period. The results of our experiments showed that salinisation did not increase decomposition rates but rather hampered aerobic decomposition (ca 50% reduction) while anaerobic decomposition remained unchanged. Peat origin (fen peat and bog peat) and land use (nature reserve and dairy meadow) did not affect the response of decomposition rates to salinisation. However, water quality might be differentially affected by salinisation. We also showed that salinisation leads to higher phosphate concentrations and lower DOC concentrations; in addition to higher ammonium concentrations in nature reserves and higher nitrate concentrations in agricultural sites. In our 16-week experiment, no signs of adaptation of the microbial community to the more brackish conditions were detected. This is a result of biogeochemical interactions associated with the higher salinities, these will probably also affect the surface water quality in the peat meadow areas.




## Acknowledgements

This study was supported by the research program Knowledge for Climate (Kennis voor Klimaat), hotspot 'shallow waters and peat meadow areas' project code HSOV1a. We are grateful to Natuurmonumenten for facilitating peat sampling in the nature reserves Nieuwkoopse Plassen and Fochteloërveen. In addition, farmer Gert Diever and the Veenweide Innovatiecentrum (peat meadow innovation centre) are greatly thanked for allowing us to take soil samples at their sites. We would like to acknowledge Gerrit Rouwenhorst (Utrecht University) and Richard van Logtestijn (VU University, Amsterdam) for their help with chemical and gas analyses.

# Chapter 5

# Peat origin and land use effects on microbial activity, respiration dynamics and exo-enzyme activities in drained peat soils in the Netherlands

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*Accepted for publication in Soil Biology & Biochemistry*

## Abstract

This study assessed the risk of decomposition-driven soil subsidence in drained peat soils in the Netherlands, contrasting in peat origin and land use. In a full factorial design, fen peat and bog peat were sampled from sites in use for nature conservation and for dairy farming, which contrast in history of drainage and fertilisation. In these four common peat types, the microbial activity and respiration dynamics were studied in samples from superficial oxic peat layers by measuring Substrate Induced Respiration (SIR) and Substrate Induced Growth Response (SIGR). Total and active microbial biomass, microbial growth potential and potential exo-enzyme activities were determined in unamended samples and after nitrogen and/or glucose amendments.

Remarkably, peat origin and land use did not affect basal respiration rates. The high metabolic quotient ($qCO_2$) and low respiration quotient (RQ) show a low energy use efficiency, which clearly indicates that the peat in our study areas is rather recalcitrant, regardless of peat origin and land use. This is relevant for predictions of the effects of climate change, as the temperature sensitivity of the OM increases with its recalcitrance. Land use affected microbial biomass and potential growth rates as they were quadrupled in dairy meadows compared to nature reserves. This may be attributed to the pulses of manure and chemical fertiliser that are being supplied in agricultural peatlands. Potential activities of oxidative exo-enzymes (phenol oxidase, POX, and phenol peroxidase, POD), in contrast, depended more on peat type, indicating a difference in peat substrate quality. Basal respiration rates were not related to enzyme activities. The activity of the oxidative enzyme POX and the concentration of phenolic compounds, which are considered the main regulators of peat decomposition according to the enzymic latch theory, were not related to respiration rates. It was concluded that decomposition theories like the enzymic latch theory cannot be applied one-to-one in the drained peat soils in the Netherlands. Phosphorus enrichment was identified as a potential driver of increased peat decomposition.

## 5.1 Introduction

Currently, 450-550 Pg of carbon is in a sequestered state in peat soils worldwide. This is about 70% of the global atmospheric carbon stock (Joosten, 2010). This large carbon stock in peat soils has the potential to be rapidly converted to atmospheric carbon dioxide when environmental or climatological conditions change (Laiho, 2006). Peat soils in the Netherlands have been drained centuries ago to facilitate agricultural use since centuries. This practice has converted Dutch peat soils from a net sink to a net source of carbon, through an increase in decomposition and removal of aboveground production (Joosten, 2010). In order to anticipate on the resulting soil subsidence (Schothorst, 1977) and its expected future acceleration due to climate change (Keller et al., 2004; Dorrepaal et al., 2009), insight in the characteristics of peat decomposition is important for local and regional governments.

The decomposition process in peat soils is largely controlled by soil heterotrophic microorganisms. They produce enzymes that convert complex organic matter into simpler products such as carbon dioxide, water and mineral N molecules, with various intermediary products. These enzymes enter the environment through secretion and lysis and have been called 'exo-enzymes' (Sinsabaugh et al., 2009; Sinsabaugh et al., 2010; Sinsabaugh and Shah, 2011). The factors determining the abundance, composition and activity of soil microbial communities include edaphic factors (soil type, moisture content, pH, nutrient availability), land management practices (drainage, fertilisation) and the vegetation composition (Borga et al., 1994; Fenner et al., 2005; Bougon et al., 2009; Eisenhauer et al., 2010). Most peat in the Netherlands originates from fens and bogs. Fen peat develops in eutrophic conditions and consists of the remains of *Carex* spp., *Phragmites* spp. and/or woody species such as *Betula* and *Alnus* spp. On the other hand, bog peat is formed in ombrotrophic conditions and largely consists of *Sphagnum*-derived material. The decomposition of *Sphagnum*-derived organic matter (OM) is generally slower than that of OM formed by *Carex* spp. (Aerts et al., 1999; Scheffer et al., 2001). These differences have been attributed to the lower pH in bog peat compared to fen peat (Bergman et al., 1999) and to differences between the two peat types in chemical composition, which are associated with the botanical composition of the peat.

The presence of Sphagnum-derived OM restricts decomposition (Verhoeven and Toth, 1995). This effect has been shown to be related to the presence of inhibiting substances like the phenolic compound sphagnum acid (Verhoeven and Toth, 1995; Verhoeven and Liefveld, 1997). These phenolic compounds bind to exo-enzymes such as the hydrolytic enzymes phosphatase and β-glucosidase, which are thereby inactivated (Wetzel, 1992; Verhoeven and Liefveld, 1997; Freeman et al., 2001; Fenner and Freeman, 2011). The enzyme phenol oxidase (POX) catalyses non-specific oxidation reactions, stimulating the degradation of the inhibiting phenolic substances. POX is principally active in the presence of oxygen. Releasing the oxygen constraint by drainage will lead to increased POX activity, which in turn accelerates the decomposition of soluble phenolic compounds and reduces their inhibitory effect on soil exo-enzymes. This hypothesis is called the 'enzymic latch mechanism' (Freeman et al., 2001). Hence, POX is considered an important regulator of decomposition rates in peat (Freeman et al., 2001; Freeman et al., 2004).

Apart from peat origin, land use is another important factor determining peat decomposition rate (Table 1.2). In the Netherlands, there are near-natural peatlands with a high groundwater table and a species-rich vegetation, as well as peatlands in use for agriculture, mainly dairy production, with a highly productive species-poor vegetation of grasses growing in fertilised and drained peat soils. The possible effects of land use on the decomposition process are complex, because land use differences encompass an array of different aspects. Firstly, one could expect that the long history of drainage and occasional ploughing has stimulated POX activity leading to lower concentrations of phenolic compounds remaining (Table 1.2). This would facilitate the decomposition process, according to the mentioned enzymic latch theory (Freeman et al., 2001; Fenner and Freeman, 2011). It is possible that this results in peat soils with a high proportion of amorphous and recalcitrant OM, as virtually all labile OM has been decomposed (Berg and Meentemeyer, 2002). Secondly, plant biodiversity is lower in agricultural meadows and fields than in nature reserves. The differences in plant community composition affect soil microorganisms as each plant species has a unique contribution to the functioning of the belowground system through their particular litter quality and root exudates (Eisenhauer et al., 2010). Furthermore, in the Dutch peat meadows, primary production is mostly removed by mowing and/or grazing, so that the material available for decomposition has a large proportion of roots and rhizomes. Lastly, OM decomposition is potentially affected by the large amounts of fertiliser applied in agricultural peatlands. Nitrogen addition stimulates the decomposition of easily degradable OM and hampers the decay of recalcitrant organic compounds, such as lignin and other phenolic compounds, as described in the nitrogen mining theory (Berg and Meentemeyer, 2002; Knorr et al., 2005a; Moorhead and Sinsabaugh, 2006; Craine et al., 2007). Consequently, nitrogen addition reduces recalcitrant OM decomposition rates (Craine et al., 2007). This was demonstrated experimentally with cores of *Carex*-derived peat, which showed lower decomposition rates after nitrogen amendment (Aerts and Toet, 1997). This differential response (stimulating as well as retarding) to nitrogen is also observed in potential activity of hydrolytic and oxidative enzymes (Carreiro et al., 2000). The exo-enzymes cellobiohydrolase (CBH) and β-1,4-glucosidase (BG), both involved in the degradation of easily degradable compounds, have been found to be stimulated after nitrogen addition to peat. At the same time, the activity of POX, which is involved in the degradation of recalcitrant (phenolic) compounds, is reduced after nitrogen addition (Carreiro et al., 2000; Sinsabaugh, 2010).

In this study, the effects of peat origin (fen vs. bog) and land use (agriculture vs. nature) on OM degradation in terms of respiration and exo-enzyme activities of peat soils were studied in a full factorial design (Tables 1.1 and 1.2). We determined respiration rates and potential enzyme activities upon ammonium and/or glucose addition as sources of nitrogen and energy. An increase in respiration rate after the addition of nitrogen and/or glucose will tell whether one of these factors was limiting peat decomposition. Furthermore, total microbial biomass and glucose-responsive microbial biomass were determined. Additionally, measuring the activity of extracellular enzymes involved in the C, N and P cycling can significantly increase our understanding of the relation between resource availability, microbial community structure and functioning, and ecosystem processes (Caldwell, 2005). Besides POX, CBH and BG, we also measured potential activities for leucyl aminopeptidase (LAP) and N-acetylglucosaminidase (NAG), enzymes that are mainly associated with combined nitrogen and carbon release. Furthermore, acid phosphatase (AP) was measured, which liberates phosphate through the breakdown of organic phosphate compounds. This enzyme is highly relevant in OM decomposition (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012).

Given the high concentration of phenolic compounds in living *Sphagnum* spp. and *Sphagnum*-derived peat, we hypothesised that the bog peat from nature reserves has the lowest respiration rates despite its fibric character. Furthermore, low respiration rates in drained hemic fen peat in agricultural land use were expected, because it was thought that the facilitation of decomposition by drainage of agriculturally used peat has resulted in amorphous peat with a higher recalcitrance of the remaining OM in comparison to the OM in nature reserves. The peat in agricultural use is, therefore, expected to show low decomposition rates in general, which are even further reduced after nitrogen amendment.

5

## 5.2 Material and methods

### 5.2.1 Study sites

In this study, the distinction is made between peat that originates from minerotrophic fens, consisting of the remains of *Carex* spp., *Phragmites* spp., *Betula* spp. and *Alnus* spp., and peat formed in ombrotrophic bogs, with a large proportion of *Sphagnum*-derived material. These are two major classes that can currently still be detected in drained peat meadow soils in the Netherlands. Both these peat types were collected in agriculturally used meadows as well as in nature reserves (Table 1.2), resulting in a full factorial design with four peat types. We will refer to these peat types as fen peat or bog peat under agricultural or natural land use. The agricultural sites have a monocultural vegetation of *Lolium perenne*, which is grazed by dairy cattle and mown for hay production. Fertilisation with manure, and occasionally artificial fertilizers, takes place four to five times a year through injection into the soil (ca 150 kg N·ha$^{-1}$·yr$^{-1}$ and 30 kg P·ha$^{-1}$·yr$^{-1}$). The sites with hemic fen peat were located in the peat meadows of Zuid-Holland and Utrecht and encompassed a *L. perenne* meadow in agricultural use near Zegveld as well as a mesotrophic hay meadow with a Calthion palustre vegetation type (Zuidhoff et al., 1996) which had never been fertilised in a nature reserve near Nieuwkoop. At the time of sampling, the agricultural meadow and the hay meadow had ditch water levels of 55 and 20 cm below soil surface, respectively. The agricultural meadow with oligotrophic *Sphagnum*-derived hemic peat was located in the province of Friesland and had a *L. perenne* monoculture. Here, the water table was approximately 30 cm below soil surface. The natural *Sphagnum* fibric bog peat was located in the nature reserve Fochteloërveen. This site had a water table at the soil surface level and vegetation characterised by *Sphagnum* spp. and *Molinia caerulea*.

### 5.2.2 Soil sampling and preparation

Peat samples were collected on 26 and 29 April 2013. The soils were sampled at a depth where year-round oxic conditions could be expected and below the zone with high root density (Table 5.1). In the nature reserve Fochteloërveen we sampled fibric peat just below the living *Sphagnum* spp. tissue. At each location, five replicate samples were collected over an area of approximately 400 m$^2$ using an Edelman corer with length 10 cm and diameter 7 cm. The samples were transported to Utrecht in a cool box and were stored at 4°C until the start of the experiment, which was within one week after sampling. In preparation for the experiment, samples were manually homogenised and cleared from coarse roots (>2 mm Ø) and large wood particles.

**Table 5.1:** Characteristics of the study sites.

| Site | Land use | Peat type | Location | Highest-lowest water table [1] | Sampling depth (cm) |
|---|---|---|---|---|---|
| **Zegveld** | Agriculture | Fen peat | N 52.138818, E 4.835930 | 35-80 | 20-30 |
| **Echtenerbrug** | Agriculture | Bog peat | N 52.874654, E 5.805269 | 10-75 | 25-35 |
| **Nieuwkoopse Plassen** | Nature | Fen peat | N 52.140689, E 4.798340 | 5-40 | 20-30 |
| **Fochteloërveen** | Nature | Bog peat | N 52.990897, E 6.394049 | 0-25 | 10-20 |

[1] highest-lowest, cm below soil surface (BIS Nederland, 2013). Location was determined using Google maps.

### 5.2.3 Edaphic characteristics

Each reference to the weight of the soil samples refers to dry weight. Dry weight was determined by oven-drying a subsample from each replicate (70 °C, 48 h). OM content was determined by loss-on-ignition (550 °C, 5.5 h). pH was determined by adding 100 mL of demineralised water to 10 g of fresh soil, shaking for 2 hours (rotary shaker, 100 rpm) and measuring pH in the soil suspension (WTW Measurements Systems, Ft. Myers, FL, USA). Water extractions were performed by adding 100 mL of demineralised water to 7-8 g of fresh peat, shaking on a rotary shaker (1 h, 100 rpm) and filtering the samples (Whatmann GF/C, Dassel, Germany). The concentrations of $NO_3^-$, $NH_4^+$, $PO_4^{3-}$ and dissolved organic carbon (DOC) in the extracts were determined on a Continuous Flow Analyser, (Skalar, Breda, the Netherlands) after storage at -20 °C. The concentration of soluble phenolic compounds in the peat extracts was determined with the Folin-Ciocalteau reagent, in a procedure adapted from Box (1983). 30 µL Folin-Ciocalteau reagent was added to 100 µL of the extract. After eight minutes, 100 µL Na2CO3 (10.6 g·100 $mL^{-1}$) was added. After 40 minutes, absorbance at 760 nm was measured (Spectrostar, BMG Labtech, Ortenberg, Germany). A calibration curve was made based on tannic acid. Carbon and nitrogen contents of the samples were determined using an EA/110 CHNS-O analyser (Interscience BV, Breda, the Netherlands). Field capacity is the soil water content after the soil has been saturated and allowed to drain freely for about 24 to 48 hours. In our case, the field capacity of the peat at the four sample sites was determined in the year 2010. Soil samples were placed on a Whatman 595½ filter with pore size 4-7 µm, the samples were saturated with demineralised water and weighed after 30 hours to determine field capacity in g water per g dry soil.

### 5.2.4 Fumigation-extraction procedure

The overall microbial biomass ($C_{fum}$) was determined using fumigation-extraction procedure (Vance et al., 1987). Duplicate samples of 5 g of soil with field moisture content were subjected to a 24 h treatment with an ethanol-free chloroform atmosphere followed by extraction with 50 mL of a 0.5 M $K_2SO_4$ solution. After the extraction, DOC and dissolved organic nitrogen (DON) concentrations were measured in fumigated and non-fumigated control soils using a continuous flow auto analyser (Skalar SA-40, Breda, the Netherlands). Before analysis, extraction samples were stored at -20 °C. Following Vance et al. (1987), microbial carbon was estimated by multiplying the amount of DOC liberated by fumigation by an empirically derived factor of 2.64 reflecting the relative amount of non-extractable to extractable carbon in microbial biomass. We calculated microbial N as the difference in extractable DON before and after fumigation divided by 0.54 (Brookes et al., 1985). Molar C:N ratios of microorganisms were calculated based on these C and N data.

### 5.2.5 Respiration

The homogenised samples at field moisture content were allowed to acclimatise for three days at 20 °C in a dark box covered with a moist cloth to minimise evaporation. Soils were incubated to measure Substrate Induced Respiration (SIR) (Anderson and Domsch, 1978) and Substrate Induced Growth Response (SIGR) (Keuskamp et al., 2013). Respiration was measured after amending the samples with ammonium (N), glucose (G) or both or plain demineralised water (control), to investigate if decomposition was nitrogen- or energy-limited. 0.06 mg N·g $FW^{-1}$, as $NH_4Cl$, was added to the N treatments. $D^+$-glucose was added as a source of labile organic carbon in the G treatment (0.6 mg C·g $FW^{-1}$) following the procedure of Keuskamp et al. (2013). To start the incubations, 2 mL treatment solution was thoroughly mixed through 20 g FW of soil. By adding 2 mL of treatment solution to the samples, the evaporation that took place during acclimatisation was compensated. Consequently, respiration measurements took place at field moisture content. 10 g of the amended soil was put in 600 mL flasks that were connected to a respiration monitor equipped with optical $CO_2$ and $O_2$ sensors (Biometric Systems, Germany). By using these small soil samples, it was assumed that diffusion differences between the four peat types were small.

Soils were incubated for 6 days at 20 °C. During incubation, $CO_2$ production was measured at intervals of 130 min. The flasks were flushed with fresh air whenever $CO_2$ levels exceeded 4.5 mL·$L^{-1}$ or $O_2$ levels decreased to less than 180 mL·$L^{-1}$.

### 5.2.6 Potential enzyme activity assays

Potential enzyme activity assays of two oxidative and five hydrolytic enzymes were conducted on soil subsamples from the various treatments (control, N, G, GN) after 5 days of incubation. As summarised by Sinsabaugh et al. (2008), these enzymes have different functions. The oxidative enzymes POX and POD are non-specific and attack C bonds in complex (phenolic) structures such as tannins and lignin. CBH and BG are involved in the depolymerisation of cellulose, resulting in free glucose as a carbon and energy source. CBH hydrolyses cellobiose dimers from molecules of cellulose, whereas BG further degrades cellobiose and other substrates to glucose. LAP catalyses the hydrolysis of leucine residues from peptides and proteins. NAG catalyses the terminal reaction in the hydrolysis of chitin, and liberates both C and N. LAP and NAG are mainly associated with nitrogen release although also carbon is being released. AP liberates phosphate through the breakdown of organic phosphate compounds (Sinsabaugh et al., 2008; Sinsabaugh and Follstad Shah, 2012). Potential enzyme activities were determined based on absorbance measurements in 96-well microplates, following a protocol modified from (Allison and Vitousek, 2004) (Appendix 5.A). 2 gram FW of the soil samples was suspended with acetate-buffered demineralised water (60 mL, 50 mM, pH 5.5). All substrates and reference solutions were prepared in this buffer solution. For each soil sample, 8 wells of a 96 wells plate were filled with 150 µL of the soil suspension. To four of these wells, we added 50 µL of a substrate solution (assay wells), to the other four wells a plain buffer solution (homogenate control) was added. All microplates were incubated in dark conditions at 20 °C on a microplate shaker (600 rpm), the details are listed in Appendix 5.A. Thereupon, the soil particles were allowed to settle for several minutes. 100 µL of particle-free solution was transferred to a new 96 wells plate.

Besides the homogenate control, we also included wells containing substrate solutions and buffer solution without additions as controls. To improve coloration, 10 µL 1 M NaOH was added to the NAG, CBH, AP and BG assays. After that, absorbance was measured with a platereader (Spectrostar, BMG Labtech, Ortenberg, Germany). Specific wavelengths for each assay can be found in Appendix 5.A.
Additionally, the micromolar absorption coefficients of the products pNP and pNA were determined by running a standard curve by making dilutions of a 1 nM pNP or pNA solution in buffer. To determine the absorption coefficient of L-DOPA, a reaction mixture of 10 µL mushroom tyrosinase (0.1 $mg \cdot mL^{-1}$), 0.3 mL buffer and 0.1 $mL \cdot L^{-1}$-DOPA (1 mM) was used. The absorbance of aliquots of this mixture was measured after 6 h of incubation at room temperature. Potential activity was calculated using the difference in absorbance between reaction and reference wells, corrected for change of absorbance through substrate consumption (Keuskamp et al., 2015):
$\Delta[P]=(\Delta ABS-\alpha_s[S]_{t0})/(\alpha_p-\alpha_s)$
ΔABS is the difference of the median absorbance measured in the reaction and the reference wells. $\alpha_s$ and $\alpha_p$ represent the absorption coefficients of the substrate and product. The potential enzyme activity was expressed as $\Delta P \cdot g^{-1}$ soil $DW \cdot h^{-1}$.

### 5.2.7 Data analysis and statistical analysis

*Respiration*
$CO_2$ production rates increased exponentially after glucose amendment, indicating microbial growth. The rate of increase was quantified according to Keuskamp et al. (2013). A logarithmic growth function was fitted to the initial, rising part of the respiration peak:
$R_{CO2}(t)=p \cdot e^{\mu max(t-b)}$

Here, p is the initial respiration rate, b is the lag time before exponential growth starts, $\mu_{max}$ represents the relative growth rate. The fitting was done using a least squares procedure in R. Furthermore, respiration rates were used to calculate initial microbial biomass ($C_{SIR}$) according to Anderson and Domsch (1978):
$C_{SIR} =81.84 \cdot R_c +3.7$

$C_{SIR}$ is the microbial biomass as µg microbial $C \cdot g$ soil $DW^{-1}$. $R_c$ is the average respiration over the first 5 hours as µg $CO_2$-$C \cdot g$ soil $DW^{-1} \cdot h^{-1}$. In addition, we calculated the metabolic quotient ($qCO_2$) which represents the relative respiration rate for microorganisms. This was calculated by dividing the basal respiration rate over the first 24 h, which is the average respiration rate in this period ($BR_{0-24}$), by the $C_{SIR}$ (Anderson and Domsch, 1978). The average respiration rates in the period between 100 and 150 hours after start of the experiment are also presented; by focusing on this period, the disturbing effects and associated $CO_2$

peak of the soil preparations (sieving, mixing) prior to the respiration measurements are excluded. In control samples, the microbial respiratory quotient (RQ) was calculated as the production of $CO_2$ divided by the consumption of $O_2$ over the first 100 h of incubation. An RQ value smaller than 1 indicates that recalcitrant carbohydrates with a large proportion of double bindings or ring structures are being decomposed, whereas an RQ larger than 1 is indicative for decomposition of labile carbohydrates, such as root exudates or anaerobic decomposition (Dilly, 2001).

