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ECOLOGICAL STOICHIOMETRY IN WATERSHEDS:

FROM LAND TO WATER IN THE QINGHAI LAKE BASIN

By

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Dissertation

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in Organismal Biology, Ecology, and Evolution

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Missoula, MT

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ABSTRACT

REN, ZE, Ph.D, December 2019 Organismal Biology, Ecology, and Evolution

Ecological Stoichiometry in Watersheds: From Land to Water in the Qinghai Lake Basin

Chairperson: Dr. James J. Elser

We examined the influences of grassland status (as indexed by normalized difference vegetation index, NDVI) on carbon (C), nitrogen (N), and phosphorus (P) concentrations and stoichiometry, nutrient limitation, as well as microbial community structure in soil, stream, and/or lake ecosystems in the Qinghai Lake watershed, where grassland is the dominant landcover and more than half of the grassland is degraded.

Chapter 2 showed that grassland degradation decreased C and N concentrations as well as C:N, C:P, and N:P ratios in soil. Moreover, grassland degradation decreased C, N, and P concentrations and influenced C:N and N:P ratios in soil microbial biomass. Soil microorganisms exhibited strong homeostatic behavior while variations of microbial biomass C:N and N:P ratios suggest changes in microbial activities and community structure. The soil became relatively more P rich and thus N limitation is anticipated to be more apparent with grassland degradation. Chapter 3 provided a picture of potentially differential influences of grassland degradation on DOC, TN, and TP in streamwater. The imbalances of C:N:P stoichiometry between streamwater and biofilms and the non-isometric relationships between biofilm C and P suggest that stream biofilms might be limited by P and sensitive to P variation. Chapter 4 indicated that grassland degradation has the potential to differentially influence the nutrients delivered to streams with substantial increases in P but decreases in N and N:P, alleviating P limitation of stream periphyton and, ultimately, stimulating P-limited phytoplankton growth in the lake. Chapter 5 revealed that grassland degradation shifted bacterial diversity and communities in soil, likely by changing soil moisture, soil organic carbon, total nitrogen, and total phosphorus. Chapter 6 showed that the variation of bacterial communities in stream biofilms was closely associated with rate of change in NDVI, pH, conductivity, as well as C, N, P contents and C:N ratio in biofilms *per se*. Alpha diversity was positively correlated with C, N, and P in biofilms. Abundant subcommunities of microbes were more strongly associated with environmental variables.

Overall, my dissertation revealed strong impacts of grassland degradation on several aspects of nutrient dynamics and limitation as well as on microbial communities in terrestrial and aquatic ecosystems in the Qinghai Lake watershed.

Acknowledgements

It would have been to have completed this dissertation without the help and support of many people. At this moment at the end of the thesis, I would like to thank them sincerely.

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Chapter 1 Introduction

Grassland Degradation

Grasslands account for ~40% of the world's land surface (excluding Greenland and Antarctica) and are among the largest terrestrial biomes in the world (Suttie et al., 2005). However, these grasslands are experiencing widespread degradation due to human impacts (Gang et al., 2014). Grassland degradation is a process of retrogressive succession (Dyksterhuis, 1949; Li, 1997), in which aboveground vegetation coverage and biomass decline. This leads in turn to simultaneous impairment of soil physical, chemical, and biological properties, such as declines in soil moisture, soil nutrients, perturbation of microbial communities, as well as increases in soil erosion (Abril and Bucher, 1999; Viragh et al., 2011; Li et al., 2012a; Dlamini et al., 2014). Anthropogenic impacts on grasslands also affect aquatic systems because terrestrial and aquatic ecosystems are highly linked in watersheds (Williamson et al., 2008). Thus grazing-induced grassland degradation likely affects the cycling of multiple key chemical elements that regulate the structure and function of aquatic and terrestrial ecosystems in the watershed (Schlesinger and Bernhardt, 2013).