*Statistical analysis*
Edaphic properties were compared using one-way ANOVA, with a Tukey post-hoc test corrected for the number of comparisons. Repeated measures ANOVAs (Greenhouse-Geisser corrected) were used to analyse the effect of N on respiration rates, half-lives, RQ, μmax and potential enzyme activities. Outliers, as identified by Grubbs tests, were removed from all analyses and graphs. Homogeneity of variances was assessed by Levene's test, normality was tested with the Shapiro-Wilk test and residuals were visually explored by P-P plots to check if residuals were scattered about a straight line without systematic deviations. Data were log or square-root transformed if needed to stabilise variances. All analyses were done in IBM SPSS 20 (IBM software, Armonk, New York, United States).

## 5.3 Results

### 5.3.1 Edaphic properties

The bog peat from the nature reserve Fochteloërveen was distinctly different from the other peat types in terms of the edaphic properties. The bog peat from the nature reserve had the highest OM content, moisture content, C:N ratio and concentration of soluble phenolic compounds (Table 5.2). In contrast, pH values were similar amongst all peat types with values between 5 and 6. Nutrient availability was elevated in the agricultural (dairy) meadows as compared to the nature reserves. Nitrate and phosphate concentrations were highest in the dairy meadow on the bog peat, and ammonium concentrations peaked in the dairy meadow on the fen peat.

### 5.3.2 Respiration

*Respiration rates after glucose and nitrogen amendments*
The respiration rates of soil incubations in the unamended and the nitrogen-amended treatments showed rather stable respiration rates in time, except for the first hours where disturbance of the soil samples due to weighing and mixing temporarily increased $CO_2$ emissions (Figure 5.1 panels a,b,c,d for control treatments and e,f,g,h for nitrogen treatments).

**Table 5.2:** Edaphic characteristics of the peat samples from our four study sites, mean ± standard error. Letters indicate significance of differences between peat types (ANOVA $p$<0.05).

| Property | Fen peat, agriculture | Bog peat, agriculture | Fen peat, nature | Bog peat, nature | Unit |
|---|---|---|---|---|---|
| **OM** | 0.72±0.14 $^{ab}$ | 0.70±0.11 $^{a}$ | 0.70±0.013$^{a}$ | 0.95±0.015 $^{b}$ | fraction |
| **pH** | 5.3±0.20 $^{a}$ | 6.0±0.10 $^{b}$ | 5.9±0.05 $^{ab}$ | 5.3±0.16 $^{a}$ | |
| **Moisture content** | 0.77±0.006 $^{a}$ | 0.67±0.06 $^{a}$ | 0.84±0.006 $^{b}$ | 0.91±0.002 $^{c}$ | fraction |
| **C:N ratio** | 10±0.1 $^{a}$ | 13±0.2 $^{b}$ | 12±0.3 $^{ab}$ | 22±1.1 $^{c}$ | |
| **Field capacity** | 3.3±0.07 | 2.3±0.04 | 4.6±0.08 | 22±1.2 | g water· g dry soil$^{-1}$ |
| **Soluble phenolic compounds** | 2.0±0.1 $^{ab}$ | 1.15±0.2 $^{a}$ | 2.2±0.3 $^{b}$ | 5.1±0.2 $^{c}$ | mg·g DW$^{-1}$ |
| **$NO_3^-$** | 1.72±0.74 $^{b}$ | 12.29±3.12 $^{c}$ | 0.05±0.04 $^{a}$ | 0±0 $^{a}$ | mg·kg DW$^{-1}$ |
| **$NH_4^+$** | 43.2±8.9 $^{c}$ | 7.7±2.4 $^{b}$ | 1.0±0.3 $^{a}$ | 6.0±1.1 $^{ab}$ | mg·kg DW$^{-1}$ |
| **$PO_4^{3-}$** | 1.2±0.26 $^{a}$ | 49.3±33 $^{b}$ | 7.2±6.6 $^{a}$ | 4.5±1.0 $^{ab}$ | mg·kg DW$^{-1}$ |
| **$SO_4^{2-}$** | 1610±100 $^{bc}$ | 1060±161 $^{a}$ | 1130±114 $^{ab}$ | 1870±113 $^{c}$ | mg·kg DW$^{-1}$ |

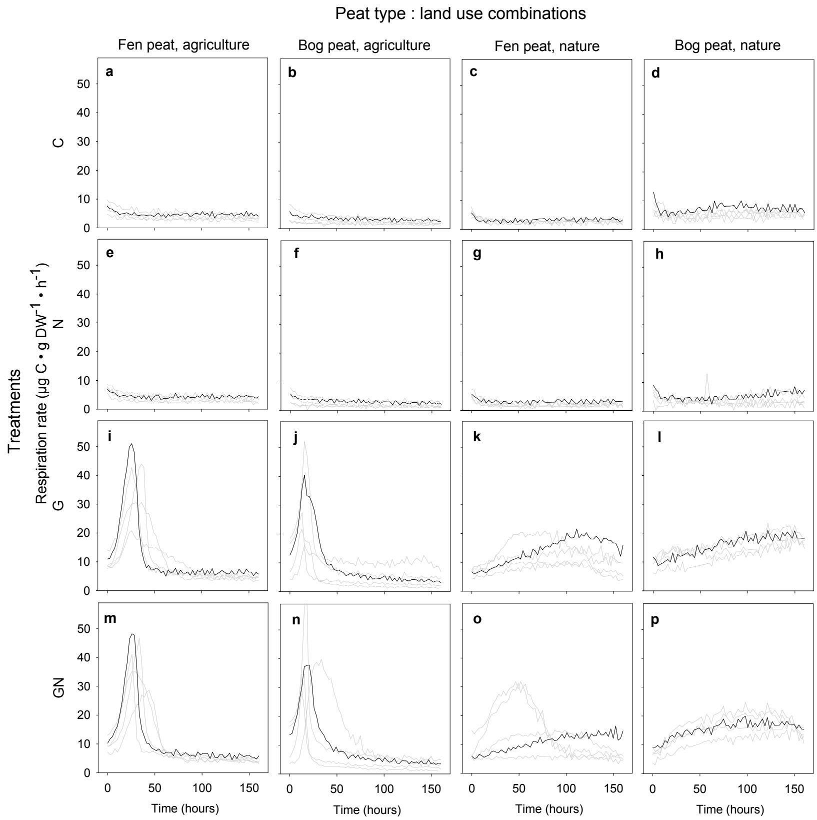


**Figure 5.1:** Respiration rates (µg $CO_2$-C·g DW$^{-1}$·h$^{-1}$) in time (h) of samples from agricultural peat meadows and nature reserves of peat originating from eutrophic or oligotrophic vegetation, in control treatments (C) and after amendment of ammonium (N) and/or glucose (G). In each panel, one of the five replicates is plotted in black, the other four replicates are plotted in grey.

At first sight, there is no effect of N addition on respiration. Glucose addition stimulated respiration rates in all peat types. Upon glucose addition, a clear peak in respiration was seen in the agricultural peat samples (Figure 5.1 panels i,j,m,n), whereas this peak was more stretched out in the samples from nature reserves (Figure 5.1 panels k,l,o,p,). Furthermore, within-site differences in the response to the combined glucose and nitrogen amendment of the fen peat samples with 'nature' land use were remarkable: two samples clearly gave a peak in respiration rates while other samples showed a steady increase, a low and wide peak or no response to this treatment in terms of $CO_2$ emission rates.

*Effect of nitrogen addition on respiration at the end of the incubation*

The average respiration rates for the control and nitrogen treatments in the period between 100 and 150 hours after the start of the experiment are presented in Figure 5.2. Here, nitrogen addition significantly reduced respiration rates (RM ANOVA F=21.061, $p<0.001$). RM ANOVAs per combination of peat type and land use indicated that in all combinations the nitrogen treatment showed lower respiration rates, except for the fen peat samples with agricultural land use. The interaction of land use and treatment was significant (RM ANOVA F=10.856, $p<0.05$); the decrease in respiration rates was strongest in the samples from nature reserves, which did not have a recent history of fertilisation. Peat origin did not affect the response to nitrogen addition.

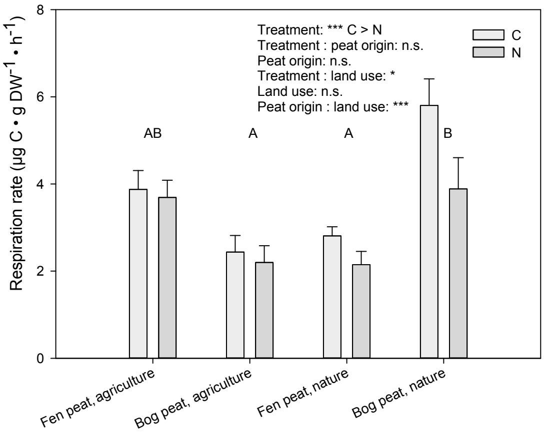


**Figure 5.2:** Respiration rates of the control and nitrogen-amended samples in the period 100-150 hours. Error bars reflect standard errors. Main results of RM ANOVA are shown (***$p<0.001$, ** $p<0.01$, * $p<0.05$). Capital letters indicate significance of differences between combinations of peat origins and land uses (ANOVA $p<0.05$).

### 5.3.3 Microbial properties

*Microbial biomass and activity*

Remarkably, the respiration rates in the first 24 h of the respiration measurements ($BR_{0\text{-}24}$) were not affected by peat origin or land use (Figure 5.3a). $qCO_2$, the respiration rate per unit of microbial biomass, was higher in nature reserves compared to dairy meadows (Figure 5.3b). The active (glucose-responsive) and total microbial biomass in the peat samples are shown in Figure 5.3c. Both active and total microbial biomasses were higher in samples from the agricultural soils than in the samples from nature reserves. The active microbial biomass fraction ranged from 12% of the total microbial biomass in the fen peat under agricultural land use, to 58% of the microbial biomass in the bog peat under natural land use. The other peat types showed intermediate values (31% and 29% respectively).

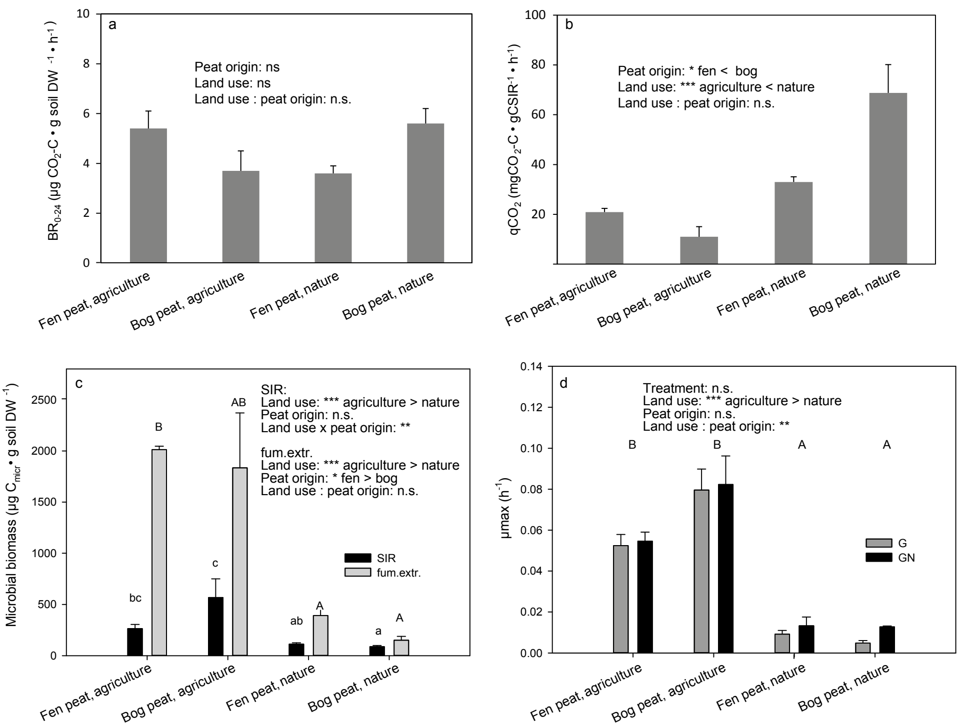


**Figure 5.3:** a. Basal respiration rate in the first 24 h of incubation. b. Relative respiration rate for microorganisms ($qCO_2$). c. Microbial biomass determined by SIR (active, glucose responsive) and by fumigation-extraction (total). d. Microbial growth rate response after amendment with glucose or with glucose and ammonium ($\mu_{max}$). Error bars reflect standard errors. Main results of ANOVA are shown (***$p$<0.001, ** $p$<0.01, * $p$<0.05). Capital letters indicate significance of differences between combinations of peat origins and land uses (ANOVA $p$<0.05).

Figure 5.3d presents the potential growth rate of the microbial biomass ($\mu_{max}$), which varied between 1-8% per hour. $\mu_{max}$ was larger in dairy meadows than in nature reserves (RM ANOVA F=80.082, $p$<0.001). Overall, nitrogen amendment in combination with the glucose treatment did not increase $\mu_{max}$, with the exception of the bog peat from nature reserve Fochteloërveen; here, $\mu_{max}$ was significantly higher in the GN treatment than in the glucose treatment (RM ANOVA F=939.834, $p$<0.001).
The C:N ratio of the microbial community in the bog peat from the agricultural site (10.7 ± 3.3) was higher than in the other peat types (6.6 ± 0.7, 6.8 ± 0.3 and 6.3 ± 1.0 respectively).

*Respiration quotient, a proxy for OM quality*
The RQ of all samples was generally well below 1, which is expected for aerobic consumption of reduced carbon substrates without growth (Dilly, 2003). The RQ of control samples in the fen peat from the nature reserve Nieuwkoopse Plassen was 0.14± 0.01 (Figure 5.4). This was significantly lower than the RQ of the other samples (RM ANOVA F=24.362, $p$<0.001).

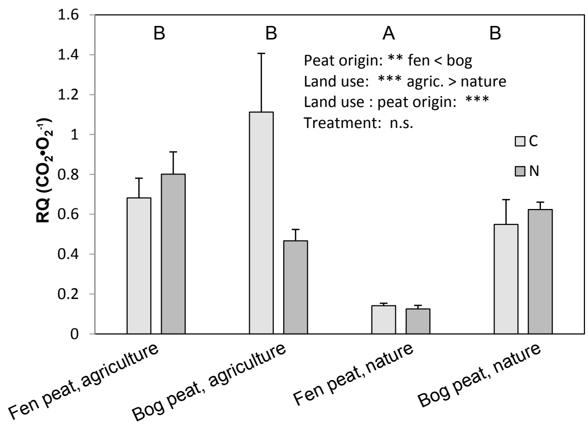


**Figure 5.4:** Respiration quotient (RQ) in mol $CO_2$ produced per mol $O_2$ used over the first 100 h. Error bars reflect standard errors. Main results of RM ANOVA are shown (***$p$<0.001, ** $p$<0.01, * $p$<0.05). Capital letters indicate significance of differences between combinations of peat origins and land uses (ANOVA $p$<0.05).

### 5.3.4 Potential enzyme activities

Peat origin significantly affected the potential activities of oxidative enzymes POX and POD, and the hydrolytic enzyme NAG which is involved in N acquisition. The potential activities of POX, POD and NAG (Figure 5.5a,b,f) were significantly higher in the fen peat samples than in the samples of bog origin (RM ANOVA F=48.591, $p$<0.001; F=1690.841, $p$<0.001; RM ANOVA F=12.792, $p$<0.01 for POX, POD and NAG respectively). For POX, the interaction between peat origin and land use was significant (RM ANOVA F=379.665, $p$<0.05).

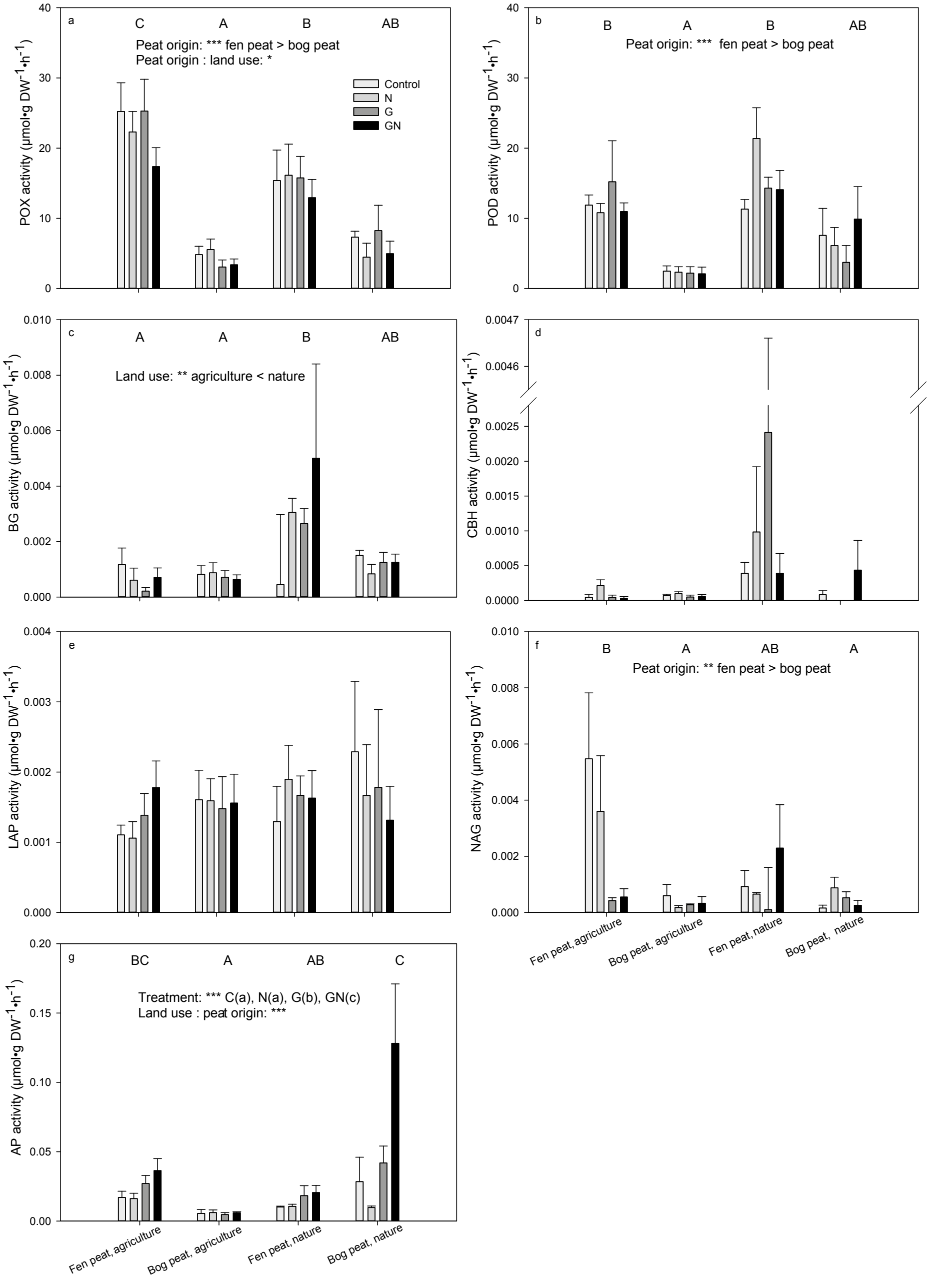


**Figure 5.5:** Potential enzyme activities of each land use and peat type combination and treatment (C, N, G, GN). a=phenol oxidase, b=peroxidase, c=β-1,4-glucosidase (acquiring C), d=cellobiohydrolase (acquiring C), e=leucyl aminopeptidase (acquiring N), f=N-acetylglucosaminidase (acquiring N), g=acid phosphatase (acquiring P). Main results of RM ANOVA are shown (***$p$<0.001, ** $p$<0.01, * $p$<0.05). Capital letters indicate significance of differences between combinations of peat origins and land uses (ANOVA $p$<0.05).

The fen peat samples from a dairy meadow showed a higher enzyme activity than the fen samples from the nature reserves, which is opposite to the findings in Chapter 3. The potential POX activities from the bog peat samples did not differ between land use types. No such interaction was found in the POD enzyme assays. There were no indications that pH influenced POD and POX activities.
The activity of BG was significantly affected by land use as the activity appeared to be larger in the samples from the nature reserves (RM ANOVA F=10.420, $p<0.01$). AP was the only enzyme showing a treatment effect (Figure 5.5g). The combined glucose and nitrogen addition, and to a lesser extent the single glucose addition, led to an increased activity. CBG and LAP did not show any treatments, peat origin or land use effects.

## 5.4 Discussion

In contrast to the hypotheses, aerobic decomposition rates of the peat samples from both major peat origins in the Netherlands (fen vs. bog) were not affected by land use despite the long history of deep drainage and fertilisation in the agriculturally used sites and the absence of such a history in the nature reserves. Although basal respiration rates between peat types were similar, the microbiota was affected by land use as indicated by microbial biomass, relative respiration rates and microbial growth response upon glucose addition. On the other hand, exo-enzyme activities were mostly affected by peat origin, rather than by current land use. The recalcitrant nature of the peat studied here is characteristic and reflected in the high metabolic quotient ($qCO_2$) and low respiration quotient (RQ), revealing low energy use efficiency. Phosphorus enrichment turned out to be a potential driver of increased peat decomposition, while nitrogen addition reduced decomposition rates.

### 5.4.1 Biomass and activity of microbiota and exo-enzyme activities

The total microbial biomass was larger in the agricultural meadows. This phenomenon has been attributed to the fertilisation with manure that takes place in the agricultural peat meadows. This results in a pulse of nutrients and OM four to five times per year, stimulating plant and microbial growth (Walsh et al., 2012). Peat origin did not evidently affect microbial biomass in the present study. In addition, relative respiration rates per unit of microbial biomass ($qCO_2$) in the control samples of agricultural peat soils were low compared to those of the nature reserve soils.

Besides the higher microbial biomass and low relative respiration rates in samples from agricultural sites, the microbial growth response of the microbiota in these soils was generally stronger in the samples from agricultural peat soils compared to samples from nature reserves. The high increase in microbial growth upon glucose amendment indicates a uniformly present energy limitation in the microbial communities in dairy meadows (Keuskamp et al., 2013). In contrast to the rather uniform response to the glucose addition in the samples from dairy meadows, the response of samples from the nature reserves showed much more variability. This indicates that the spatial heterogeneity of microbial communities in the studied nature reserves is larger than in dairy meadows. This is possibly a result of the stronger heterogeneity of the vegetation in nature reserves (Eisenhauer et al., 2010).