Important grasslands are found on the Qinghai-Tibet Plateau (QTP), which is known as the "Third Pole" of the Earth and which is extremely vulnerable to anthropogenic activities and global climate change (Jin et al., 2005; Zhao et al., 2006; Qiu, 2008; Yao et al., 2012). On the QTP, grassland covers more than 60% of the plateau, accounting for 44% of China's grasslands and 6% of the world's grasslands (Scurlock and Hall, 1998; Tan et al., 2010). In recent decades, however, the QTP grassland has experienced serious degradation due to various driving forces, but especially climate change and overgrazing (Harris, 2010). In the study area, the Qinghai Lake watershed, half of the grassland was classified as degraded in 2010 (Luo et al., 2013).

To study this degradation, researchers often employ the normalized difference vegetation index (NDVI) derived from satellite observations as a vegetation index (Thiam, 2003; Yengoh et al., 2014). It has been consistently demonstrated that NDVI is closely related with above-ground vegetation biomass and coverage (Carlson and Ripley, 1997; Eastwood et al., 1997) and thus NDVI is commonly used to estimate regional vegetation coverage (Carlson and Ripley, 1997). Indeed, the China National Standards (GB19377-2003) classify the degree of grassland degradation based on grassland vegetation coverage as indicated by NDVI. Thus, NDVI has been widely used to estimate vegetation status and indicate grassland degradation (Thiam, 2003; Akiyama and Kawamura, 2007; Li et al., 2013; Hilker et al., 2014). In our study area, NDVI had strong relationships with *in situ* measured aboveground biomass, fractional vegetation cover, and grass species richness (Ren et al., 2019). Moreover, various grassland vegetation factors (including aboveground biomass, fractional vegetation cover, grass species richness, and NDVI) were not associated with elevation although the study area across a large space (Ren et al., 2019). In addition, NDVI also exhibited large spatial variation across our study region. Thus, the spatial variation of NDVI permits us to use an approach known as "space-for-time" substitution (Figure 1.1) to study impacts of grassland degradation by comparing sub-basins differing in NDVI. To assess the possibility of non-independence of the sampling sites, we tested if differences between NDVI values were correlated with the linear distance between them (Figure 1.2). This relationship was statistically significant (P=0.002) but quite weak (R^2=0.039), suggesting that spatial non-independence would not contribute strongly to our space-for-time approach.

"Space-for-time" substitution is an alternative strategy to study long-term phenomena in ecology (Pickett, 1989; Blois et al., 2013). It has been used widely and successfully, especially in systems that experience strong successional dynamics (Pickett, 1989). In this approach, temporal processes (past or future trajectories) of ecosystems are studied and inferred via contemporary spatial patterns (Blois et al., 2013). A good example for the application of "space-for-time" substitution is in studying the impact of natural disturbance on ecosystems (Pickett, 1989). Response to natural disturbance has been recognized as a long-term process. However, systematic long-term studies are usually absent (Sousa, 1984). Thus, the spatial distribution of disturbances has been widely used to construct disturbance temporal regimes (Pickett, 1989). A variety of studies using the "space-for-time" substitution approach indicate that the approach provides consistent estimates of realistic long-term variations (Meurant, 2012; Blois et al., 2013). Grassland degradation is a natural and anthropogenic disturbance. However, intensive and systematic long-term studies of grassland degradation are lacking. Thus, according to “space-for-time” substitution, spatial patterns of grassland status were used to study the impacts of grassland degradation in the Qinghai Lake watershed in this study (Figure 1.1).

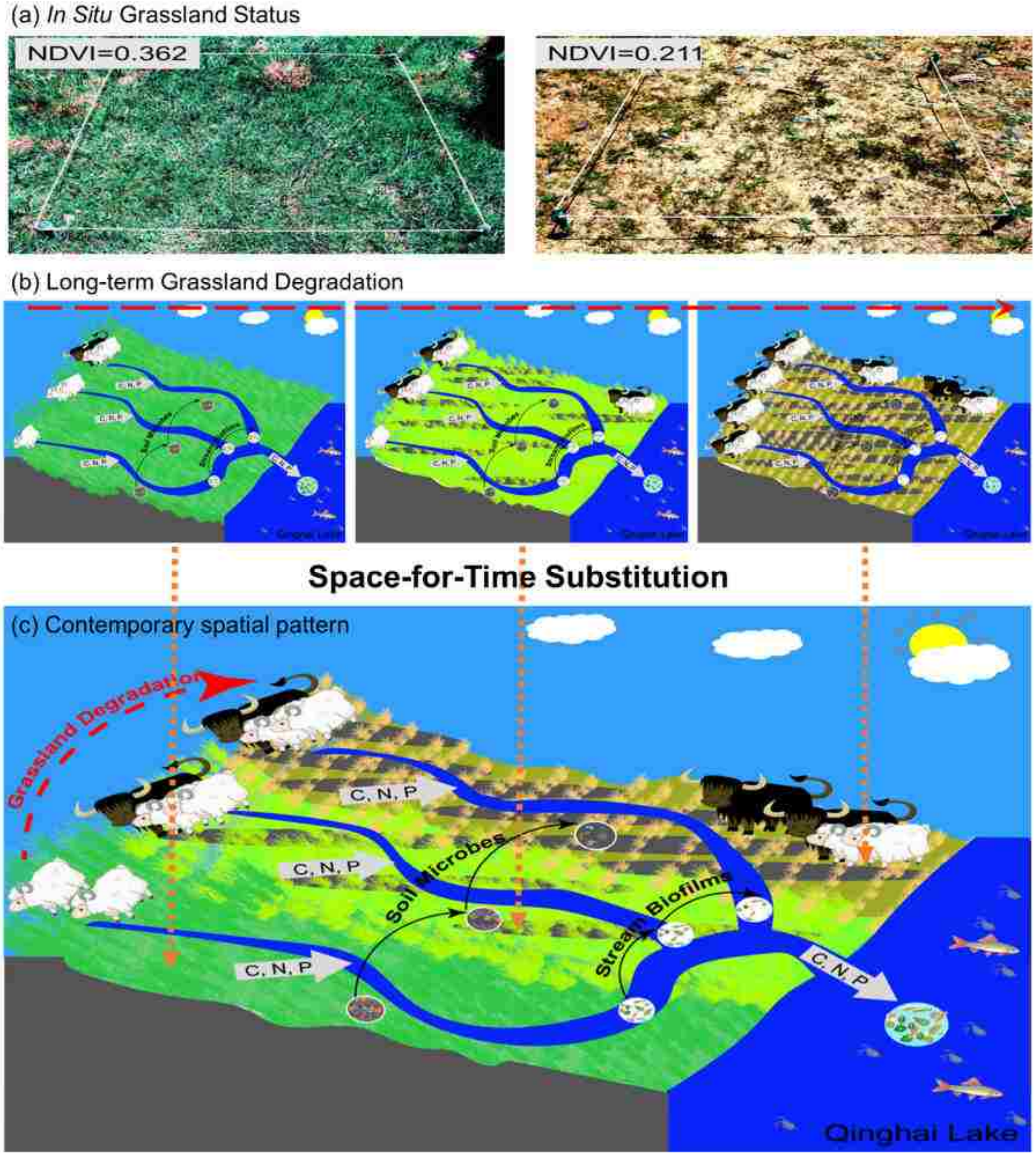

Figure 1.1 (a) Field photos showing *in situ* grassland status. (b) Long-term grassland degradation process. (c) According to “Space-for-Time” substitution, the contemporary

spatial pattern of grassland status was used to study grassland degradation in Qinghai Lake watershed.