Overall, it appears that the reclamation and conversion of peatlands to agricultural land has changed the resource limitations experienced by the microbiota and most likely also the composition of the microbial community. The large difference between active and total microbial biomass in combination with the low relative respiration rates and large growth response upon glucose addition suggest that a part of the large microbial community in the dairy meadows is inactive, but is able to respond quickly to changing conditions such as the supply of manure, consisting of (labile) organic carbon and nutrient compounds. This behaviour indicates that microbial communities in the agricultural peat meadows contain more r-strategists, i.e. opportunists that react rapidly to changing conditions, than the communities in the nature reserves. The microbial communities in nature reserves, in contrast, generally consist more of slowly growing organisms and spatial heterogeneity seems to be larger than in agricultural soils. Consequently, more detailed studies on the microbial community composition in these case areas contrasting in origin and land use would be a very interesting follow-up study.



Peat origin, to a larger extent than land use, affected the potential activities of the oxidative enzymes POX and POD. The potential activities of POX and POD generally were higher in fen peat than in bog peat, which is not parallel to the variation in respiration rates, neither there were analogies found in abiotic factors. The potential enzyme activities measured here are in contrast with previous findings by Fenner and Freeman (2011) and Sinsabaugh (2010) who found that both nutrient concentrations and phenolic compound concentrations affect the potential enzyme activities and respiration rates. The biogeochemical cascade describes the degradation of phenolic compounds by POX, after which the concentration of phenolic compounds decreases, which in turn stimulates microbial growth and results in the release of carbon dioxide. These higher POX and POD activities should lead to lower concentrations of phenolic compounds and higher respiration rates (Fenner and Freeman, 2011). However, this was not confirmed in the comparison of the peat types studied here; phenol concentrations, nutrient concentrations and pH did not affect POX activity or decomposition rates.

The dynamics of phenolic compound degradation and POX have not been completely unravelled yet. Although POX has a much higher activity in oxic conditions, the enzyme has been reported to be active in anoxic conditions as well (Elder and Kelly, 1994; Freeman et al., 2001; Freeman et al., 2004; Fenner and Freeman, 2011). Furthermore, POX activity generally increases with pH (Pind et al., 1994; Toberman et al., 2010), while negative as well as positive correlations between POX activity and soluble phenolic compound availability have been found in literature (Pind et al., 1994; Williams et al., 2000; Toberman et al., 2010). In the latter case, the causal relation remains unclear, as high concentrations of soluble phenolic compounds could have resulted from either low enzyme activity or high production of secondary products during the degradation of condensed phenolic compounds. This would explain the occurrence of both positive and negative correlations between POX activity and the concentration of soluble phenolic compounds.

### 5.4.2 Peat as a substrate for microbiota

The degree of recalcitrance of the peat for microbial activity can be deduced from the metabolic quotient ($qCO_2$) and respiration quotient (RQ). The $qCO_2$ was generally between 10 and 70 mg $CO_2$-C·g $C_{micr}^{-1}$·$h^{-1}$. These values are substantially higher than in other wet organic soils (Hartman and Richardson, 2013). The RQ was rather low, which means that the $O_2$ consumption is rather high compared to $CO_2$ production, which is caused by the relatively small quantities of $O_2$ that are incorporated in recalcitrant OM. The high metabolic quotient ($qCO_2$) and low respiration quotient (RQ) show a low energy use efficiency, which clearly indicates that the peat in our study areas is rather recalcitrant, regardless of peat origin and land use (Anderson and Domsch, 1993; Dilly and Munch, 1998; Dilly, 2001; Keuskamp et al., 2013). The hypothesis that the peat from dairy meadows would be in a further stage of decomposition (more amorphous) and thus more recalcitrant, which would have been reflected in a low RQ and high $qCO_2$ was not confirmed. A low RQ was also expected in the bog peat because of the high content of phenolic compounds, which are generally considered to be rather recalcitrant and inhibit decomposition (Freeman et al., 2004; Fenner and Freeman, 2011). However, the results are in contrast with this hypothesis as RQ values that were measured in the peat from a natural bog had intermediate to high RQ values compared to the other peat types, whilst phenolic compound concentrations where highest. Fen peat showed the lowest RQ, which we attribute to pyrite oxidation (Van Gaans et al., 2007).

The respiration rates of the samples from the natural bog reserve were higher than the rates of the peat meadows, which was also opposite to the presumed recalcitrant nature of this type of peat. Still, all respiration rates fit well within the range of other peat incubation studies (Aerts and Toet, 1997; Moore and Dalva, 1997; Basiliko et al., 2007; Kechavarzi et al., 2010). Based on the recalcitrant nature of bog peat we had expected it to have lower decomposition rates than the fen peat (Verhoeven and Toth, 1995; Verhoeven and Liefveld, 1997). The results gave no indication for a correlation between RQ and edaphic soil properties such as phenolic content, pH and nutrient concentrations, as was also found by Dilly (2001). This indicates that respiration rates cannot be deduced easily from edaphic characteristics.

There are several possible explanations for the deviation of the respiration rates of the samples from the natural bog Fochteloërveen from the other samples. Firstly, all measurements are expressed per unit of dry soil, the samples from the Fochterloërveen however, have a higher organic matter content. So, expressed per unit of organic matter they are ca 30-35% lower. In addition, the samples were taken in a shallow peat layer in a peat-forming bog, which means that the proportion of easily degradable carbon compounds in this rather fresh organic material has typically been high. As young material still contains material with high turnover rates, the $CO_2$ production is expected to be relatively high (Berg and Meentemeyer, 2002; Glatzel et al., 2004). The relatively young and fresh OM that accumulates near the soil surface has in general higher respiration rates than peat samples from greater depth (Szafranet-Nakonieczna and Stêpniewska, 2014). However, the high metabolic quotient ($qCO_2$) and low respiration quotient (RQ), both suggest that the organic material in Fochterloerveen is recalcitrant rather than labile (Anderson and Domsch, 1993; Dilly, 2001). Most of the variability in the biological response would depend on abiotic constraints, mainly physical properties, nutrient status and concentration, and maturity of the OM. Under field conditions, the water filled pore space in the natural bog samples is significantly higher than in the other samples. A plausible cause for the high respiration rates compared to the other peat types is an increase in oxygen availability during incubation in comparison to the field conditions. The Fochteloërveen is a nature reserve with water levels near soil surface, whereas the other areas are slightly to moderately drained. Therefore, it is likely that the availability of oxygen increased during incubation most in the samples from the Fochteloërveen. High basal respiration rates in bog peat compared to fen sites have been found previously and were attributed to an increase in oxygen supply as well (Fisk et al., 2003).

This indicates that the peat soils in the Netherlands, of which the majority has a very long history of drainage and agricultural use, are in an advanced stage of decomposition. Natural peatlands, especially bogs, are generally associated with harsh conditions for microbiota, which results in low decomposition rates (Verhoeven and Liefveld, 1997), although the microbial community living here is adapted to these conditions (Kulichevskaya et al., 2007). The combination of low RQ and high $qCO_2$ values indicates that the microbial communities of the studied soils are severely energy-stressed and largely composed of K-strategists: slowly growing microorganisms oxidizing mainly recalcitrant materials (Keuskamp et al., 2013). Clear exceptions are the inactive microorganisms in the agricultural peat soils, which are adapted to the pulses of labile organic compounds supplied with the manure.

### 5.4.3 Nutrient limitations in peat decomposition

The fact that the nitrogen-amended treatments had lower respiration rates than the control treatments between 100 and 150 hours of incubation suggests that the microorganisms were degrading recalcitrant OM to obtain nitrogen in the absence freely available nitrogen and/or labile organic compounds. These results confirm the recalcitrant nature of the peat OM and fit the 'nitrogen mining theory' (Neff et al., 2002; Moorhead and Sinsabaugh, 2006; Craine et al., 2007).

The effect of nitrogen on decomposition rates was larger in the samples from the nature reserves, where the nitrate and ammonium concentrations in pore water were lowest. It was previously found that the soil microorganisms from natural *Sphagnum* bogs metabolised less carbohydrates and invested more in N acquisition by metabolizing amino acids, compared to forest and fen peat samples (Fisk et al., 2003). However, caution is required when extrapolating this finding up to the ecosystem scale and longer time-scale because short-term effects of depletion of labile carbon sources after increased decomposition facilitated by nitrogen enrichment cannot be ruled out completely.

Cumulative $CO_2$ production in the experiments with glucose additions (data not shown) suggested that not all glucose was respired during the respiration peak or the experimental period as a whole. This indicates that not energy but another factor was limiting the microbial community after glucose addition. However, it is also possible that the glucose is sequestered in additional microbial biomass. In literature, enzyme activities are often used as an indicator of nutrient limitations (Sinsabaugh, 2010; Sinsabaugh et al., 2011). In our experiment, acid phosphatase (AP) was the only investigated enzyme that increased in activity upon adding glucose and even more after adding glucose and nitrogen. Therefore, we assume that the treatments induced phosphate limitation, which led to higher activities of AP. Additionally, lowest AP activities were found in the bog peat samples from an agricultural meadow, which had the highest soluble $PO_4^{3-}$ concentrations, whereas, the highest activities of AP were found in the sample types with lowest $PO_4^{3-}$ concentrations. P limitation of the microbial community decomposing *Carex* OM has previously been reported (Amador and Jones, 1993; Aerts and De Caluwe, 1997). Additional decomposition measurements after P addition are needed to verify if P is indeed limiting decomposition in field conditions.

Surprisingly, glucose addition did not lower the potential activities of cellobiohydrolase (CBH) and β-1,4-glucosidase (BG), which are involved in the depolymerisation of cellulose resulting in glucose monomers. The treatments did not affect the nitrogen-acquiring enzymes either. Perhaps, the half-life of these enzymes is too high to detect a decrease in activity during incubation, or nitrogen was still limiting the soil microbes.

## 5.5 Conclusion

This study aimed to unravel the effects of peat origin and land use on decomposition characteristics, with special attention to microbial biomass, activity and growth, and growth limitations. Unexpectedly, we did not find a uniform effect of land use (agriculture vs. nature reserves) or peat origin (fen vs. bog) on respiration rates. Nevertheless, substantial differences in microbial activity, microbial biomass and respiration dynamics were found between land uses, whereas enzyme activities differed mostly between peat types. SIR measurements led to the conclusion that agricultural land use has significantly modified the microbial community. There are strong indications that the microbial communities in the agricultural peat meadows contain more r-strategists, i.e. opportunists that react rapidly to changing conditions, than the communities in the nature reserves.

POX is considered the main regulator of peat decomposition (Freeman et al., 2004; Fenner and Freeman, 2011). This study showed that the activities of these oxidative enzymes were higher in fen peat than in bog peat. Concentration of phenolic compounds or pulses with nitrogen and/or glucose did not affect POX activity. Unlike previously reported in literature, neither POX activity nor the concentration of phenolic compounds did correlate to respiration rates. We did not find an effect of resource availability on POX. This provides no support for the enzymic latch theory or 'biogeochemical cascade' proposed by Fenner and Freeman (2011). Furthermore, we found, in line with the 'nitrogen mining theory' (Craine et al., 2007), that nitrogen limits the decomposition of the recalcitrant OM as decomposition rates were lower after nitrogen addition, although it is also possible that labile carbon sources were depleted after a period of increased decomposition. As AP was the only enzyme that responded to the treatments, it is recommended to further investigate the role of phosphorus in relation to peat decomposition.

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## Acknowledgements

This study was supported by the Dutch National Research Programme Knowledge for Climate (Kennis voor Klimaat), as part of the hotspot 'shallow waters and peat meadow areas' (project code HSOV1a). We are grateful to Natuurmonumenten for facilitating peat sampling in the nature reserves Nieuwkoopse Plassen and Fochteloërveen. In addition, farmer Gert Diever and the Veenweide Innovatiecentrum (peat meadow innovation centre) are greatly thanked for allowing us to take soil samples at their sites. Furthermore, we would like to thank Dr. Heike Schmitt (Institute for Risk Assessment Sciences, Utrecht University, the Netherlands) for lending us the respiration monitor.

# Appendix

**Appendix 5.A:** Soil enzymes assays for potential exo-enzyme activity.

| Abbreviation | Substrate | Product | Substrate concentration (mM) | Incubation time (h) | Wavelength (nm) |
|---|---|---|---|---|---|
| **POX** | L-Dopa | dicq | 2.5 | 3 | 450 |
| **POD** | L-Dopa + peroxide | dicq | 2.5 | 3 | 450 |
| **BG** | pNP-β-glucopyranoside | pNP | 5 | 3 | 405 |
| **CBH** | pNP-cellobioside | pNP | 2 | 5 | 405 |
| **LAP** | leucine p-nitroanilide | pNA | 1.67 | 16 | 405 |
| **NAG** | pNP-β-N-acetylglucosaminide | pNP | 2 | 4 | 405 |
| **AP** | pNP-phosphate bis (tris) salt | pNP | 54 | 2 | 405 |

# Chapter 6

# Spatial analysis of soil subsidence in peat meadow areas in Friesland in relation to land and water management, climate change, and adaptation

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*Environmental Management 55 (2015) 360–372*

## Abstract

Dutch peatlands have been subsiding due to peat decomposition, shrinkage and compression since their reclamation from the fifth century onwards. Currently, subsidence amounts to 1-2 $cm \cdot yr^{-1}$. Water management in these areas is complex and costly, greenhouse gases are being emitted, and surface water quality is relatively poor. In addition, the Netherlands Royal Meteorological Institute predicts higher temperatures and drier summers, which both are expected to enhance peat decomposition. Regional and local authorities and landowners responsible for peatland management have recognised these problems. Stakeholder workshops have been organised in three case study areas in the province of Friesland to exchange knowledge on subsidence and explore future subsidence rates and the effects of land use and management changes on subsidence rates. Subsidence rates were up to 3 $cm \cdot yr^{-1}$ in deeply drained parcels and increased when we included climate change in the modelling exercises. This means that the relatively thin peat layers in this province would have shrunken or even would have disappeared by the end of the century when current practices continue. Adaptation measures were explored, such as extensive dairy farming and the production of new crops in wetter conditions, but little experience has yet been gained on best practices. The workshops have resulted in useful exchange of ideas on possible measures and their consequences for land use and water management in the three case study areas. The province and the regional water board will use the results to develop land use and water management policies for the next decades.

## 6.1 Introduction

Centuries of drainage and peat cutting have resulted in a major loss of peat soils in the Netherlands. Peatland ecosystems once covered a major proportion (40%) of the Dutch land surface, but the area of peat soils has been reduced to less than 8% since drainage started in the 11th century (Schothorst, 1977; TNO, 2007). Drainage has enabled agricultural use by optimising oxygen and nutrient availability and has allowed access of heavy machinery. However, deep drainage to facilitate intensive agriculture currently causes rapid soil subsidence, generally up to 2 $cm \cdot yr^{-1}$ (MNP, 2005; Querner et al., 2012). As a consequence of the rapid subsidence, regular adjustments of the water level are needed enforcing subsidence rates. These peat soils are emitting on average 19 tonnes of $CO_2 \cdot ha^{-1} \cdot yr^{-1}$ (Van den Akker et al., 2008) and often lead to poor surface water quality (Van Beek et al., 2007). Subsidence of peat soil is the result of a combination of processes, i.e., shrinkage, compression, and oxidation, which are all caused by lowering of the water table. The shrinkage process is a reduction in volume caused by the withdrawal of water from the upper soil layer. The loss of the buoyant force of water in the upper layers also leads to the compression of deeper layers. The microbial oxidation of soil organic matter under oxic conditions leads to a major peat loss after drainage. In fact, up to 85% of subsidence can be attributed to oxidation (Schothorst, 1977).

It has been predicted that the peat areas in the Netherlands will subside between 40 and 60 cm between 1999 and 2050 (Hoogland et al., 2012). Moreover, continuation of the current land use in the Dutch peat meadow areas will lead to the disappearance of most peat within 200 years and all peat within 500 years (Rienks et al., 2002; Querner et al., 2012). The national government, provinces and water boards increasingly realise that a continuation of this management will have problematic side effects such as damage to building foundations, desiccation of nature reserves, emission of greenhouse gasses, increasing costs for water management and infrastructural maintenance, deterioration of surface water quality and, finally, loss of the characteristic landscape. In the Dutch peat areas, the regional governments are reaching out to stakeholder groups representing the various interests (farmers, recreation entrepreneurs, nature conservation agencies) to discuss the future of the peat areas and the adaptation measures needed for a sustainable management avoiding very high costs and threat to human settlements from potential flooding at later stages.

The problems indicated have been familiar to regional and local stakeholders for decades; however, recent concerns about, and research into, the effects of climate change have suggested that the situation will most probably be aggravated in the near future. Besides a rise in average temperatures of 1-2 °C in the period 1990–2050, two of the climate change scenarios put forward by the Netherlands Royal Meteorological Institute (i.e., the G+ and W+ scenarios) predict drier summers due to a change in atmospheric circulation (Van den Hurk et al., 2006). The predicted temperature rise, changes in precipitation, and the more frequent occurrence of extreme episodic events will potentially have strong additional effects on organic matter decomposition, see also Chapter 2 of this thesis and a body of literature (Laiho, 2006; Witte et al., 2009; Hellmann and Vermaat, 2012; Querner et al., 2012). Experimental studies have overwhelmingly shown that soil organic matter decomposition increases at higher temperatures. A temperature increase of 10 °C usually leads to a tripling of the peat decomposition rates (Berglund et al., 2008; Dorrepaal et al., 2009). Furthermore, it is plausible that dry summers enhance long-term decomposition rates (Fenner and Freeman, 2011). It has been estimated that the combination of temperature rise and lower groundwater levels in summer in peat meadow areas will lead to a 70% increase in subsidence in the W+ scenario (Querner et al., 2008).

Here, the current management strategy of regularly adapting water levels to the subsiding land surface is being re-evaluated in the context of the development of a policy plan by the province of Friesland for future management of their peat meadow areas. In order to facilitate the decision-making and increase the public support for land use and water management changes, spatial visualisations of the effect of climate change on soil surface level were generated and the consequences for agriculture, nature, and environmental targets were analysed and discussed in stakeholder workshops in three case study areas. These meetings involved the use of a spatial model presented on an interactive mapping device (the 'touch-table', Figure 6.1) which was used as a common interface. This allowed the participants to learn about the mechanisms behind soil subsidence and the consequences. The participants were asked to change land use and water levels to observe the impacts on soil subsidence and to inspect relevant map layers such as expected agricultural yields (Janssen et al., 2013; Janssen et al., 2014). This article summarises the way in which spatial information on peat soil characteristics, subsidence rates, and water resource management was presented to stakeholders. It also summarises what overall conclusions can be drawn from the peat responses to different climate scenarios and from the effectiveness of various adaptation measures.

**Figure 6.1:** Use of the 'touch table' during the workshop in Hommerts (province of Friesland, the Netherlands).

## 6.2 Materials and methods

In three case study areas, workshops were conducted to provide detailed, spatially explicit knowledge to the local stakeholders. The stakeholders were able to apply measures in the form of land use and water management changes using the 'touch-table' tool. A questionnaire was used to identify the opinion and knowledge gains of the stakeholders (Janssen et al., 2014). 19 stakeholders representing different interest groups and authorities (agriculture, recreation, water board, province, municipalities, nature conservation organisations) participated in the workshops.

### 6.2.1 Modelling soil subsidence

Soil subsidence maps have been generated by modelling spatially explicit information on soil, land use, and ditch water and groundwater levels. Factors determining the rate of soil subsidence are the presence and thickness of peat, the presence of a clay cover, and the height of the ditch water level relative to the soil surface. The models provided in the Guidelines for Soil Protection were taken as the starting point (Van den Akker et al., 2007). These models are based on long-term data of land subsidence in the western and northern Netherlands (Figure 1.3). Furthermore, the soil maps of the province of Friesland are ca 40-50 years old. For the workshops, we were able to use the still unpublished new soil maps produced by the research institute Alterra, which is linked to the Wageningen University and Research centre.

*Groundwater level*

Detailed information on the relationship between soil subsidence and ditch water level has originated from the experimental farm 'Zegveld' in the province of Utrecht (Van den Akker et al., 2007). At the experimental farm, two pairs of blocks were created in 1969 with ditch water levels of 35 and 70 cm below ground surface, respectively. We define this vertical distance between Ground surface and Ditch water Level as GDL. Later on, these water levels were adjusted to 20 cm (high GDL) and 55 cm (low GDL), respectively. The monitoring results for the period 1972-2006 (Figure 1.3) show that the difference in subsidence between the plots with high and low GDL has been consistent, i.e., 4.4. and 11 $mm \cdot yr^{-1}$, respectively (Van den Akker et al., 2007). The relationship between subsidence and drainage depth as defined in Zegveld is confirmed by a long-term study of subsiding peat meadows in Friesland. In the period 1920–1960, the average land subsidence was 5 $mm \cdot yr^{-1}$, while it increased to an average of 12 $mm \cdot yr^{-1}$ between 1960 and 1995 after increasing the GDL (Nieuwenhuis and Schokking, 1997). Another example is a study in which data on surface water levels and land subsidence from the Frisian peat meadow areas were shown to be significantly correlated (Janssen, 1986).

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In the Netherlands, groundwater levels are generally being monitored twice a month. The three highest (HG3) and the three lowest (LG3) groundwater levels measured in each year are averaged. The Mean Highest Water table (MHW) and Mean Lowest Water table (MLW) are then determined as the mean HG3 and LG3 for at least 8 years, respectively. Research has shown that the MLW provides the best correlation with soil subsidence rates (Van den Akker et al., 2007), because this relates to the thickness of the unsaturated peat layer that is being oxygenated in summer (Fenner and Freeman, 2011). In general, the lowest groundwater levels occur in August and September, coinciding with highest soil temperatures, providing optimal conditions for peat oxidation in during these months (Wesseling, 1985; Hoving et al., 2004). Although MLW corresponds best with subsidence rates, we used the average GDL in the workshops with local stakeholders. This GDL term is easier to implement and interpret than MLW and still gives reliable estimates mimicking the models based on MLW (Van den Akker et al., 2007). Hydrological modelling was done with the SIMGRO model by research institute Alterra; this model quantifies the hydrology on a local scale. The equations given in Table 6.1 are based on the long-term monitoring data described above and on expert judgment on the quantitative effects of the major factors driving the subsidence rates, (i.e., presence of a clay cover, MLW, and GDL).

*Clay cover*

Due to alternating periods of rapid and slower sea level rise during the Holocene, periods of undisturbed peat formation alternated with periods where the peatlands were flooded by nearby rivers or estuaries and covered by a marine or riverine clay layer. Peat meadows with presence of a clay cover have a slower subsidence because oxygen intrusion in the peat soil is limited below the clay layer, and less organic material is present to decompose in the soil profile (Van den Akker et al., 2007). Therefore in the soil subsidence models, a distinction was made between peat and peat with clay cover, where the clay cover thickness is less than 40 cm. Soils with a clay cover thicker than 40 cm are not considered peat soils in these workshops as their subsidence is marginal.

*Land use, peat origin and thickness*

All study areas are characterised by grassland parcels, with occasionally some fields where maize is grown. Based on measurements in Friesland, the subsidence rates of arable land have been estimated to be 1.5x faster than that of grassland (Janssen, 1986). The peat soils in the Netherlands are composed of various peat origins, ranging from fen peat, which was formed in eutrophic conditions, to bog peat, which was formed in oligotrophic conditions. While the fen peat has a slightly faster rate of decomposition, it also has a higher bulk density, so that land subsidence rates of nutrient-rich and nutrient-poor peat are almost equal (Janssen, 1986; Van den Akker et al., 2007). Peat thickness is taken into account in the model predictions of land subsidence. It was assumed that once the MLW has become deeper than the thickness of the peat layer, subsidence would not increase if a further MLW drop occurs.

*Climate change scenarios*

The Royal Netherlands Meteorological Institute has constructed four climate change scenarios for the Netherlands, which have an equal probability of occurrence (Van den Hurk et al., 2006). The G and G+ scenarios predict a moderate temperature change (+1 °C), the W and W+ scenarios predict higher temperature (+2 °C). The '+' indicates a modified atmospheric circulation, resulting in drier summers. As a result of higher temperature and drier summers, the W+ scenario has been modelled to result in an increase up to 70% for soil subsidence rates in peat meadow areas with peat lacking a clay cover in 2050 (Querner et al., 2012). In addition, substantial amounts of surface water need to be supplied to prevent desiccation of the peatx (Querner et al., 2012). Pressures on freshwater resources are, however, increasing and the question is whether sufficient good quality freshwater will be available in future (MNP, 2005).

The study presented here is based on the W+ scenario. For the purpose of our study, we used a 1.5x faster rate in our model simulations to calculate the subsidence rates at higher temperatures, based on literature of the total effect of higher temperatures (Andriesse, 1988; Davidson and Janssens, 2006; Berglund et al., 2008; Dorrepaal et al., 2009).

**Table 6.1:** Equations for land subsidence of grassland parcels (S, mm·$yr^{-1}$) in relation to the average lowest groundwater level (MLW, cm) and related to the vertical distance between ground level and ditch water level (GDL, cm) for peat meadows with or without clay cover. The second set of equations has been used in this study. Furthermore, we multiplied the subsidence rates with the factor 1.5 for arable fields and also for the higher temperatures predicted in the W+ scenario.



| Soil conditions | Relation to | Equation | Remark |
|---|---|---|---|
| **Peat** | groundwater level (ALGL, cm) | S(i)=0.2354*ALGL-6.68 | Generally applicable in the Netherlands |
| **Peat with clay cover** | groundwater level (ALGL, cm) | S(i)=0.2354*ALGL-10.47 | |
| **Peat** | ground-ditch level (GDL, cm) | $S(i)=0.538*GDL^{0.776}$ | Adapted equations used for 'touch table' workshops in Friesland |
| **Peat with clay cover** | ground-ditch level (GDL, cm) | $S(i)=-4*10-6*GDL^3+12*10^{-4}*GDL^2+439*10^{-4}*GDL)$ | |

*Modelling the effect of adaptation*

A new interactive tool, a 'touch-table' with implemented GIS applications, was used during the workshops, enabling the visualisation of land use, subsidence rates, and groundwater or ditch water levels characteristics in a spatially explicit and readily understandable way for the study area under investigation. The learning-by-doing aspect of this type of tool was found effective in supporting the exchange of information between stakeholders with

different backgrounds (Janssen et al., 2014). Soil subsidence rates under the current climate and the W+ scenarios were likewise visualised per parcel. Various adaptation measures, (e.g., higher surface water levels, change of land use, or turning over land into open-water systems) could also be evaluated as they affect GDL and, in turn, affect land subsidence rates which were visually presented.
The consequences of climate change and adaptation measures on land subsidence could immediately be calculated and displayed in a spatially evident way by running the models in this interactive tool. Although the model does describe 0 subsidence if the water level is at the soil surface, it does not include the possibility of peat formation for even wetter situations and, hence, excludes rising surface levels; on the other hand, the history of drainage could have a continuing stimulating effect even once anoxic conditions have established (Chapter 2). During the workshops, we did discuss such options and the risks of adverse effects on water quality after rewetting agriculturally used peat soils (Zak et al., 2008). In addition to the effects of adapted water regimes on subsidence, their effects on agricultural yields were also displayed using the parameters defined in by the Dutch Foundation for Applied Water Research (Stowa, 2005).

### 6.2.2 Study areas in the province of Friesland

In collaboration with the provincial government and the water board (Wetterskip) of Friesland, the Netherlands, three study areas were selected, together covering the range of variation in Friesland in types of peat meadows (peat origin, thickness of peat layer, land use, ditch water level management). These study areas were Hommerts, Groote Veenpolder, and Buitenveld, as examples of polders with a peat soil with clay cover, a thick peat layer (generally >1.0 m) and a thin peat layer (<1.0 m), respectively (see Figure 6.2).

*Hommerts (1)*
In the western part of Friesland, there are clay-covered peat areas which in the past have been little cut-over, and thus retain substantial depths of peat in places. The Hommerts polder is an example of such an area, covering 2,400 ha. A land reallotment scheme in the 1970s restructured the area and resulted in deeper drainage leading to summer groundwater levels deeper than 1 m below soil surface. The whole area consists of 'clay-covered peat' soils. According to recent measurements, the thickness of the clay cover ranges between 10 and 40 cm. An east–west orientated sand ridge cuts through the area. The old course of the Drylster Ie, a former bog stream that ran west of the building ribbon and discharged into the former Middelsee, is still recognisable in the landscape. The peat layer beneath the clay cover is continuous and does not contain further clay deposits. The peat thickness is mostly around 40–60 cm, with thicker layers up to 2 m in the northern part of the area (Figure 6.3).

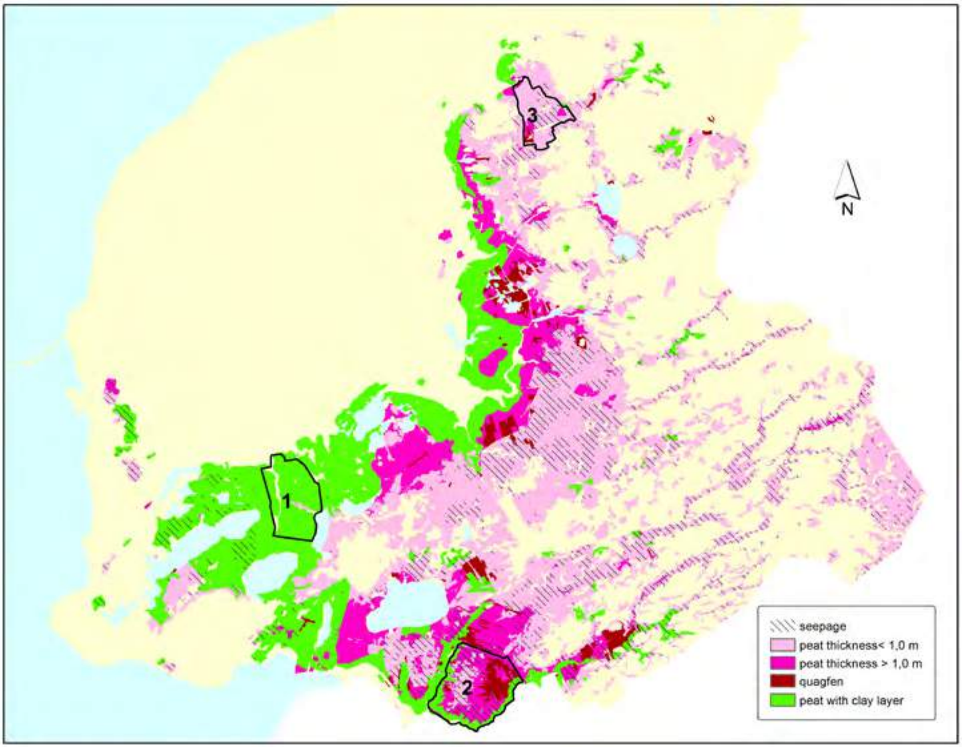


**Figure 6.2:** Location of three study areas in Friesland, the Netherlands; 1. Hommerts; 2. Groote Veenpolder; 3. Buitenveld.



*Groote Veenpolder (2)*

This polder is an example of the area in the southern part of Friesland, where clay cover is mostly absent and large parts of the peatlands have been superficially cutover for fuel in the mid-19$^{th}$ century. These areas were reclaimed for agriculture at a later stage and turned into peat polders around 1900. Because of the peat extraction, these areas now lie significantly below sea level. The ground level in the Groote Veenpolder is now approximately -2.50 m NAP (Dutch Ordnance Datum, reflecting the average sea level). Of the 3,450 ha, over two-thirds is in agricultural use, whereas ca one-third comprises the nature reserve of the Rottige Meente, which consists of ditches, swamp forest, species-rich grasslands and associated habitats. The Groote Veenpolder is very diverse in terms of peat soil type: ranging from fen peat formed in eutrophic conditions (including the remains of trees) to bog peat, formed in nutrient-poor conditions, and from weakly decomposed fibric to 'earthified' amorhous sapric peat. The peat thickness of the peat deposit is more than 2 m in the nature reserve, but significantly less than that in the agricultural grasslands (Figure 6.3).

*Buitenveld (3)*

The Buitenveld (Frisian: Butenfjild), which covers an area of 1,525 ha, is the most north-eastern part of the Frisian peat area. It lies between clay and sandy soils. The peat soils have always been relatively thin here, but they have undoubtedly subsided during the course of the past century. The northern part of the area was reallotted and restructured in the 1950s and is now in agricultural use for dairy production. The southwestern part is a nature reserve and has the highest elevation because surface water levels have been kept higher here. The low groundwater levels due to the drainage of the agricultural part have led to seepage of water from the nature reserve, thus creating desiccation of the reserve as well as inconveniently high groundwater levels in the pastures. The peat layer is thin or even absent, with the thickest layers (up to 1.20 m) in the wetland reserve in the southwest (Figure 6.3). In the agricultural pastures, the peat layer is often thinner than 40 cm and is no longer classified as peat soil.

## 6.3 Results

### 6.3.1 Land subsidence with different climate scenarios

Maps of the three study areas displaying the rate of land subsidence calculated per agricultural parcel are shown in Figure 6.4. The parcels are separated by drainage ditches or canals indicated in blue. The colour of the parcels represents current land subsidence rates, whereas the colour of the dots in each parcel represents subsidence rates under climatic conditions of the W+ scenario. It is clear that subsidence rates differ between the three study areas. Rates are relatively low in the Hommerts and Buitenveld areas (1–15 $mm \cdot yr^{-1}$) compared to the Groote Veenpolder (6–30 $mm \cdot yr^{-1}$, with many parcels in the more rapid categories. These differences are caused by the different conditions of the peat soils in the three study areas, namely the relatively thick peat layer without clay cover in the Groote Veenpolder, the presence of a clay cover at Hommerts, and the thin peat layers at Buitenveld (see also Figure 6.3).

There are also clear differences in subsidence rates within the study areas. These are again associated with differences in thickness of peat and the presence of a clay cover, but are also related to current land use. In the Hommerts area (Figure 6.4a), the parcels with the lowest subsidence are those with the thinnest peat layers (i.e., in the southern half of the area). The within-area differences in subsidence rates are the largest in the Groote Veenpolder with rapid subsidence rates in parcels located in the northeast and south of the area (Figure 6.6b), where the peat layer is thick and drainage is deep. Parcels in the southwest corner have a lower rate of subsidence because locally high groundwater discharge gives rise to higher groundwater levels. The eastern part of this area is a nature reserve and mostly has a moderate subsidence rate because ditch water levels are deliberately kept higher than in the rest of the polder while soils are not submerged. The Buitenveld (Figure 6.6c) shows quite low subsidence rates (lower than 5 mm·yr$^{-1}$) but this are thin peat layers, so, there is little organic material to decompose. Counter-intuitively, the nature reserve in the southeast, which still has a somewhat higher elevation and has a thicker peat layer, has faster subsidence, up to 15 mm·yr$^{-1}$.

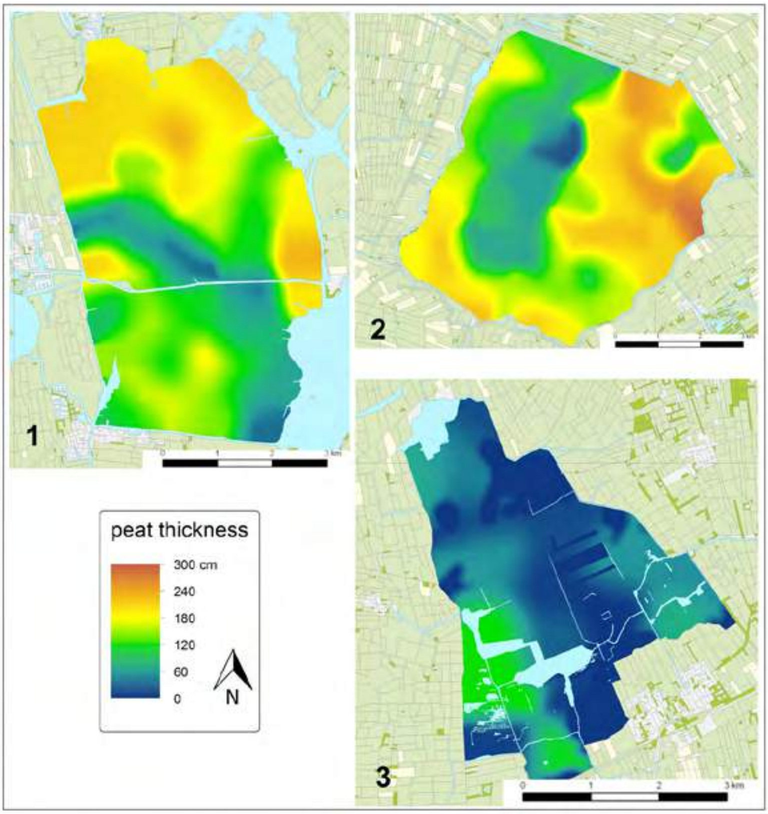


**Figure 6.3:** Peat thickness for the three study areas Hommerts (1), Groote Veenpolder (2) and Buitenveld (3).

**Table 6.2:** Mean soil subsidence rates for the study areas for the current situation and under climate change (subsidence rates in 2050).

| Soil conditions | Mean soil subsidence (mm·yr$^{-1}$) | Mean soil subsidence under climate scenario W+ (mm·yr$^{-1}$) |
|---|---|---|
| **Hommerts** | 6.3 | 6.9 |
| **Buitenveld** | 5.8 | 6.7 |
| **Groote Veenpolder** | 13.6 | 15.6 |

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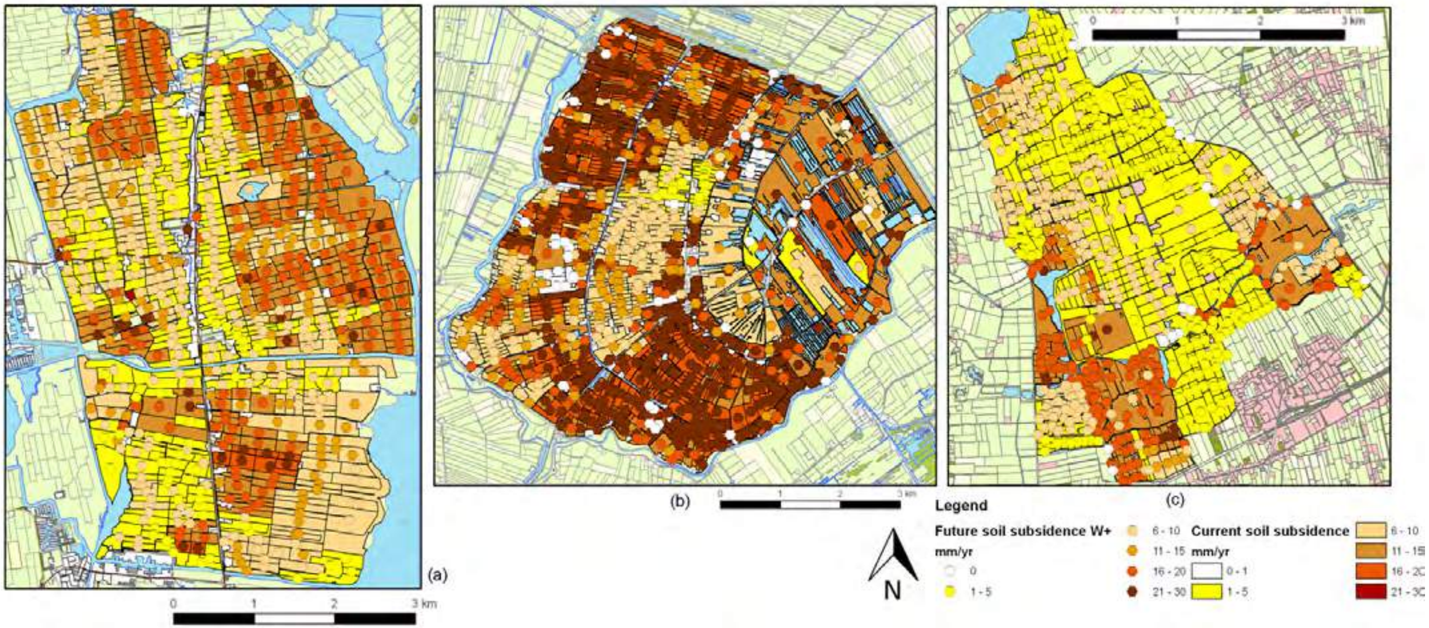


**Figure 6.4:** Maps of subsidence rates in current conditions and for the W+ climate change scenario in the study areas Hommerts (a), Groote Veenpolder (b) and Buitenveld (c).

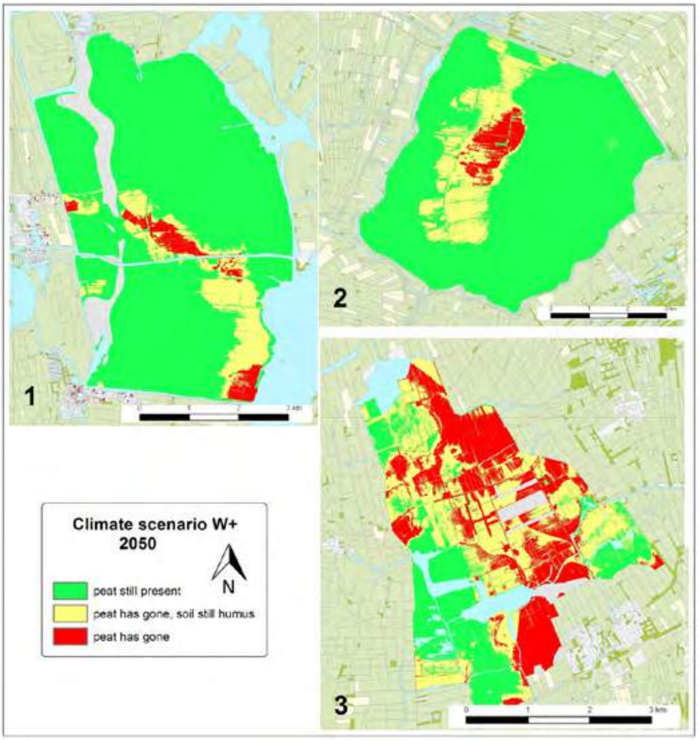


**Figure 6.5:** Status of peat in 2050 at climate scenario W+ for the three study areas Hommerts (1), Groote Veenpolder (2) and Buitenveld (3).

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More drastic changes in land surface levels emerge if subsidence rates are calculated for conditions predicted by the W+ scenario, which imply lower summer groundwater levels and higher mean temperatures (Figure 6.4). Subsidence rates show relatively small increases in the Hommerts area, where the clay cover does not change its behaviour and in the Buitenveld area, where peat layers are mostly thin. Almost all parcels in the Groote Veenpolder show a distinctly faster subsidence rate in the W+ scenario. In both areas, many parcels reach the highest level of subsidence, i.e., 20-30 $mm \cdot yr^{-1}$. The average climate-induced increases for the three areas are ca 0.5 $mm \cdot yr^{-1}$ (Hommerts), ca 1 $mm \cdot yr^{-1}$ (Buitenveld), and ca 2 $mm \cdot yr^{-1}$ (Groote Veenpolder, see Table 6.2).

### 6.3.2 Predicted peat cover in 2050 and 2100

If the high subsidence rates calculated for the W+ scenario are extrapolated for the next 35 or 85 years, assuming a gradual increase starting from the current rates over the years, it is clear that the proportion of the areas where peat soil disappears becomes substantial. In the predictions for 2050, about half of the Buitenveld area has lost its peat soil entirely and 20% will only have a humic mineral soil, with the nature reserve as the sole remaining part with a true peat soil (Figure 6.5). In the other two areas, these proportions are distinctly lower and mostly limited to the parcels where the peat soil is thin (see also Figure 6.3).

If the subsidence rates for the W+ climate are extrapolated to 2100, the areas without peat soils become much larger (Figure 6.6). In Buitenveld, more than 80% of the area would lose its peat soil, with the remnants only in nature reserves. The Groote Veenpolder would lose its peat soils across the major proportion of the dairy meadow areas. Only the nature reserve Rottige Meente in the east and the peat meadow area with high groundwater discharge in the southwest would still have peat soils in 2,100. Hommerts would lose around 70% of its peat soils, with the greater part of its area even lacking humic remains.

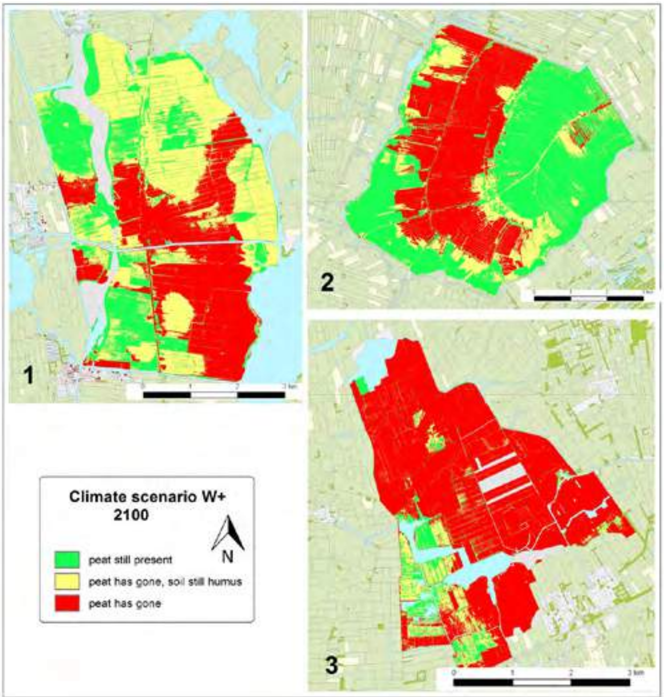



**Figure 6.6:** Status of peat in 2100 at climate scenario W+ for the three study areas Hommerts (1), Groote Veenpolder (2) and Buitenveld (3).

### 6.3.3 Effects of adaptation measures to reduce land subsidence

The way in which the effectiveness of adaptation measures was investigated during workshops using the 'touch-table' is illustrated by the examples generated during the stakeholder workshop on the Groote Veenpolder. Figure 6.7a shows the current land use on the parcels in this area: the eastern, dark-green coloured area is the nature reserve; all other parcels (light green) are in use as agricultural dairy meadows.

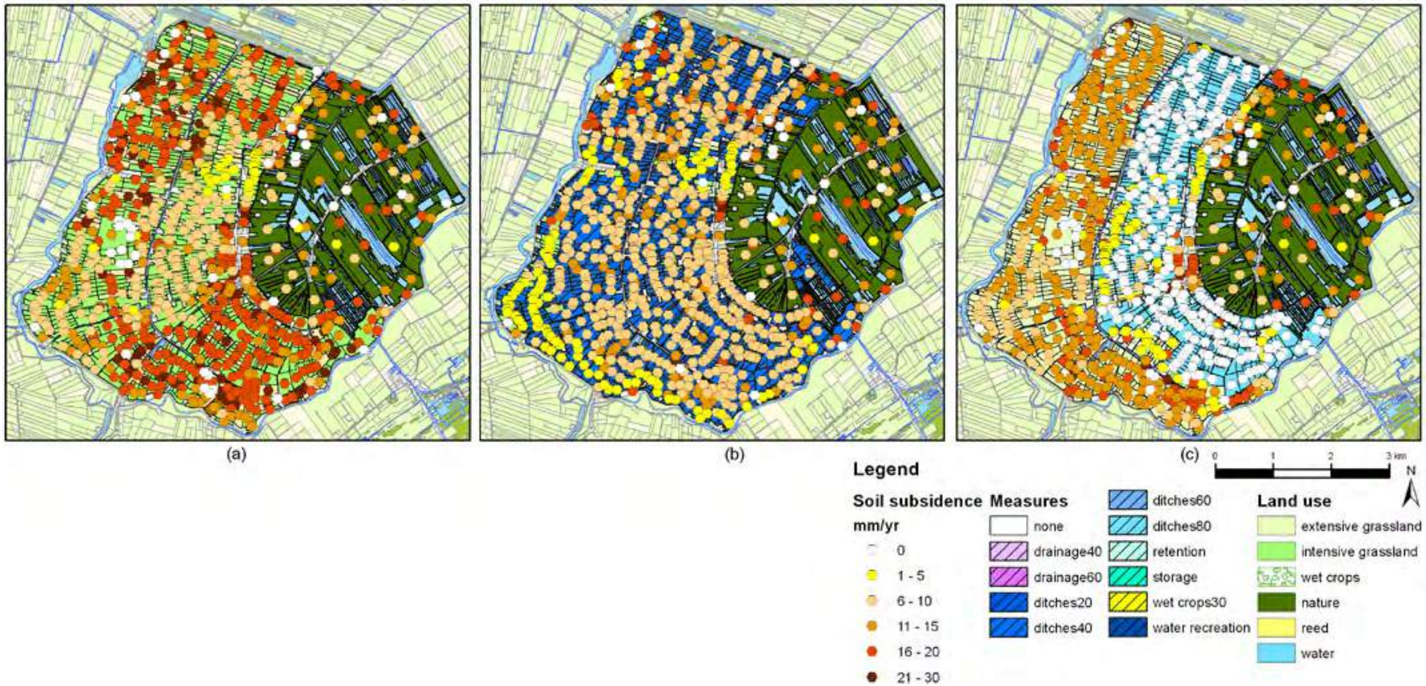



**Figure 6.7:** Examples of measures to reduce the rate of subsidence in the Groote Veenpolder; (a) current land use and water level management (ditch water level 1 m below ground level); (b). Higher ditch water level (ditch water level 0.4 m below ground level) in the western (blue) part of the polder and (c) extensive grassland use (westernmost section) and open water (mid section, blue area) as buffer for the nature area (green). The dots indicate land subsidence rates in each parcel.

6

The land subsidence data in this map are the same as those in Figure 6.4. The stakeholders have raised the ditch water levels in the dairy meadows from 100 cm below surface level towards 40 cm below ground level in Figure 6.7b (dark blue area). This measure, which would reduce the grass and maize growth to such an extent that agricultural targets are no longer met, has a strong inhibiting effect on subsidence in all parcels, reducing it from 21 to 9.5 mm·yr$^{-1}$ (Figure 6.7b). In some parcels, however, subsidence rates will increase as these sites are currently quite wet, and this measure would, therefore, lower the ditch water levels which leads to increased subsidence rates. Another set of measures was implemented in Figure 6.7c: the western section with peat meadows was transferred into 'extensive' dairy meadows with a somewhat higher water level in the ditches (70 cm below ground level) and less intensive fertiliser use. The midsection (light blue) was turned into a superficial open-water area as a buffer zone to prevent water loss from the nature reserve in the east. It is clear that the land use change towards extensive dairy meadow did reduce subsidence rates, but not as strongly as in the situation depicted in Figure 6.7b. The open-water measure, however, effectively halted soil subsidence to almost 0, although the uncertainties on the predictions of the models in these wet conditions are large. When averaged for these two sections, the subsidence had decreased to 7.5 mm·yr$^{-1}$, which is similar to the subsidence in the nature reserve in the eastern part of the polder.

## 6.4 Discussion

### 6.4.1 Quality of the spatial information

The current rates of soil subsidence in typical peat meadows in Friesland were estimated in this study to be up to 3 cm·yr$^{-1}$ for sites with a thick peat layer without clay cover and a ditch water level 1 m below ground surface. The higher subsidence values are estimated for parcels grown with maize. These values have been based on long-term monitoring of land level in peat meadows in this region and on the extensive soil level measurements in the experimental farm in Zegveld (Janssen, 1986; Van den Akker et al., 2007). Similar subsidence rates were found in long-term peatland monitoring studies in Norfolk, England (Dawson et al., 2010), extensive drained peatlands south of the Venice lagoon (Gambolati et al., 2005; Camporese et al., 2006), peat meadow areas in northeast Germany (Egglesman, 1976; Gebhardt et al., 2010), and peatlands in agricultural use in New Zealand (Schipper and McLeod, 2002). A recent evaluation of the actual water levels maintained in the study area in the past 20 years, which are adapted every 10–15 years to follow soil subsidence, indicates a drop of about 40 cm (J. Schouwenaars, personal communication), which concurs nicely with the data from the soil subsidence monitoring. Therefore, we consider this modelling exercise valuable for the exploration of future predictions of subsidence rates and peat thickness.

The data for Zegveld indicate that subsidence is affected by short-term variation in climate: the decline was relatively fast in the dry years 1976 and 1996, whereas in wet years, the surface levels even raised, showing that shrinkage and compression are partially reversible. This is consistent with the observation that subsidence rates have been found to be highest in the period immediately after the reclamation, and to slow down to a constant rate thereafter (Schipper and McLeod, 2002; Gambolati et al., 2005; Dawson et al., 2010). Consolidation and possibly the degradation of easily degradable organic components are responsible for this early response to reclamation or water level drop (Schothorst, 1977; Berg and Meentemeyer, 2002). For the situation in Friesland, the very long history of drainage, as well as the new water management structures implemented during the reallotment schemes in the 1990s (Schouwenaars, 2002) might have resulted in a slightly more rapid subsidence during the late 1990s, later stabilising to the current rate. Our model did not include short-term changes in subsidence rates associated with weather influences or temporarily high subsidence rates directly after lowering water levels.

The spatial information used in the maps indicating current peat soil thickness, land elevation, and land use in the province of Friesland has been derived from recent updates of the information for these characteristics by the research institute Alterra and can be considered representative for the situation in 2012. The algorithms used to calculate current soil subsidence in a spatially explicit way have been based on the data for (1) mean lowest ditch level in summer (GDL), (2) thickness of the peat layer, (3) presence or absence of a clay cover, and (4) agricultural land use. Furthermore, Alterra has modelled lower water levels in summer, and the effects of higher temperatures on peat decomposition were incorporated. These data were presented for each parcel, thereby covering part of the spatial variability. This level of spatial differentiation was of fundamental importance in the stakeholder workshops, because measures can often be taken at the scale of individual parcels, and land ownership boundaries generally coincide with boundaries between parcels.



### 6.4.2 Characteristic aspects of soil Subsidence in Friesland and climate change effects

Compared to the peat meadows in the western part of the Netherlands, the Frisian peat meadow areas are characterised by larger parcels and deeper drainage. While in the western Netherlands peat meadow use often has combined targets for agricultural production and biodiversity enhancement, agricultural production is the main or even the only pursue in peat meadows used for agriculture/dairy production in Friesland. Land use planning policies in the province of Friesland have sought for spatial separation of agricultural use and nature management, so that large sections are being managed for dairy production, while other areas have become nature reserves with targets for the European Natura-2000 framework and tailored land and water management. The deeper drainage has led to a 1.5x

faster rate of subsidence and the much smaller populations of meadow birds than in the peat meadows in the provinces of Noord- and Zuid-Holland.
The acceleration of soil subsidence due to climate change will clearly lead to distinctly lower soil levels and loss of the entire remaining peat layer in parts of the study areas during the course of this century. Areas with thin peat layers, such as most of Buitenveld and some parts of the centre of the Groote Veenpolder, will only have mineral or humic soils by 2050. Subsidence will continue in other areas and by 2100 large proportions of the Groote Veenpolder and Hommerts will also have lost their peat layers. The central part of the Groote Veenpolder will be deprived of peat by the end of the century. The nature reserve Rottige Meente will still have peat, and differences in elevation between the nature reserve and the neighbouring agricultural area will increase.
The local variations in peat thickness will finally result in differences in land elevation, when parts of a polder will stop subsiding while the remainder continues to sink. This will create problems for infrastructure (roads, water control structures) and buildings, and will certainly also lead to desiccation of nature reserves, which generally have a much slower rate of soil subsidence and will lose water toward the lower sinking agricultural areas surrounding them. Issues related to the greenhouse gas balance of peat meadow areas were also discussed, but not in a spatially explicit, quantitative sense. Although there are reasonably reliable estimates of the $CO_2$ emissions associated with subsidence, much less is known on the emissions of methane and nitrous oxide. For interpreting environmental effects of subsidence, we assumed that a subsidence rate of 10 $mm \cdot yr^{-1}$ is associated with a $CO_2$ emission of 22,600 kg $CO_2$ $ha \cdot yr^{-1}$ (Van den Akker et al., 2008). Although an elaborate study of GHG emissions in the Dutch peat meadow areas has resulted in well-based insights on the GHG balance of a number of polders with different management in the western peat district (Schrier-Uijl et al., 2010), these results cannot be transferred one-to-one to the proposed changes in land use in the polders in Friesland that we studied.

### 6.4.3 Adaptation measures

Designing a climate-proof and economically feasible plan for the Frisian peat areas was outside the scope of these workshops. The main objective was to provide knowledge on consequences of climate change and the effectiveness of adaptive water level and land use measures. Decision-making on regional policies in the Netherlands is usually done in a consensus-oriented way, which requires that local stakeholders have sufficient knowledge on the topics being discussed. These workshops and the 'touch-table' did prove effective in exchanging, validating, and correcting information among local and regional stakeholders (Janssen et al., 2014). During the workshops, the effect of adaptation measures on soil subsidence rates were explored using the 'touch-table' and discussed among the stakeholders. The 'touch-table' simulations during workshops showed to what degree subsidence rates can be slowed down by specific measures, such as raising ditch water levels is a spatially explicit way. In the examples of measures explored by stakeholders, a complete halt to subsidence only occurred after transforming a drained peat meadow area into an open-water lake. Actually, as described in Chapter 2 of this thesis and in other literature (Best and Jacobs, 1997; Fenner and Freeman, 2011), peat formation could take place again with such high-water levels, although the history of drainage possibly has a long-term stimulating effect on decomposition. Furthermore, peat soils with a history of fertilisation can release vast amounts of phosphate and ammonium (Olde-Venterink et al., 2002; Van de Riet et al., 2013), which poses a risk of eutrophication of the regional surface water after rewetting. In the current situation, the micro-economic gains at the level of individual farms are in contrast with macro-economic (societal) costs of upholding current forms of land use. Rewetting to create optimal conditions for peat formation would be a costly measure initially, because all farmers have to be bought out. However, calculations of long-term costs and benefits of rewetting peat meadows and buying out the farmers in the central part of the province of Noord-Holland have shown that the economic balance could be positive over a period of 50 years because of avoided costs, if the costs of mitigation of $CO_2$ emissions were to be included (Provincie Noord-Holland, 2012).

In Hommerts and Groote Veenpolder, the areas with thickest peat layers, the participants concluded that only drastic increases in GDL in combination with alternative crops such as reed, hemp, and duckweed would significantly reduce subsidence rates. However, a possible change in income is considered worrisome. The conclusion of the workshop in Buitenveld was that raising water levels to reduce the degradation of the already thin peat layers would be troublesome for infrastructure, especially houses located at the banks of watercourses. Some participants suggest to accept subsidence, and at the same time, stop adapting water levels to the subsided soil surface levels. Doing so, subsidence is gradually reduced and conditions gradually become wetter. In the meantime, farms can change their main source of income.



Solutions where ditch water levels would be raised would substantially reduce subsidence rates. However, it is uncertain whether formerly drained peat soils would recover into peat-forming systems in a short time-span. An example of rewetting is found in De Veenkampen, Gelderland, the Netherlands, where an intensively used dairy meadow was rewetted from water levels 40 cm below soil surface to an average water level within 20 cm below soil surface. 20 years after rewetting, combined $CO_2$ and $CH_4$ emission rates were about 2500 kg $C{\cdot}ha^{-1}{\cdot}yr^{-1}$ in the rewetted field and 3000 kg $C{\cdot}ha^{-1}{\cdot}yr^{-1}$ in the control field (Best and Jacobs, 1997). This indicates that substantially reducing drainage depth does not lead to equivalent reductions in carbon loss, and hence decomposition and subsidence. This might be caused by the long-term effect of oxygenation (Chapter 2 of this thesis; Fenner and Freeman, 2011). Raising ditch water levels would necessitate a restructuring of the agricultural land use.

Farmers would need to find alternative practices to compensate for the lower productivity of their grasslands. Such innovations could include (1) the use of new crops adapted to the wetter conditions (e.g., those that can be used as a resource in bioplastics), (2) the combination with non-agricultural activities such as facilitating recreation or medical care. The attempt to test a water level management still enabling agricultural practice (average summer ditch water level 70 cm below ground surface) resulted in only a 20% reduction in subsidence rates in the Groote Veenpolder. Agricultural targets are at risk of not being met for most parcels in this exercise. It can be concluded that raising ditch water levels to strongly reduce subsidence will inevitably result in quite drastic changes in the forms of economic land use to which these areas may be put in the future.
The participating stakeholders were enthusiastic about the workshops; they appreciated the format of information exchange and the ability to explore the future of the peat meadow areas. The results of the questionnaire showed that the level of knowledge about soil subsidence was higher after the workshops. The stakeholders also stated that they were able to provide their own knowledge and ideas. The combination of the knowledge from researchers, stakeholders, and from the maps was considered useful. Several participants mentioned that this approach to the problem of climate change and soil subsidence was instructive and that especially experimenting with different solutions provided new insights. More information on the exchange of thoughts and opinions among stakeholders regarding the various simulations can be found in other publications (Janssen et al., 2013; Janssen et al., 2014).

## 6.5 Conclusion

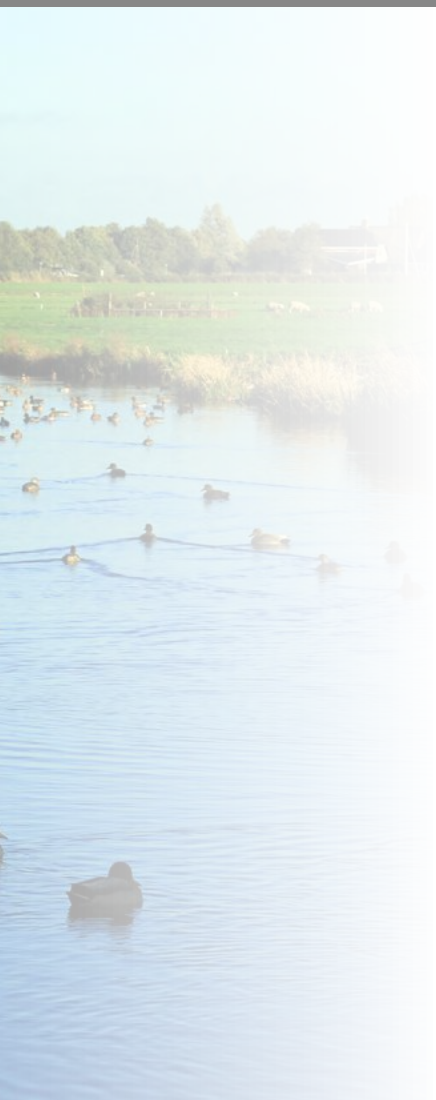
In this study, spatially explicit information on the effect of peatland management and climate change on subsidence rates were provided and validated in stakeholder workshops using an interactive mapping device. Subsidence rates were up to 3 $cm \cdot yr^{-1}$ in deeply drained parcels and increased when we included climate change in the modelling exercises. Because peat layers in Friesland are generally relatively thin (less than 1.5 m), most peat will have disappeared from the province by the end of the century when current practices continue. This would lead to the loss of characteristic landscape features with a long cultural history. The national government, provinces and water boards increasingly realise that a continuation of this management will have problematic side effects such as damage to building foundations, desiccation of nature reserves, emission of greenhouse gases, increasing costs for water management and infrastructural maintenance, and deterioration of surface water quality. In peat polders with thin peat layers (several decimeters) such as Buitenveld, moderate changes in drainage depth do not reduce subsidence rates substantially. In peat polders with thicker peat layers (over 1 m in this case) such as Hommerts or Groote Veenpolder, the degradation rate of the organic soil could be reduced. There, the use of new crops and exploring other sources of income in peat polders with thicker peat layers (over 1 m in this case) needs deliberation. Participants suggest deliberating the option of where the water board stops adapting the water levels to the subsided soil surface levels. In this process, subsidence is gradually reduced and conditions gradually become wetter. In the meantime, farms can change their main source of income.

## Acknowledgements

This study was supported by the research programme Knowledge for Climate (Kennis voor Klimaat), hotspot 'shallow waters and peat meadow areas' project code HSOV1a. We would like to acknowledge Dr. Ir. M.M. Hefting for reviewing the manuscript and making valuable suggestions.

# Chapter 7

# Synthesis



Peatlands around the world have sequestered 450-550 Pg of carbon since the last glaciations ended 11,700 yr BP; this is a large carbon stock compared to the 750 Pg of C that is currently in the atmosphere (IPCC, 2013). However, peatland carbon stocks can turn into carbon sources due to land use change and climate change, resulting in a positive feedback between climate change and carbon loss from peatlands (Laiho 2006). Therefore, peatlands are of major importance for the global C budget. Insight in the feedback between climate change and decomposition in fen and bog peatlands under different land use will elucidate the role of peatlands in current climate change scenarios and will form a basis for new climate adaptation strategies.

In the Netherlands, the peat surface area reached its maximum around 2500 years BP, covering almost half of the total land area. This vast peatland expanse has declined ever since. From the year 1000 AD, reclamation and drainage to facilitate agriculture started to turn these natural ecosystems into strongly modified land areas in which peat no longer was formed but instead was lost through oxidation. In the course of the past millennium, the Dutch peatlands turned from a sink into a source of carbon and the soil surface started to subside. Currently, water management in Dutch peat areas is highly specialised and fragmented to effectively remove excessive water in winter and supply additional water during dry periods to facilitate agricultural practices and housing.

Environmental issues in Dutch peat areas are soil subsidence, greenhouse gas emissions and poor surface water quality due to leaching of nutrients and dissolved organic carbon (Van Beek et al., 2007; Van den Akker et al., 2007; Vermaat and Hellmann, 2009; Querner et al., 2012). It is expected that higher temperatures and irregular precipitation patterns associated with future climate change will accelerate peat decomposition (Witte et al., 2009) and, hence, worsen the environmental conditions in peatland areas. In this thesis, the effects of climate change on the decomposition of peat soils in the Netherlands were studied. In this final chapter, the general conclusions are summarised and discussed from both a scientific and a practice-orientated point of view. First, the obtained mechanistic insights on decomposition rates and the effects of peat origin (fen or bog) and land use (agriculture or nature) are reviewed (§7.1) and extrapolation of the results is discussed (§7.2). After that, general effects of summer drought, salinisation and temperature increase on peat oxidation are discussed (§7.3). Finally, the management implications (§7.4) and suggestions for further research (§7.5) are discussed to end with a general conclusion (§7.6).



## 7.1 Peat origin and land use effects on peat decomposition

In this thesis, the question was addressed whether peat origin and land use affect the peat's response to climate change. The approach consisted of laboratory incubation experiments focussing on oxygenation of normally anoxic peat, salinisation, microbial activity, respiration dynamics and exo-enzyme activities, in addition to measurements on vertical soil profiles and mapping exercises. This approach resulted in insight in decomposition dynamics.

### 7.1.1 Effects of peat origin on decomposition

At the start of this doctoral research, higher decomposition rates were expected for fen peat than for bog peat as it was assumed that fen peat has a lower concentration of recalcitrant phenolic compounds, which supposedly hampers decomposition (Hättenschwiler and Vitousek, 2000; Freeman et al., 2001; Freeman et al., 2004). Comparing the sites with agricultural and nature management on fen and bog peat, indeed, an indication for higher decomposition rates in fen peat than in bog peat can be distinguished, as previously found using cotton strips (Verhoeven et al. 1990). Table 7.1 summarises the decomposition rates as measured in this doctoral research in $\mu gC \cdot gOM^{-1} \cdot h^{-1}$, this carbon loss per unit of organic matter (OM) per hour facilitates comparison amongst peat types. The main conclusion regarding the effect of peat type on decomposition is that, in accordance to literature, fen peat tends to decompose faster than bog peat.
Potential phenol oxidase (POX) and phenol peroxidase (POD) activities are higher in fen peat where the concentration of phenolic compounds, both soluble and condensed, are lower than in bog peat. The ranges of concentrations of soluble and condensed phenolics match the ranges found in literature (Djurdjevic et al., 2003). However, the oxygenation experiment did not affect the concentrations of phenolic compounds while decomposition rates were clearly affected by oxygenation; no clear effect of ground water levels on the concentrations of phenolic compounds in vertical soil profiles was found either. Hence, the link between the phenolic compounds and decomposition rates in the areas we studied is not straightforward. It is suggested here to look further into the effects of the quality of the soluble and condensed phenolic compounds, instead of only the quantity. Possibly, the peat soils have experienced oxygenation more often than was presumed, leading to a change in the quality of the phenolic compounds, perhaps leading to a reduced latch function.

*Pyrite oxidation*

2-8% of the dry weight in fen peat consists of pyrite, which is more than in bog peat (Vermaat et al., 2012). Pyrite is formed in an anoxic environment when iron, sulphide and organic matter are present. Pyrite concentrations were not measured in this research. However, the combination of increasing sulphate concentrations and acidification indicates pyrite oxidation, as this is a process during which $Fe^{2+}$, $SO_4^{2-}$ and $H^+$ are being released. Pyrite oxidation is usually associated with oxic conditions, but can also take place in anoxic conditions, coupled to $NO_3^-$ or $Fe^{3+}$ reduction (Jørgensen et al., 2009). For example, in the salinisation experiment (Chapter 4), sulphate concentrations increased in time in the anoxic incubations of fen peat. In the respiration measurements (Chapter 5), part of the fen samples showed a low respiration quotient (RQ), indicating that the $CO_2$ production relative to $O_2$ consumption was low. This could be caused by the oxygen consumption associated with pyrite oxidation. Pyrite oxidation may have several consequences. For example, in periods with low concentrations of $O_2$, or $NO_3^-$ in anoxic conditions, the $SO_4^{2-}$ that had been formed in oxic conditions can function as a TEA and facilitate decomposition. On the other hand, pyrite oxidation is associated with acidification, which might lead to lower POX activity as this enzyme has a pH optimum around 8 (Pind et al., 1994; Williams et al., 2000; Freeman et al., 2004). Another possible effect of increasing sulphate is the release of phosphate from the soil adsorption complex, because sulphate and phosphate compete for binding places (Beltman et al., 2000).

## 7.1.2 Effects of land use on decomposition

Throughout this study, the functioning of drained peat-meadow soils in intensive agricultural use was compared with soils of undrained peatlands and only slightly drained wet meadows in nature reserves. In this way, the consequences of long-term land use effects, such as drainage, fertilisation, occasional ploughing and seeding of the monospecific grasslands for decomposition characteristics were evaluated (Table 1.2). Nutrient analyses confirmed that ammonium, nitrate and phosphate concentrations were generally, but not consistently, higher in the agricultural sites.

It was expected that phenolic compound concentrations are lower in the aerated part of soil profiles, so that a transition zone in vertical soil profiles would be detectable above which the concentration of phenolic compounds is low due to high oxygen availability (Chapter 3). Below this transition zone, higher concentrations of phenolic compounds can be expected. This transition zone would be located higher in the soil profiles of nature reserves, where ground water levels are usually higher, than in the drier agricultural sites. However, no transition zone was detected and phenolic compounds only marginally increased with depth.

Despite similar basal respiration rates between peat types, the functioning of microbiota was clearly affected by land use as indicated by significant differences in microbial biomass, relative respiration rates, microbial growth response upon glucose addition (Chapter 5), and nitrification rates (Chapter 4). The peat samples from agricultural parcels had a larger microbial biomass that grew rapidly upon glucose amendment compared to the samples from nature reserves. This is in line with the higher potential activity of β-glucosidase (BG) in nature reserves, BG is involved in the depolymerisation of cellulose. Apparently, the availability of energy is higher in agricultural peat soils. We did not use radio-labelled glucose, so it is not clear if the origin of the $CO_2$ is peat decomposition, glucose decomposition, or a priming effect. It became apparent that not only carbon dynamics but also nitrogen dynamics differ between agricultural sites and nature reserves. In the oxic incubations of the salinisation experiment, ammonium was accumulating in the samples from the agricultural sites and nitrate in those from the nature reserves. This shows that (potential) nitrification rates are higher in agricultural sites than in nature reserves (Chapter 4). The microbial communities in agricultural peat soils are apparently adapted to the fertilisation practices. Four to five times a year a pulse of nutrients and organic matter is applied via the injection of manure and/or chemical fertilizer. It is assumed that the microbial communities in the agricultural peat meadows contain more r-strategists, i.e. opportunists that react rapidly to changing conditions, than the communities in the nature reserves.

It was observed that decomposition rates declined after nitrogen addition (Chapter 5 and Table 7.1). The treatment effects were larger in the samples from the nature reserves, where nitrate and ammonium concentrations in pore water were lowest. This suggests that the microorganisms are degrading recalcitrant organic matter to obtain nitrogen, indicating that freely available nitrogen and labile organic compounds do not supply sufficient amounts of nitrogen to fully satisfy microbial needs. These results confirm the recalcitrant nature of the peat organic matter and fit the 'nitrogen mining theory', in which recalcitrant organic matter is degraded to obtain nitrogen (Craine et al., 2007).

## 7.2 Extrapolating laboratory-based decomposition rates to the ecosystem scale

The effects of changing environmental conditions can be estimated by performing incubation experiments, although one should be cautious in translating the results to long-term effects on an ecosystem scale (Laiho, 2006). The summer drought experiment, salinisation experiment, and the respiration and enzyme analyses were all short-term incubation studies. These studies allowed us to differentiate between shallow and deep peat layers, origin of the peat and land use. Furthermore, short-term effects of fertilisation were studied by amending the soil samples with nitrogen and/or glucose. On the other hand, long-term effects of land use (fertilisation, drainage, vegetation in monoculture) were evaluated by comparing peat from nature reserves and from dairy meadows both in experimental work and in the descriptive field study showing vertical soil profiles of 19 different sites. The $CO_2$ production in the respiration measurements (Table 7.1) is higher than in the other experiments, which is probably caused by differences in the length of the experimental period. As seen in the respiration curves in Chapter 5, respiration rates are higher at the beginning of the experiment than later in the experiment, which is attributed to disturbance. The decomposition rates measured in this research fit well in the range of peat decomposition rates reported in literature ($CO_2$ in oxic conditions: 1-30 $\mu gC \cdot g\ DW^{-1} \cdot h^{-1}$, combined $CO_2$ and $CH_4$ production in anoxic conditions: ca 5 $\mu gC \cdot g\ DW^{-1} \cdot h^{-1}$) (Aerts and Toet, 1997; Geurts et al., 2010; Hilasvuori et al., 2013).

**Table 7.1:** Comparison of decomposition rates that were measured in this thesis.

| | Peat origin and peat type | | | | Decomposition product | Unit |
|---|---|---|---|---|---|---|
| | **Fen, agriculture** | **Bog, agriculture** | **Fen, nature** | **Bog, nature** | | |
| **Summer drought (Chapter 2)** | | | | | | |
| **Anoxic conditions** | | | | | | |
| **$CO_2$ production** | 2.64 ± 0.48 | 1.15 ± 0.47 | 0.71 ± 0.16 | 1.17 ± 0.18 | $CO_2$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **$CH_4$ production** | 0.54 ± 0.03 | 0.53 ± 0.04 | 0.54 ± 0.23 | 0.27 ± 0.04 | $CH_4$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Oxic conditions** | | | | | | |
| **$CO_2$ production** | 8.45 ± 0.70 | 0.93 ± 0.22 | 1.73 ± 0.27 | 0.68 ± 0.05 | $CO_2$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **$CH_4$ production** | 0.55 ± 0.09 | 0.27 ± 0.07 | -0.04 ± 0.11 | -0.22 ± 0.21 | $CH_4$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Effect of oxygenation on C loss in whole experimental period** | +352 | +83 | +182 | +159 | $CO_2 + CH_4$ | % increase in carbon loss (C/C) |
| **Salinisation (Chapter 4)** | | | | | | |
| **Anoxic conditions** | | | | | | |
| **Control** | 1.33 ± 0.10 | 1.03 ± 0.15 | 7.12 ± 1.62 | 2.99 ± 0.73 | $CO_2 + CH_4$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Salt** | 1.21 ± 0.27 | 1.20 ± 0.33 | 10.45 ± 5.00 | 2.36 ± 0.41 | $CO_2 + CH_4$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Difference** | n.s. | | | | | |
| **Oxic conditions** | | | | | | |
| **Control** | 2.45 ± 0.14 | 1.54 ± 0.11 | 1.90 ± 0.23 | 10.8 ± 0.86 | $CO_2 + CH_4$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Salt** | 1.25 ± 0.15 | 0.89 ± 0.4 | 0.91 ± 0.22 | 4.30 ± 0.80 | $CO_2 + CH_4$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Difference** | -49 | -43 | -52 | -60 | $CO_2 + CH_4$ | % |
| **Respiration (Chapter 5)** | | | | | | |
| **Oxic conditions** | | | | | | |
| **Control** | 5.32 ± 0.51 | 3.75 ± 0.56 | 4.00 ± 0.29 | 6.12 ± 0.60 | $CO_2$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Nitrogen** | 5.07 ± 0.47 | 3.41 ± 0.58 | 3.05 ± 0.39 | 4.09 ± 0.74 | $CO_2$ | μgC·gOM$^{-1}$·h$^{-1}$ |
| **Difference** | n.s. | -9 | -24 | -33 | | % |

7

The experimental work presented in this thesis shows that anaerobic decomposition rates in incubation studies are not that much lower than aerobic decomposition rates, whereas, aerobic decomposition is more energy-efficient than anaerobic decomposition due to differences in thermodynamic yield. In the bog samples from the nature reserve, the $CO_2$ production was not even stimulated by oxygenation. High potential anaerobic decomposition rates compared to aerobic decomposition rates have been found before (Basiliko et al., 2007). When there is no lack of electron acceptors, anaerobic decomposition rates can still be relatively high because the microorganisms present there are specialised and adapted to these conditions (Szafranet-Nakonieczna and Stêpniewska, 2014). From our measurements and other reported studies, it appears that anaerobic decomposition should not be ignored (Moore and Dalva, 1997; Wright, 2011). Moore and Dalva (1997) found anaerobic decomposition rates approximately 2.5 times lower than aerobic decomposition rates. Drainage depth in the western part of the Netherlands is usually about 50 cm; however, there are several meters of water-saturated peat below, which could contribute to subsidence substantially. It is expected that the availability of TEAs is a major factor influencing anaerobic decomposition rates, due to slow water flows in peat (Morris and Waddington, 2011). Nevertheless, when discussing soil subsidence, only the water levels are discussed in order to reduce the aerobic decomposition and, hence, subsidence. To more accurately model subsidence rates in peat areas varying in peat thickness it seems important to pay attention to the contribution of anaerobic decomposition to soil subsidence.

## 7.3 Expected consequences of climate change on Dutch peatlands

### 7.3.1 Summer drought

*Effects of summer drought on decomposition rates*

In the near future, frequency and severity of droughts are predicted to increase at high latitudes, where much of the world's peat resides (IPCC, 2007b; KNMI, 2014). In the Dutch drained peat meadows, this will result in a drop of summer water tables as the lateral flow of water from the ditches into the peat meadow soil is too slow to make up for the high evaporative water loss (Hoving et al., 2008). Consequently, deeper peat layers that have not previously been exposed to oxygen will be aerated. This process of enhanced decay of peat in deeper normally water-saturated layers is called secondary decomposition (Tipping, 1995; Borgmark and Schoning, 2006). The peat in these water-saturated deeper peat layers has other characteristics than the peat in the topsoil, which has been exposed to oxygen for a long time (Hansson et al., 2013). For example, the C:N ratio of deeper peat layers are lower, due to higher carbon loss compared to nitrogen loss. This was also seen in the vertical peat profiles in the areas that have not been cut over (Chapter 3, data not shown).

Because of the differences between peat quality and composition from well-aerated layers and peat from deep, anoxic peat layers, it is not realistic to compare aerobic and anaerobic decomposition rates of the same peat samples to estimate the effects of oxygenation on deep peat layers. So, in the summer drought lab experiment samples from permanently anoxic peat layers were oxygenated for zero to eight weeks (Chapter 2). This experiment showed that short duration oxygenation leads to a steep increase in the rate of decomposition. Over the whole experimental period, carbon loss rates where highly stimulated by oxygenation (83-352%). The first explanation for this effect is the higher rate of the decomposition process in oxic than in anoxic conditions, as the most favourable terminal electron acceptor (TEA), oxygen, is not present in the latter conditions (Moore and Dalva, 1997). Moreover, the summer drought experiment showed that in the anoxic period after the oxygenation the decomposition rate remained higher than in the samples that did not experience oxygenation. Hence, the results indicate that oxygenation stimulates decomposition, even after anoxic conditions have returned. This is in line with the enzymic latch theory and previous findings (Freeman et al., 2001; Fenner and Freeman, 2011). In addition, the experiment showed that even a short, one-week, period of oxygenation stimulated carbon loss in the post-oxygenation period and that peat origin and land use do not affect the reaction to summer drought with respect to decomposition rates.

*Biogeochemical framework*

Fenner and Freeman (2011) propose a 'biogeochemical cascade whereby constraints on decomposition are removed by severe drought in oligotrophic peatlands'. In short, this cascade involves a positive effect of oxygen on POX synthesis and activity; consequently, POX degrades phenolic compounds, which in turn enables higher activities of hydrolytic enzymes leading to a release of $CO_2$, easily degradable carbon compounds, nutrients and an increase in pH. Then, the conditions for soil microbial organisms improve and the production of hydrolytic enzymes and POX increases, leading to increased decomposition rates. In the study of Fenner and Freeman (2011), executed with monoliths from oligotrophic, ombrotrophic, mesotrophic and eutrophic peatlands, $CO_2$ production increased within sixteen to twenty-four days after drought (Fenner and Freeman, 2011).

The biogeochemical cascade as described by Fenner and Freeman (2011) was not detected in the summer drought study presented in Chapter 2, as the concentrations of phenolic compounds were not affected by the oxygenation treatments and the concentration of soluble nutrients did not change due to oxygenation either. In the experiment of Fenner and Freeman (2011), POX activity increased within four to sixteen days of drought and soluble phenolic compound concentrations decreased within days after that. In our experiment, the samples did not experience drought, but they were exposed to oxic conditions. In our view, this fits the aim of that study, viz, to unravel the effects of oxygenation on the decomposition and mineralisation of anoxic peat layers that are prone to be aerated during summer droughts. During a dry summer, these peat layers will become exposed to oxygen, but will not experience drought.

There are several explanations for the lack of change in the concentrations of phenolic compounds and nutrients. Soluble phenolic compounds could have been degraded further and condensed phenolic compounds could have been cleaved from the organic matter complex and released as soluble phenolic compounds, resulting in a stable concentration of soluble phenolics. However, the concentration of condensed phenolic compounds was not affected by the oxygenation either. Furthermore, it is possible that the quality of the phenolic compounds changed and not the quantity, i.e. that phenolic compounds are being degraded but secondary degradation products still contain inhibiting phenolic groups. Besides an explanation in terms of the enzymic latch theory, oxygenation can bring about a renewal of TEAs (Knorr and Blodau, 2009). Indeed, in the fen peat samples, a clear increase in the sulphate concentrations was seen after oxygenation probably as a result of pyrite oxidation. This TEA increase might facilitate anaerobic decomposition when oxygen is depleted, however, also $CH_4$ production peaked after the oxygenation period.
Despite the lack of clarity regarding the exact mechanism(s) behind the enhanced decomposition after short-term oxygenation and role of the enzymic latch theory in the decomposition in Dutch peat soils, the summer drought experiment indicates that dry summers will drastically enhance peat decomposition, not only during the drought period itself but also after anoxic conditions have returned.

*Phenolic compounds and POX in vertical soil profiles*
To explore long-term effects of oxygen availability on the concentration of soluble and condensed phenolic compounds and potential phenol oxidase (POX) activity, vertical peat soil profiles were studied (Chapter 3). The vertical soil profiles were considered to reflect long-term effects of water levels on phenolic compounds and potential activity of POX. It was hypothesised that there would be a transition zone with low concentrations of phenolic compounds in the aerated part of peat profiles towards higher concentrations of phenolic compounds in deeper, anoxic parts of the profiles. This would be explained by higher (current or past) POX activity in oxic conditions (Freeman et al., 2001; Sinsabaugh, 2010). The concentration of soluble phenolic compounds turned out to generally increase with depth, as expected. The condensed phenolic compounds, however, did not show such a pattern, with the exception of one of the three study areas (Tjeukemeer area) where a positive correlation between condensed phenolic compounds and depth was found. Unexpected was the positive correlation between potential POX activity and depth. Even at great depths in permanently anoxic peat layers, the enzyme phenol oxidase was detected and potentially active. Most of the locations studied are characterised by downward seepage. Hypothetically, this could transport the oxidative enzyme to deeper peat layers. However, the locations with upward seepage or neutral water flow did not show profiles different from locations where downward seepage was dominant. Therefore, it is unlikely that hydrology is a major factor explaining the presence or absence of POX in deeper layers.

Zibilske and Bradford (2007) found that the removal of oxygen reduced POX activity by only 50%, while other studies did not find straightforward correlations between potential POX activity and oxygen availability or water table depth either (Pind et al., 1994; Williams et al., 2000). This result shows that the effect of oxygen availability on the activity of this enzyme is not as straightforward as previously thought. Peat origin-dependent chemical characteristics of the organic matter or associated microbial community could also affect POX activity (see §7.1.1). More research is needed to unravel or disentangle the contribution of POX to peat decomposition, especially in deeper, saturated peat layers (§7.5)

### 7.3.2 Salinisation

During summer, the water level in Dutch peat areas drops. To prevent drying out of the peat soils, river water is supplied to many Dutch peat meadow areas in summer. However, seawater intrusion and evaporation may locally lead to slightly brackish surface water during severe drought periods; this may even lead to periods during which water supply to polders with peat meadows will be stopped (MNP, 2005) as salt intrusion threatens crop production and livestock performance. The closure of water inlet during summer leads to enhanced peat desiccation and higher decomposition rates due to higher oxygen availability. In addition, brackish seepage (wells) can surface more easily during these dry periods (De Louw et al., 2011).

The effects of salinisation on aerobic and anaerobic decomposition and mineralisation of superficial and deep peat samples were discussed in Chapter 4. In the experimental work presented, a salt concentration of ca 4 ‰ was used (to compare: seawater has a salinity of 35 ‰). The experimental salt concentration, 2000 mg $Cl^{-}\cdot kg^{-1}$, is substantially higher than the maximum salt concentration for the surface water that is currently supplied (250 $mg\cdot L^{-1}$). On the other hand, chloride concentrations in brackish water in wells and groundwater seepage show concentrations well above 1000 $mg\cdot L^{-1}$. The brackish water used in the experiments had a chemical composition reflecting sea salt, with high concentrations of sodium, chloride and sulphate.

Aerobic decomposition rates were reduced by 50% after salinisation, independent of peat origin and land use, whereas anaerobic decomposition rates (both in terms of $CO_2$ and $CH_4$) remained unchanged. We had hypothesised that the production of $CO_2$ in anoxic conditions would increase after salinisation because of the addition of $SO_4^{2-}$ as an alternative TEA and that suppression of $CH_4$ production upon $SO_4^{2-}$ addition would be observed, the latter being a common phenomenon in peatlands (Dise and Verry, 2001). The lack of an effect of salinisation on anaerobic decomposition might be due to the decline in dissolved organic carbon (DOC) concentration as a result of precipitation of DOC into particulate carbon because of the high (chloride) salt concentration (Evans et al., 2006; Hruska et al., 2009). The availability of small organic compounds like acetate, involved in the processes of

sulphate reduction and methanogenesis, control the final steps in the degradation of organic matter (Rydin and Jeglum 2006). A low availability of these soluble organic compounds due to precipitation might have cancelled out a positive effect of higher sulphate concentrations. In addition to the effect of salinisation on decomposition rates, water quality can be affected as a result of desorption and adsorption of ions to the soil complex and changes in mineralisation processes. The experiments indicated that with higher salt concentrations, inorganic nitrogen became more available as ammonium and nitrate in the soils of nature reserves and agricultural meadows, respectively. Furthermore, soluble phosphate concentrations increased as well as sulphate concentrations, the latter is attributed to pyrite oxidation. The interaction between phosphate and sulphate can be attributed to internal eutrophication mechanisms (Beltman et al., 2000; Smolders et al., 2006).
Summarising, the salinisation experiment summarised here and presented in Chapter 4 of this thesis indicates that aerobic decomposition is hampered with salinisation while anaerobic decomposition remains unchanged. However, in field situations adaptation of the microbial community to the more brackish conditions could take place with time, resulting in a less hampering effect. Furthermore, in the brackish treatments, higher soluble nutrient concentrations were present which could affect surface water quality in the peat meadow areas.

### 7.3.3 Temperature increase

Apart from summer droughts and salinisation, temperature increase is another consequence of predicted climate change with large impacts on peat decomposition rates. In previous studies, it was shown that a slight increase in temperatures stimulated peat decomposition and soil subsidence significantly (Berglund et al., 2008; Dorrepaal et al., 2009). $Q_{10}$ values represent the relative change in respiration rates over a 10 °C temperature interval. $Q_{10}$ varies with the recalcitrance of the organic matter, it differs between oxic and anoxic conditions, and also with the height of the temperature interval considered. Firstly, according to enzyme kinetics, the more chemically recalcitrant the organic matter, the greater the temperature sensitivity of microbial respiration (Davidson and Janssens, 2006). This leads to relatively high $Q_{10}$ values for peat organic matter, which is rather recalcitrant as shown by the high metabolic quotient and low respiration quotient (Chapter 5). The low respiration quotient could, in part, also be attributed to the use of oxygen in the process of pyrite oxidation. $Q_{10}$ values in literature ranged from 2.1 for easily degradable organic matter to 3.8 for highly recalcitrant peat (Davidson and Janssens, 2006; Conant et al., 2008). Vertical *Sphagnum* peat profiles showed that, within the oxic part of the profile, the temperature sensitivity of decomposition increases with depth, and hence age, of the organic matter (Hilasvuori et al., 2013). Secondly, $Q_{10}$ values are higher for aerobic decomposition than for anaerobic decomposition (Szafranet-Nakonieczna and Stêpniewska, 2014); suggesting that a temperature increase and desiccation in summer show a positive interaction. Lastly, the studies of Berglund et al. (2008) and Dorrepaal et al. (2009), show that the temperature sensitivity is higher in a low temperature range.

### 7.3.4 Overall effects of climate change on peat decomposition

The combined climate change effects (temperature increase, summer drought and salinisation) will have large consequences for peatlands in the Netherlands. Temperature increase and summer drought are expected to increase peat decomposition rates. This combination of aspects will result in higher peat decomposition and subsidence rates as a result of climate change. On the other hand, climate change driven supply of brackish water and salinisation due to brackish seepage could reduce decomposition rates. This implies that salinisation could partly mitigate the effects of higher temperatures and drier summers. Salinisation is however not an appealing mitigation strategy as it has adverse effects on water quality and crop production; furthermore, salinisation will not take place uniformly either. Brackish seepage can surface in wells and brackish inlet water will not spread uniformly throughout peat polders when water boards decide to supply brackish water to peat area. In this thesis, it was shown that salinisation could lead to higher ammonium and phosphate concentrations and lower DOC concentrations in the pore water. These higher pore-water concentrations can potentially be leached during periods of intense precipitation after drought, which will deteriorate the surface water quality in the peat meadow areas.

In Dutch modelling studies, it has been assumed that a temperature increase of 2 °C results in an increase in decomposition rates by 25% (Hendriks, 1992; Querner et al., 2012). This corresponds with a $Q_{10}$ of just over 2. Given the recalcitrant character of peat organic matter this estimated $Q_{10}$ is rather conservative. The combined effects of higher temperatures and dryer summers are estimated to result in a total increase in subsidence of around 70% in the W+ (currently coined $W_H$) KNMI climate change scenario (Querner et al., 2012). In the workshops presented in Chapter 6, a model was used in which the peat decomposition rates in the most extreme KNMI climate change scenario was multiplied by a factor 1.5 compared to current decomposition rates, this approaches a $Q_{10}$ of around 3 which seems relevant for peat OM (Davidson and Janssens, 2006; Conant et al., 2008). Alterra research centre modelled Mean Lowest Water levels for the climate scenario W+. These data were combined with subsidence formula that led to a prediction of distinctly faster subsidence rate in the W+ scenario for almost all parcels in the case study areas.

Currently, spatial differences in subsidence rates already lead to challenges for peatland management. At the moment, numerous parcels are drained more deeply by small, privately owned pumps (´onderbemalingen´). This contributes to local spatial differences in elevation and this effect will become more prone with climate change. Hence, the spatial differences in subsidence rates will most likely increase, even at the parcel scale, leading

to more complex water management issues and problems for infrastructure (roads and houses). Deploying subsurface drainage in order to distribute the water that is available more evenly throughout a peat parcel is a promising development, which deserves further testing at the field and polder scale (Hoving et al., 2008).
To sum up, temperature increase and summer drought will enhance soil subsidence; the latter has both a direct effect during the dry period, but also a prolonged effect as shown in Chapter 2. On the other hand, the episodic supply of brackish water via groundwater or inlet water will perhaps slightly decrease subsidence rates, whereas adverse effects on water quality are expected.

## 7.4 Management suggestions

The work presented here focuses on summer drought and salinisation in peat meadow areas as consequences of climate change. Summer drought and salinisation, together with a higher demand of surface water supply in summer, were indicated as main consequences of climate change in Dutch peat meadow areas (Witte et al., 2009). The substantially increased decomposition rates of deeper, normally anoxic peat layers, both in the period during and after a relatively short period of drought, are expected to lead to higher subsidence rates. On the other hand, salinisation, e.g. because of slightly brackish water that is supplied to peat areas in dry summers to prevent desiccation, is expected to hamper decomposition, while nutrients such as phosphate and ammonium are more likely to be released from the soil complex. This latter aspect potentially affects water quality. In addition, salinisation threatens crop production and livestock performance.
Measures to avoid critical situations focussing on water supply are the creation of reservoirs for (fresh) water storage and the instalment of subsurface drainage in order to distribute the water more easily throughout a peat parcel during dry summers. Although subsurface drainage slows down subsidence rates, peat oxidation of the aerated part of the peat profile still takes place (Jansen et al., 2009; Kemmers and Koopmans, 2009). Consequently, the subsurface drains become located closer to the soil surface as the overlaying peat decomposes, so that the parcels are getting wetter and current agricultural practices become less and less profitable, stressing the need for long-term peatland management strategies.
Additional measures to be considered to reduce soil subsidence are related to the local agricultural practices. For instance, maize cultivation results in higher decomposition rates because of deep root systems (FAO, 1998) and deep ploughing, which facilitate oxygen intrusion into the soil. Maize cultivation should be situated on parcels where peat preservation is not relevant (e.g. on thin peat layers). Additionally, it is suggested to raise water levels in areas with very deep drainage. Locally, ground water levels are lower than 90 cm below soil surface, particularly in the Frisian peat meadows. It is expected that

raising these deep water levels by a few decimetres does not significantly harm agricultural practices, while at the same time, peat degradation is reduced. Also higher summer water levels are a measure to reduce subsidence rates.

It is stressed here that long-term planning of (water) management in peat areas is necessary, and, if peat conservation is considered important, changes in peatland management are inevitable. Although the process of soil subsidence in drained peat areas has been well-known from the 1960's onwards (Schothorst, 1969; Schothorst, 1974), peatland management in many regions of the Netherlands is still directed towards the cultivation of conventional crops with high yields, this goes together with low water levels, while peat conservation has less priority. Current segmentation of gains of individual farms and societal costs of water management and greenhouse gas emission impedes agricultural innovation and land use change. Calculations of long-term costs and benefits of rewetting peat meadow areas have shown that the economic balance over a period of fifty years is positive when drastic changes in peatland management are implemented (Provincie Noord-Holland, 2012).
The province of Friesland has been active recently in formulating an 'Outlook for the Frisian peat meadow areas' ('Veenweidevisie Friesland'). Many promising statements have been put forward in the documents of this outlook, such as discouraging maize cultivation, protecting nature values on peat soils, applying subsurface drainage, reducing or stopping subsidence, higher (summer) water levels, etc. (Gedeputeerde Staten Provincie Friesland, 2014). The next steps, in which, besides other activities, 'wetter crops' are being tested, are crucial for carrying through changes in peatland management.
To increase the level of awareness of local stakeholders, workshops were organised under the auspices of the Kennis voor Klimaat programme in five peat areas in the Netherlands. The workshops used spatial analysis tools to effectively inform stakeholders on the process of subsidence, the controlling factors involved and the consequences for agriculture, infrastructure, residential areas and water management. It became clear during the workshops in case study areas that awareness on soil subsidence was initially rather low, but many stakeholders became cooperative and curious about alternative land use options in peat meadow areas. Some farmers suggested stopping adapting water levels to the subsided soil surface levels. Doing so, subsidence is gradually reduced and conditions gradually become wetter. In the meantime, farms can change their main source of income and orient themselves on alternative crops like reed, hemp and duckweed for energy or bioplastic production. It is recommended here to welcome this idea with open arms, to be receptive to new ideas and tailor-made measures, and to stimulate practical research on the possibilities of alternative peatland management options.

## 7.5 Suggestions for further research

The ´enzymic latch theory´ was a source of inspiration for this doctoral research (Freeman et al., 2001; Fenner and Freeman, 2011). In line with this theory was the result that higher decomposition rates were found in fen peat, with higher potential phenol oxidase activities, than in bog peat. A result contrasting the enzymic latch theory is that the concentrations of phenolic compounds were not affected by drought, both in short-term experimental work and in comparative field studies. Another point of interest is the discovery of potential POX activity in deep peat layers with presumed permanently anoxic conditions. Therefore, a continuation of the work on phenolics and POX would enable the further unravelling of the dynamics in phenolic compounds and phenol oxidase in Dutch peat meadow areas in relation to decomposition rates.

The measurements of respiration and enzyme activities (Chapter 5) raised the question if phosphate is limiting peat decomposition. Phosphorus fertilisation has been found to stimulate decomposition of unfertilised peat and litter samples (Aerts and De Caluwe, 1997; Hobbie and Vitousek, 2000; Craine et al., 2007). However, water and nutrient budgets in thirteen Dutch agricultural peat polders indicate that mineralisation and fertilisation cause a surplus of phosphorus in these peat soils, so that P is accumulating in peat soils (Vermaat and Hellmann, 2009). It is therefore questionable whether phosphorus is indeed limiting decomposition in these mineralising and fertilised peat soils.

A more practice-oriented continuation of this research could focus on the balance between decomposition and organic matter accumulation in only slightly drained (agricultural) peat areas, following the information presented in §7.3 and §7.3.4. Modelling studies do not estimate subsidence rates in very wet conditions very accurately (Laiho, 2006; Van den Akker et al., 2007), while more accurate information on subsidence could facilitate decision-making. There are several factors involved in the estimation of subsidence rates in wet or rewetted peatlands, such as: input of organic matter, decomposition rates in oxic and anoxic peat layers, and history of drainage which affects decomposition rates in rewetted conditions. In natural peatlands, waterlogged conditions lead to peat accumulation, as primary production exceeds decomposition. However, in peat meadows in the Netherlands, the greater part of primary production is removed by grazing or mowing, resulting in little input of organic matter. Another important point shown by this thesis and other recent literature (discussed in §7.3) is that the contribution of anaerobic decomposition to soil subsidence is possibly higher than was previously thought in situations with sufficient supply of alternative TEAs. In addition, rewetting of currently drained peat soils results in decomposition rates higher than expected purely based on groundwater levels (Best and Jacobs, 1997; Fenner and Freeman, 2011; Brouns et al., 2014c). Forced, immediate rewetting should be compared further with the option to stop adjusting groundwater levels to the subsiding soil surface level, not only in terms of the nutrient release associated with rewetting (Olde-Venterink et al., 2002; Van de Riet et al., 2013) but also with respect to subsidence rates.

Further research on soil subsidence in wet and rewetted conditions with little organic matter input, because of harvesting, is desirable. This is in line with the suggestion of workshop participants in Friesland who suggested stopping adapting water levels to the subsiding soil level, which would finally drive farmers to change their business. There are already a number of projects heading in this direction. The project 'omhoog met het veen' is currently examining whether peat growth can be initiated on degraded peatlands (Van de Riet et al., 2013). Also the project PeatCap evaluates the possibilities of establishing conditions for peat formation (PeatCap, 2015). In addition, the potential of growing alternative crops in wet conditions such as reed, hemp or duckweed that can be used for the production of bioplastics is being explored (Wageningen U.R., 2014). Various alternative cultures, such as cranberries and even *Sphagnum* have recently been proposed as well (Van de Riet et al., 2014). Finally, a study on the public administration and governance, specifically on approaches for implementing large changes in peatland management is recommendable in order to make a smooth transition to a different peatland management.

## 7.6 Conclusion

This study on the effects of climate change on the peat oxidation and subsidence of peat meadows in the Netherlands has resulted in some new insights. Dry summers enhance the decomposition process of peat in drained peat meadows substantially, both during and after the dry period. This is caused by stronger aeration of the peat soil and higher temperatures; the continuation of high decomposition rates even after rewetting is probably due to faster anaerobic decomposition because of higher TEA availability and perhaps lower phenolic concentrations. On the other hand, salinisation could potentially hamper decomposition. However, a higher salinity might increase the flux of nutrients (ammonium and phosphate) from the peat soils to surface and ground water; on the other hand, nitrate and DOC concentrations might decrease in conditions that are more brackish.

The most important differences between fen peat and bog peat are that fen peat had slightly higher decomposition rates than bog peat, lower concentrations of phenolic compounds and higher potential oxidative enzyme activities. Although land use (intense agricultural use vs. nature management) did not significantly affect decomposition rates, the microbial community showed distinct adaptation to land use. In the agricultural peat soils, the microbial community responded very fast to changing conditions, which can be attributed to the regular pulses of nutrients and carbon compounds that are supplied with the application with slurry as fertiliser.

Peatland management in the light of adaptation to a changing climate was discussed during stakeholder workshops. It became evident that a large part of the peat surface area in the province of Friesland will have disappeared by the end of this century when current practices continue. Changing peatland management towards wetland and aquatic system restoration to reduce the loss of peat soils could result in the loss of a characteristic landscape, but the continuation of current management also eventually leads to the loss of peat soils. Meanwhile, while decisions on these issues are still lacking, environmental issues such as greenhouse gas emissions are not solved and problems with water management, infrastructure and buildings will become more eminent. It is suggested here to follow one of the remarks made during stakeholder workshops: stop adapting the drainage depth to the subsiding soil levels, which urges farmers to change their business gradually over a long period of time, e.g. alternative crops, recreation or medical care. Practice-oriented research is necessary to inform farmers on best practices for cultivating alternative crops and innovative studies on public administration and governance are desirable in order to support peatland management.

# Abstract

Peat is formed in wet and acidic conditions, where net primary production exceeds the decomposition of organic matter. Currently, peatlands cover only 3% of the earth's land surface but hold approximately one third of the world's soil organic carbon (450-550 Pg C), which is nearly the same amount as in the atmosphere. However, land use change (e.g. reclamation) and climate change can turn this large carbon sink into a carbon source, thereby generating a positive feedback for climate change.

A millennium of cutting and draining peat in the Netherlands has led to substantial loss of this organic soil type. Locally, peat layers of several meters thick have disappeared. The peat cover has decreased from over 40% of the surface area of the Netherlands to 8% in 2007. Not only the area of peat cover, but also the thickness of peat layers is decreasing in the Netherlands. The peatlands in the Netherlands have a long history of being drained to facilitate agriculture. Drainage increases the carrying capacity of the soil for heavy machinery and improves yields by optimising oxygen and nutrient availability in the soil. The downside of these practices is the oxidation of the organic soil, which leads to subsidence. Subsidence is currently the major problem in Dutch peat areas and is generally 1-2 $cm \cdot yr^{-1}$. It is common practice in the Dutch peat polders that surface water levels are adapted to the subsiding soil surface levels every 10 to 15 years. The result is an inexorable cycle of subsidence and hydrological adjustments. These practices could continue until all peat has disappeared, which would take up to 500 years in the western peat area and about one century in most of the northern peat areas.

The national government, provinces and water boards realise that a continuation of current peatland management will have increasing side effects such as desiccation of nature reserves, emission of greenhouse gasses, deterioration of surface water quality, increasing costs for water management and infrastructural maintenance, damage to building foundations, and, in the end, loss of the characteristic landscape. Climate change could aggravate the conditions in Dutch peatlands, in the first place because higher temperatures will stimulate decomposition processes. Furthermore, the Royal Netherlands Meteorological Institute predicts that the summer periods will become drier. Consequently, water tables will be lower in summer. This increased aeration of peat is expected to stimulate its decomposition. Furthermore, river water supplied to peatlands during dry periods and groundwater are expected to become more brackish with unknown effects on peat decomposition rates.

In this Ph.D. thesis, the effects of climate change on the decomposition of peat soils in the Netherlands are explored, focusing on the effects of summer drought and salinisation on the biological processes during peat decomposition. Throughout this thesis, the different peat types that are present in the Netherlands are taken into account. The distinction is made between peat that was formed in minerotrophic versus oligotrophic conditions (fen and bog peat) and between two types of land use (agriculture and nature management), generating four distinctly different peat types. The framework and background of this project is described more extensively in **Chapter 1**. **Chapter 2** focuses on the effects of a dry summer on the decomposition of normally anoxic peat. Extreme summer droughts are expected to occur more often in the future in NW Europe due to climate change. This study aimed at providing more insight in the oxidation of deep peat layers that had not previously been exposed to air. The effects of oxygenation were studied in controlled conditions in an incubation study. The peat samples were exposed to oxygen for 1, 2, 4 or 8 weeks, after which anoxic conditions returned to mimic a temporary water table drop during a dry summer. Control samples were not exposed to oxygen. The results showed that oxygenation led to a steep increase in the rate of decomposition, indicated by higher carbon loss rates during oxygenation compared to anoxic control samples. More interestingly, decomposition rates did not return to pre-oxygenation decomposition rates in the post-oxygenation period but remained significantly higher. This indicates that a dry summer period stimulates the decomposition of deep peat layers that had not previously been exposed to air and that this effect is still measurable in the period after such a dry summer. Furthermore, drought resulted in acidification and sulphate release in fen peat. Hence, low summer water levels should be avoided as much as possible to limit exceptionally high decomposition rates and associated problems such as increasing subsidence rates, greenhouse gas emissions, sulphate release and acidification.

Soluble phenolic compounds and the activity of the enzyme phenol oxidase (POX) are considered primary regulators of decomposition. In **Chapter 3**, long-term effects of water levels on the concentrations of phenolic compounds and the activity of POX, which degrades phenolic compounds, are explored. Phenolic compounds lock up organic compounds and restrain hydrolytic enzymes. The enzyme POX can release this latch, especially in the presence of oxygen. According to literature, POX is primarily active in oxic conditions. Therefore, one of the hypotheses was that potential POX activities would be considerably higher in oxic peat layers compared to anoxic peat layers. Furthermore, it was expected that there would be a transition zone with low concentrations of phenolic compounds in the aerated part of peat profiles towards higher concentrations of phenolic compounds in the deeper, permanently anoxic parts because of higher POX activity in oxic conditions. Concentrations of phenolic compounds and phenol oxidase activity were measured in vertical soil profiles to check whether there is a long-term effect of water level on the concentration of phenolic compounds and phenol oxidase activity. There was either no effect of depth or a higher potential POX activity with increasing depth. This was surprising because of the general finding that POX is mostly active in oxic conditions.

Potential POX activity significantly correlated with sulphate concentrations. It would be worthwhile to carry out further experimental work to test whether anaerobic degradation of phenolic compounds is coupled to sulphate reduction. On the other hand, it was found that POX activity is higher in fen peat, showing that other peat origin-related factors are involved as well. Our results indicate that the role of phenol oxidase on peat decomposition is not as straightforward as is often stated in literature.

**Chapter 4** focuses on the effect of salinisation on aerobic and anaerobic decomposition and nutrient release. River water is supplied to Dutch peat areas in summer to prevent drying out of the peat soils. Saltwater intrusion and evaporation make this surface water slightly brackish during drought periods. In addition, brackish seepage can surface more easily during such dry periods. In an incubation experiment, the effects of salinisation on aerobic decomposition and mineralisation of superficial peat samples and anaerobic decomposition and mineralisation of deep peat samples were studied. It is known that sulphate, a major ion after sodium and chloride in brackish and seawater, can facilitate the anaerobic decomposition as sulphate can function as a TEA. On the other hand, a sudden increase of salinity in soil porewater might (temporarily) disturb microbial communities and slow down decomposition.
The aerobic decomposition rates were reduced by approximately 50% after salinisation, whereas the anaerobic decomposition rates remained unchanged. Remarkably, the response to salinisation did not differ between the peat types and land uses. As a result of salinisation, ammonium concentrations increased in samples from nature reserves, probably due to reduced nitrification rates. On the other hand, nitrate accumulated after salinisation in samples from agricultural sites. Phosphate concentrations increased, possibly caused by changes in desorption and adsorption processes due to higher sulphate concentrations. DOC concentrations decreased in the salinised samples due to precipitation of particulates. Furthermore, the fen peat samples showed increasing sulphate concentrations, which was attributed to pyrite oxidation. Independently of salinisation, nitrification rates were higher in the agricultural, fertilised, peat soils. In conclusion, while salinisation might reduce subsidence rates (temporarily), it will have adverse effects on water quality.

A more fundamental study on aerobic respiration is presented in **Chapter 5**. The microbial community and respiration dynamics were studied by applying the techniques of Substrate Induced Respiration (SIR) and Substrate Induced Growth Response (SIGR), measuring potential enzyme activities and total microbial biomass in the four peat types on which this thesis focuses. The samples were amended with nitrogen and/or glucose to assess whether nitrogen or energy is limiting the decomposition process. Respiration

rates after nitrogen addition were generally lower than without additional nitrogen, in accordance with the nitrogen mining theory. Unexpectedly, no uniform effect of land use (agriculture vs. nature reserves) or peat origin (fen vs. bog) on basal respiration rates was found. Nevertheless, substantial differences in microbial activity, microbial biomass and respiration dynamics occurred between land uses, whereas enzymes activities differed mostly between peat types. SIR measurements led to the conclusion that agricultural land use has significantly modified the microbial community. The agricultural soils uniformly responded to glucose addition with a peak in respiration, whereas this response was absent or less distinct in samples from nature reserves. We could not establish if there was a priming effect. There are strong indications that the microbial communities in the agricultural peat meadows contain more r-strategists, i.e. opportunists that react rapidly to changing conditions, than the communities in the nature reserves. The activities of the enzymes POD, POX and NAG were mostly dependent on peat origin instead of land use. This study indicates that although soil subsidence rates highly correlate with drainage levels, peat origin does affect the dynamics of peat decomposition as well.

The results of the experimental work presented in this thesis and the existing body of literature were used to model subsidence rates in peat areas in the provinces of Utrecht, Drenthe and Friesland. The results of the workshops in Friesland are presented in **Chapter 6**. The province of Friesland has developed a policy plan to explore scenarios of climate change and their consequences for the conditions for agriculture in their peat meadow areas. This chapter describes how expert knowledge on peat subsidence has been implemented in a spatially explicit decision support system, the 'touch table', which contains an interactive GIS and has been used in workshops with stakeholders. Three contrasting study areas of 1500-3500 ha were selected for discussions among stakeholders on the current and future challenges and on the effects of possible adaptation measures. During the workshops, data on current peat soil thickness, land use and groundwater levels were shown at the scale of individual parcels and the results of soil subsidence calculations were presented for the current situation and for a situation with climate change. Maps with future projections on the thickness of the remaining peat in 2050 and 2100 showed that major parts of the areas would have lost their peat and turned into mineral soils by 2100. Raising the ditch water level from 100 to 40 cm below soil surface would reduce the subsidence rate by 30%. Only the transfer from peat meadow areas to open water would prevent further subsidence and would possibly facilitate peat formation.

In **Chapter 7** it was concluded that climate change poses peatland managers for difficult challenges. Summer drought is expected to increase subsidence rates, which are locally already very high. Salinisation is not expected to increase subsidence rates; it could hamper decomposition rates, but has adverse effects on water quality and is a threat to agricultural practices. Thoughout this thesis, the effects of peat origin (fen or bog) and land use (agriculture or nature reserve) were explored. It was found that decomposition rates in fen peat were slightly higher than in bog peat. Large effects of land use were perceived. Decomposition rates did not differ between peat from agricultural sites and from nature reserves, but the microbial community dynamics did differ. The intensive agricultural use in the dairy meadows, involving the regular application of manure, which contains labile carbon and nitrogen components, led to a microbial community that responds quickly to changing conditions in order to efficiently absorb the nutrients and carbon.

Several options for adaptive peatland management are given. For instance, freshwater storage and subsurface drainage could reduce the effects of summer droughts and subsidence rates. Ploughing and the cultivation of deeply rooting crops should be minimised. Furthermore, it was recommended, in line with the results from stakeholder workshops, to stop adapting groundwater levels to the subsiding soil surface level in order to make a gradual, long-term transition to peatland regions with virtually no subsidence, profitable agricultural businesses, lower societal costs, less greenhouse gas emissions and better water quality. In the meantime, innovative research is needed on alternative crops that can grow in wetter conditions and farmers should be trained and guided in the process of implementing changes into their operational management.

# Samenvatting

Deze Nederlandstalige samenvatting is geschreven voor degenen die niet ingewijd zijn in de veenweidenproblematiek en/of voor niet-biologen. Meer diepgaande informatie kan gevonden worden in het 'Abstract' en in de hoofdstukken waarnaar verwezen wordt.

# Veenbodems in Nederland

## Geschiedenis van de veenbodems in Nederland

In Nederland werd er tijdens de laatste ijstijd (deze eindigde ca. 11.700 jaar geleden) een laag slecht doorlatend dekzand neergelegd. Hierdoor ontstonden lokaal in depressies permanent natte omstandigheden waar moerasplanten tot ontwikkeling konden komen. Onder de natte omstandigheden stagneerde de afbraak van dood plantenmateriaal (zie Figuur 1.1). De onafgebroken plantenresten vormden een laag veen. Het veenpakket werd langzaamaan steeds dikker en de vegetatie veranderde van drijvende moerasplanten naar een vegetatie die werd gedomineerd door riet en zeggen. Nadat het open water was opgevuld met veen ontstond een moerasbos met wilgen, elzen en berken bomen. De condities werden in de loop van de tijd steeds voedselarmer omdat het veenpakket groeide en het contact met het voedselrijke grondwater wordt verbroken. In dit stadium wordt veenmos dominant en dit mos kon horizontaal uitgroeien over het drogere omliggende landoppervlak. Totdat ca. 3000 jaar geleden 40% van het huidige landoppervlak van Nederland bedekt was met dikke laag (veenmos)veen, op vele plaatsen was dit veenpakket meer dan 20 meter dik. Daarna namen het veenoppervlak en veendikte af. Eerst door natuurlijke oorzaken zoals overstromingen waardoor veen werd weggeslagen en daarna door actief menselijk ingrijpen. Vanaf de 5e eeuw na Christus werden de veengebieden ontgonnen voor begrazing en nog later werd ook akkerbouw toegepast. Greppels werden gegraven om overtollig water af te voeren, later werden ook dijken aangelegd en molens gebruikt om water af te voeren (zie Figuur 1.2). Vanaf de 9e eeuw werd veen afgestoken zodat in gedroogde vorm (turf) als brandstof kon worden gebruikt en lokaal ook voor zoutwinning. In de loop van de tijd ontstond een karakteristiek landschap met petgaten waar het veen was weggegraven en legakkers waar men de turf op te drogen legde.

## Huidige problematiek in Nederlandse veenweidegebieden

Momenteel zijn in het westen en noorden van Nederland nog veenbodems te vinden (Figuur 1.4). In West-Nederland zijn dit veel metersdikke veenlagen. Hier is het veen ontstaan in voedselrijke omstandigheden, genoemd 'laagveen' en het bestaat voornamelijk uit resten van riet, zeggen en bos. Lokaal zijn er nog resten te vinden van veenmosveen dat werd afgezet onder de voedselarme omstandigheden. Dit veen wordt 'hoogveen' genoemd. Een groot deel van deze gebieden ligt nu enkele meters onder zeeniveau en het veen is van het voedselrijke type. De drooglegging is hier enkele decimeters onder het bodemoppervlak en melkveehouderij is het belangrijkste landgebruik. In de provincies Friesland, Drenthe en Groningen zijn de veenbodems momenteel dunner. Dit zijn veel voormalige hoogveengronden. Naast melkveehouderij vindt hier ook akkerbouw plaats. In landbouwgebieden worden de veenbodems ontwaterd om de landbouw te faciliteren, hierdoor dringt zuurstof binnen in de veenbodem. Het organische materiaal in de bodem breekt dan sneller af waardoor het maaiveld daalt en de bodem letterlijk verdwijnt. Naast maaivelddaling, die gemiddeld 1-2 cm per jaar bedraagt, is de waterkwaliteit in veengebieden vaak onvoldoende doordat er veel nutriënten vrijkomen uit de bodem. Ook komen er broeikasgassen zoals koolstofdioxide ($CO_2$) en methaan ($CH_4$) vrij bij de afbraak van veen. Een toename van broeikasgassen in de atmosfeer zal leiden tot hogere temperaturen en daarmee samenhangende droogte, zoals te zien is in de meest recente klimaatscenario's van het KNMI. Deze factoren zorgen weer voor een snellere afbraak van het veen. Zo werkt afbraak van veen als een zelfversterkend proces. Naast deze milieuproblemen is het waterbeheer in deze gebieden complex. Er zijn veel verschillen in maaiveldhoogte en de grondwaterstanden worden hier steeds op aangepast zodat de bodem niet te droog is en niet te nat. Andere problemen in veengebieden zijn de schade aan infrastructuur (wegen, dijken, bruggen) en aan huizen. Door de maaivelddaling kunnen houten palen van funderingen droog boven het grondwater uit komen waardoor deze gaan rotten.

### Klimaatverandering en mogelijke effecten op veenbodems

Klimaatverandering vormt mogelijk een bedreiging voor de veenbodems. Het KNMI heeft voorspeld dat de temperaturen in de nabije toekomst hoger worden, terwijl de zomers droger worden. Dit zijn omstandigheden die veenafbraak waarschijnlijk stimuleren. Tijdens droge zomers wordt er rivierwater in de veengebieden ingelaten om verdroging tegen te gaan, maar de verwachting is dat dit rivierwater in zeer droge zomers tijdelijk brakker wordt. Ook zou dan brakker grondwater naar boven kunnen komen.

## Doelen van dit onderzoek

In dit onderzoek stonden mogelijke effecten van drogere zomers en verzilting op veenafbraak centraal. Verder is steeds rekening gehouden met het type veen. Er zijn in dit onderzoek vier veentypen onderscheiden, namelijk twee verschillende veen-origines (voedselrijk en voedselarm) en twee verschillende landgebruiken. Dus: er is onderscheid gemaakt tussen veen dat in een laagveen is ontstaan en veen dat in een hoogveen is ontstaan. Ook is onderscheid gemaakt tussen veenbodems die zich in landbouwgebied bevinden en die zich in natuurgebieden bevinden. Dit leidde in het onderzoek tot de volgende vier veentypen laagveen met landbouwkundig gebruik, laagveen uit natuurgebied, hoogveen met landbouwkundig gebruik en hoogveen uit natuurgebied.

## Resultaten van dit onderzoek

### Invloed van klimaatverandering op veenafbraak

In Hoofdstuk 2 wordt een studie gepresenteerd naar het effect van droge zomers op veenafbraak. In een droge zomer zakt het grondwater uit en worden diepe veenlagen, waar normaal gesproken geen zuurstof komt doordat het zo nat is, voor het eerst sinds lange tijd blootgesteld aan zuurstof. In het experiment zijn bodemmonsters van deze diepe veenlagen aan zuurstof blootgesteld en is de afbraaksnelheid van het veen gemeten. Het resultaat van dit experiment was dat tijdens zo'n droge periode, maar opvallend genoeg ook daarna, de afbraaksnelheden veel hoger waren dan tijdens natte en zuurstofloze omstandigheden. Dit betekent dat door zo'n droge periode de maaivelddalingssnelheden zouden kunnen toenemen.
Een belangrijke theorie over veenafbraak is de 'enzymic latch theory'. Hierin wordt gesteld dat complexe organische verbindingen, de zogenaamde 'fenolen', de afbraaksnelheid van veen blokkeren, ze vormen een soort slot dat enzymen betrokken bij de afbraak van veen de hydrolases belemmert. Een speciale groep enzymen, de fenoloxidasen (POX), kunnen deze fenolen afbreken en zo het slot openen. Bij dit proces is waarschijnlijk zuurstof nodig. Als de fenolen afgebroken zijn (bijvoorbeeld omdat er in een droge zomer zuurstof in de veenbodem is doorgedrongen) dan kunnen de hydrolases hun werk doen en het veen verder afbreken, hiervoor is verder geen zuurstof meer nodig. Wanneer na de droge zomer de grondwaterstand weer omhoog wordt gebracht is het veen dus niet beschermt tegen verdere afbraak. Wel gaat de afbraak zonder zuurstof langzamer dan in aanwezigheid van zuurstof.
In het experiment van Hoofdstuk 2 bleek echter niet dat de concentratie van fenolen lager werd door het toedienen van zuurstof. Daarom zijn ook in verticale bodemprofielen metingen gedaan aan de concentraties fenolen en de potentiële activiteit van het enzym fenoloxidase (POX) (Hoofdstuk 3). De verwachting was namelijk dat de concentraties fenolen in de bovenste lagen van de veenbodem lager zouden zijn en de activiteit van POX door de hoge zuurstofbeschikbaarheid hoger, zodat daar al veel oorspronkelijk aanwezige fenolen afgebroken zouden zijn. Er werd echter geen groot verschil in fenolconcentraties op de verschillende dieptes gevonden en, heel verrassend, er was zelfs nog potentiële POX activiteit op 3 meter diepte. Mogelijkerwijs kunnen sulfaat en nitraat de rol van zuurstof op deze diepte overnemen, maar hier kunnen we geen uitsluitsel over geven. Verder onderzoek naar de rol van deze stoffen bij de veenafbraak is nodig om goede inschattingen te maken over de toekomst van onze veenbodems.
Er is ook onderzoek gedaan naar verzilting (Hoofdstuk 4). Het bleek dat in brakkere omstandigheden de aerobe afbraak (de afbraak in aanwezigheid van zuurstof) tot 50% langzamer verloopt, in anaerobe omstandigheden (zonder zuurstof) is geen effect van verzilting gevonden. De nadelige effecten van verzilting waren dat er meer nutriënten,

zoals fosfaat en ammonium, vrijkwamen van het bodemcomplex waardoor waterkwaliteit mogelijk verslechtert.

## Invloed van veenorigine (laagveen of hoogveen) op veenafbraak

In het onderzoek is steeds onderscheid gemaakt tussen veen dat gevormd is in een laagveen en veen gevormd in een hoogveen. Hoogveen wordt gevormd door een speciaal geslacht van mossen, de veenmossen (*Sphagnum* spp.). Deze veenmossen maken veel fenolen aan, hierdoor wordt bijvoorbeeld vraat door herbivoren tegengegaan, maar tegelijkertijd bemoeilijken deze fenolen het afbraakproces. In het veen dat gevormd is in hoogvenen troffen we, zoals verwacht, een hogere concentratie fenolen aan, ook waren de afbraaksnelheden, zoals verwacht, iets lager. De activiteit van het enzym fenoloxidase, en het nauw verwante enzym fenol-peroxidase, was hoger in het laagveen.
Verder werd duidelijk dat in het laagveen veel pyriet aanwezig is ($FeS_2$). Onder invloed van zuurstof valt dit pyriet uiteen waardoor sulfaat en waterstofionen vrijkomen, wat leidt tot verzuring. Sulfaat verdringt fosfaat van de bodem, waardoor de fosfaatconcentraties in het grond- en oppervlaktewater hoger worden.

## Invloed van landgebruik op veenafbraak

Er zijn geen verschillen gevonden in de afbraaksnelheden van het veen uit landbouwgebieden en uit natuurgebieden. De verwachting was dat door de drainage in landbouwgebieden de fenolen zouden zijn afgebroken waardoor de afbraak sneller heeft kunnen verlopen. Dit had kunnen resulteren in hogere afbraaksnelheden, óf juist in lagere afbraaksnelheden doordat al het relatief makkelijk afbreekbare organische materiaal wellicht al afgebroken zou zijn.
Toch zijn er grote verschillen gevonden tussen de bodemmonsters uit landbouwgebieden en die uit natuurgebieden (Hoofdstuk 5). De microbiële gemeenschap, die door middel van het gebruik van enzymen het veen afbreekt, was namelijk heel anders in de landbouwgebieden. De microbiële biomassa in de landbouwgebieden was veel groter dan in natuurgebieden. Deze biomassa was echter niet veel actiever, totdat glucose werd toegevoegd aan de bodemmonsters. Toen werd de 'slapende' microbiële biomassa in deze landbouwbodems ineens erg actief. In een ander experiment bleek dat in de bodemmonsters uit landbouwbodem ammonium erg snel omgezet werd in nitraat. Al jarenlang worden deze landbouwbodems bemest, meestal vier tot vijf keer per jaar. Een mogelijke verklaring voor de verschillen tussen de bodems is dat de microbiële gemeenschap in de landbouwgebieden zo veranderd is dat zij optimaal gebruik kan maken van de puls aan makkelijk afbreekbaar materiaal en voedingsstoffen die in de (drijf)mest zitten.

## Suggesties voor beheer van veengebieden in Nederland t.a.v. een veranderend klimaat

Als het huidige veenweidenbeleid niet verandert dan zal het veen in de westelijke veenweidegebieden over ca. 500 jaar verdwenen zijn. De dunnere veenlagen in het noorden van Nederland zullen aan het einde van deze eeuw al voor een groot deel verdwenen zijn. Het behouden van deze veenbodems heeft voordelen: minder broeikasgasuitstoot, minder bodemdaling dus minder diepe ligging t.o.v. de stijgende zeespiegel, minder problemen met infrastructuur en bebouwing. Aan de andere kant zijn soms grote veranderingen in het beheer nodig om maaivelddaling te stoppen.
Tijdens het onderzoek hebben Karlijn Brouns en Jos Verhoeven meegewerkt aan workshops met lokale stakeholders in diverse veenweidegebieden (Hoofdstuk 6). Deze workshops werden georganiseerd door het Instituut voor Milieuvraagstukken van de VU Amsterdam. Tijdens deze workshops werd het proces van maaivelddaling besproken en werden met behulp van interactieve kaarten diverse maatregelen in een specifiek onderzoeksgebied verkend en geëvalueerd. Eén van de conclusies was dat om maaivelddaling te beperken de drooglegging sterk gereduceerd moet worden.
Een aantal agrariërs kwam met de volgende suggestie: momenteel wordt elke 10-15 jaar het grondwaterpeil aangepast aan het dalende maaiveld. Hierdoor is een vicieuze cirkel ontstaan van maaivelddaling en aanpassingen aan het waterbeheer. Als we hier mee zouden stoppen dan zou de maaivelddaling geleidelijk afnemen en de omstandigheden op de percelen geleidelijk natter worden. Conventionele landbouw, zoals intensieve melkveehouderij, is dan niet meer mogelijk. Maar wellicht kunnen er dan alternatieve gewassen verbouwd worden, bv. voor de productie van bioplastics, of kunnen andere verdienmodellen worden gevonden. Dit zou voor de lokale agrarische ondernemers een grote verandering zijn. Praktijkproeven en een goede ondersteuning van deze agrariërs zou nodig zijn om deze verandering succesvol te laten verlopen. Deze veranderingen zijn weliswaar ingrijpend, maar ze hebben de potentie om de veenbodems te behouden. Er zijn ook minder vergaande veranderingen mogelijk waardoor maaivelddaling afgeremd zou kunnen worden. Een voorbeeld is het beperken van maïsteelt omdat voor deze teelt geploegd moet worden en maïs een diep wortelstelsel vormt, waardoor meer zuurstof in de bodem gebracht wordt. Maïsteelt zou bijvoorbeeld geconcentreerd kunnen worden op plekken waar geen veenbodem is. Verder kan men denken aan waterberging in combinatie met onderwaterdrainage. Het extra water kan tijdens droge zomers via het systeem van onderwaterdrainage relatief snel de veenbodem ingebracht worden waardoor de extra veenafbraak tijdens zo’n droge periode beperkt wordt. Hier is echter veel water voor nodig.

Verder blijft het maaiveld (zij het langzamer) dalen waardoor de drainagebuizen op een gegeven moment erg dicht bij het oppervlak komen te liggen.

## Suggesties voor verder onderzoek

In Hoofdstuk 7 worden enkele suggesties gedaan voor verder onderzoek. Verder onderzoek is wenselijk naar de mate waarin de 'slot'-functie van de fenolen in Nederlandse veenbodems reeds is verdwenen vanwege de drooglegging. Ook zou nagegaan moeten worden wat dit betekent voor maaivelddalingssnelheden als deze landbouwgronden vernat zouden worden. Praktijkgericht onderzoek zou zich kunnen richten op de teelt van andere gewassen en de mogelijkheden voor overgang naar andere landbouwpraktijken in veengebieden.

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# Dankwoord

Aan het onderzoek en de totstandkoming van dit proefschrift hebben velen bijgedragen, op deze plek wil ik een aantal van hen in het bijzonder bedanken.

Een baan als onderzoeker aan een universiteit, als promovendus, een lange tijd had ik dat niet voor mogelijk gehouden. Maar het enthousiasme van Bas van de Riet, bij wie ik bij de toenmalige onderzoeksgroep Landschapsecologie stage liep, werkte erg aanstekelijk. Van het één kwam het ander en voordat ik het me goed en wel realiseerde hadden Jos en Mariet me onder hun hoede genomen. Ik leerde Jos kennen tijdens de vele uitstapjes die we gemaakt hebben: van bodemmonsters halen in de weilanden in Zegveld tot workshops in boerenschuren in Friesland. Vriendelijk, maar tegelijkertijd doortastend en relativerend, dat kenmerkt jouw begeleiding, dankjewel. Mariet vulde deze begeleiding aan met haar gedrevenheid om steeds weer een puzzelstukje te ontdekken, te verkennen en op de juiste plek te plaatsen. Mariet, ik heb bewondering voor je inhoudelijke kennis, ik heb veel van je geleerd en dankzij jouw aanwijzingen verbeterde mijn werk steeds aanzienlijk.

De leden van de lees- en promotiecommissie wil ik bedanken voor het lezen en beoordelen van mijn proefschrift en het zitting nemen in de promotiecommissie.

Collega's, jullie zijn met velen. Ik noem niet iedereen bij naam, maar ik wil wel iedereen bedanken. Met de één deelde ik lief en leed, anderen waren meer betrokken bij proefopzetten en statistiek, of juist bij praktische zaken in het lab. In het bijzonder: Joost, Jeroen, Bas, Bjorn, Annemarie, Rob, Erik, Gerrit en Bertus, maar ook alle anderen die op verschillende manieren veel voor mij hebben betekend: bedankt!

Zowel de scriptie-studenten als de masterstudenten die bij mij stage hebben gelopen bedank ik voor hun hulp en ideeën. In het bijzonder wil ik Joost van Deelen, Rosanna van Hespen, Esther Moens, Gerrit Potkamp, Rémon Saaltink en Anne Uilhoorn bedanken. Ik vond het erg leuk om betrokken te zijn bij jullie ontwikkeling.

Tijdens het veldwerk en tijdens de workshops ben ik met vele mensen in aanraking gekomen, ieder van jullie heeft het onderzoek en het proefschrift beïnvloed. De agrariërs, zoals Gert Diever en de medewerkers van het Veenweide Innovatie Centrum, wil ik bedanken voor het feit dat ik mijn onderzoek kon doen met bodemmonsters van hun terreinen. Verder bedank ik de natuurbeheerders voor de toestemming voor en de hulp bij het steken van bodemmonsters op de mooie terreinen die jullie beheren. Martijn van Schie (Natuurmonumenten Nieuwkoopse Plassen) en Martin Snip (Natuurmonumenten Fochteloërveen): wat was het altijd heerlijk om bij jullie in het veld mijn hoofd weer leeg te maken.

Mijn paranimfen Margriet en Heleen, wat ben ik blij met jullie! Margriet en ik leerden elkaar kennen als huisgenoten tijdens onze studietijd. Lekker koken, eten, daarna wel sporten. Ik heb bewondering voor je doorzettingsvermogen, zonder grootsprekerij speel je heel veel klaar. Ik wens je heel veel succes bij de opleiding tot radioloog en veel genot van je mooie gezin.
Heleen, gelijk de eerste keer dat we elkaar ontmoetten voelden we een klik. Door dik en dun ben je er voor me. Dankjewel voor deze bijzondere vriendschap.

Lieve andere vrienden, juist door weinig af te weten van onderzoek doen in het algemeen of van veenweidenproblematiek in het bijzonder hebben jullie me geholpen. Tijdens de gezellige avonden, weekendjes en (ski-)vakanties met jullie verzette ik mijn gedachten waardoor ik er daarna weer tegenaan kon.

Lieve mama, papa, Hannie en Leen, wie had dat gedacht, jullie dochter gaat promoveren. Het feit dat ik zo ver ben gekomen, heb ik voor een groot deel aan jullie te danken. Dankjulliewel voor de verschillende manieren van ondersteunen, maar allemaal met niet-aflatende vertrouwen in een goede afloop. Matthijs, lief broertje, 5,5 jaar jonger dan ik, maar zeker een voorbeeld voor me! Ik ben trots op je.

Maikel, je houdt me, voor zover dat lukt, met beide benen op de grond. Je laat me zien wat belangrijk is in het leven en dat werk niet altijd op de eerste plek moet staan. Je hebt me vele malen met het onderzoek geholpen. Bijvoorbeeld op zaterdag in het lab, als de planning niet anders toeliet. En geef toe: dat was niet ongezellig toch! Ook heb je mooie foto's voor in dit proefschrift gemaakt. Ik geniet van de momenten dat we samen zijn. Dankjewel dat je er voor me bent.

De afgelopen jaren kenden pieken en diepe dalen, zowel in de privésfeer als tijdens het onderzoek. Toch ligt dit proefschrift er nu en dat is de hobbelige weg waard geweest.
Op deze plek, aan het eind van het dankwoord, blik ik terug op het overlijden van twee bijzondere mensen. Het gedicht waar dit dankwoord mee afsluit draag ik aan hen op.

**Dankbaar om nooit te vergeten**
Vergeten doe je je sleutels,
Vergeten doe je je tas
Vergeten doe je je pincode
Maar jullie zal ik nooit vergeten

Ik ben dankbaar voor jullie vertrouwen
Dankbaar voor jullie lach
Dankbaar voor jullie liefde
Dankbaar ben ik, voor de energie, kameraadschap en verbondenheid

# Publications

## Peer reviewed publications

Brouns, K., Eikelboom, T.E., Jansen, P.C., Janssen, R., Kwakernaak, C., Van den Akker, J.J.H., Verhoeven, J.T.A., 2014a. Spatial analysis of soil subsidence in peat meadow areas in Friesland in relation to land and water management, climate change and adaptation. Environmental management 55, 360-372.

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Accepted for publication: Brouns, K., Keuskamp, J.A. Potkamp, G., Verhoeven, J.T.A., Hefting, M.M., Peat origin and land use effects on microbial activity, respiration dynamics and exo-enzyme activities in drained peat soils in the Netherlands. Soil Biology & Biochemistry

## Conference contributions

Brouns, K., Hefting, M.M., Verhoeven, J.T.A., 2014. Peatlands in a changing climate: summer droughts and salinisation. Presentation at the Rotterdam International Conference on Deltas in times of climate change. 24-26 September 2014

Brouns, K., Hefting, M.M., Verhoeven, J.T.A., 2012. Decomposition of peat during simulated summer drought. Presentation at the 14th International Peat Congress Stockholm, Sweden, 3-8 June 2012

Brouns, K., Verhoeven, J.T.A., Hefting, M.M., 2012. Do vertical soil profiles reflect the Enzymic Latch Theory? Poster presentation at BES/IUCN peatland meeting, Bangor, United Kingdom, 26-28 June 2012BES/IUCN peatland meeting, Bangor, United Kingdom, 26-28 June 2012

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## Curriculum vitae

Karlijn Brouns was born on the 9th of August 1984, in Dordrecht, the Netherlands. In 2002, she started with the study Science & Innovation (Natuurwetenschappen & Innovatiemanagement) at Utrecht University during which she discovered that biology interested her most. In February 2003, Karlijn started the bachelor Biology, also at the UU, alongside several courses on the Spanish language. After finishing the bachelor Biology, she travelled to Ecuador to work as a volunteer at a botanical garden, dairy farm and fruit farm. A visit to the Galápagos Islands was unforgettable. After that, she started with the master programme Environmental Biology, in which she followed the track Ecology & Natural Resources Management. The fascination for peat meadow landscapes started during an internship with Bas van de Riet (Ecology and Biodiversity, UU) in which nutrient limitation in species-rich Calthion meadows in Dutch peat areas was studied. The results of this research project were published (Van de Riet et al., 2010). Karlijn did a second internship at WWF Netherlands/Peru and CARE, a humanitarian organisation fighting poverty. Within a payments for watershed services programme, she evaluated various land management practices and their potential effects on soil loss in micro-basins of the Jequetepeque watershed. In 2009, she graduated the master programme cum laude and started the Ph.D. project presented in this thesis. Currently, Karlijn combines her job as a teacher at a secondary school with a master studies to become a fully qualified biology teacher.