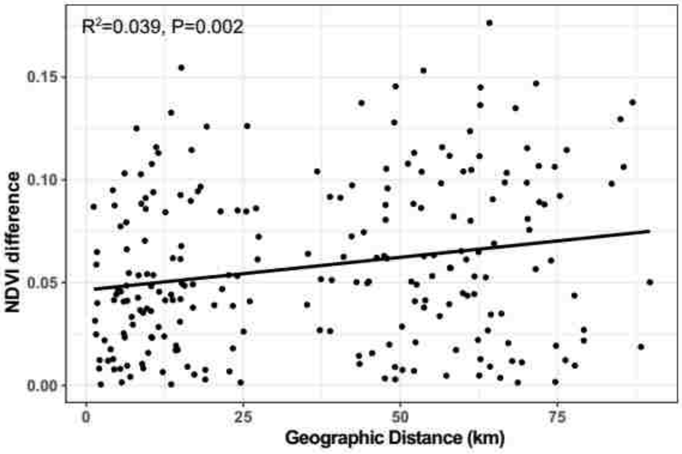

Figure 1.2 Relationships between geographic distance and NDVI difference of paired sample sites.

Landcover Change Influences Terrestrial Ecosystems

Changes in landcover significantly affect the structure, function, and elemental balances of terrestrial ecosystems (Tischer et al., 2014), potentially shifting the combined carbon (C), nitrogen (N), and phosphorus (P) stoichiometry in soil (Zhao et al., 2015) as well as affecting microbial activities, community composition, and stoichiometry (Anderson et al., 2011; Li et al., 2014; Ng et al., 2014; Tischer et al., 2014).

In grassland soil, large proportions of organic carbon and nutrient stocks are concentrated in the top layers (Gill et al., 1999). Thus, any external disturbance is likely to cause dramatic soil nutrient depletion, which in turn affects vegetation properties (Steffens et al., 2008; Ruiz-Sinoga and Romero Diaz, 2010; Dong et al., 2012; Dlamini et al., 2014). Indeed, nitrogen and phosphorus losses are higher in degraded grassland than in non-degraded grassland (Wu and Tiessen, 2002; Steffens et al., 2008). The large losses of nutrients in degraded grassland systems are primarily due to erosion, loss of vegetation cover, and soil texture changes (Dong et al., 2012; Su et al., 2015). Soil erosion is highly correlated with vegetation properties, such as vegetation density and coverage. Degraded grassland has higher soil erosion than non-degraded grassland (Zhao et al., 2011; Su et al., 2015) and topsoil erosion causes substantial loss of soil organic matter (Zhu, 2011). Moreover, soil properties influence the retention and transfer of soil nutrients (Swift et al., 2004). Degraded grassland has higher soil bulk density but lower values of porosity, infiltration, and soil moisture than non-degraded grassland (Zhao et al., 2011; Hiltbrunner et al., 2012; Su et al., 2015), leading to heavy scour from surface flow that amplifies the loss of soil organic matter and nutrients (Zhao et al., 2011). The decline of vegetation coverage, biomass, and density can influence the dynamic nature of the plant–soil interactions through the modification of the water cycle and the fluxes of other elements, potentiating depletion of soil organic carbon and organic nitrogen by soil erosion (Mchunu and Chaplot, 2012; Dlamini et al., 2014).

The overall reduction of soil organic matter and nutrients in degraded grassland have been consistently demonstrated. However, the *relative* losses of nitrogen and phosphorus during grassland degradation are still unclear (Wen et al., 2013; Wang et al., 2014), likely because many studies focus on one element at a time. While it has been suggested that soil C:N:P ratios change with variations in land use (Zhao et al., 2015), variations in C:N:P stoichiometry in soil are highly complex (Zhang et al., 2013). In general, the major sources of total soil C and N are biological (primary production, nitrogen fixation) while weathering of primary rock minerals provides the dominant source of total P (Walker and Syers, 1976; Chadwick et al., 1999). Thus, shifts in vegetation cover, plant community composition, geomorphology, as well as soil texture and geology all potentially affect nutrient stoichiometry in soil (Aponte et al., 2010; Li et al., 2012b). Since grassland degradation affects all of these primary factors, we might also expect degradation to differentially affect the accumulation or losses of N and P in soils and thus soil C:N:P ratios. This is important because soils with different C:N:P ratios as well as plants with different foliar C:N:P ratios undergo different litter decomposition processes and rates, leading to differential cycling of N and P to soil (Zhang et al., 2013). We might also expect that these differential losses of N and P from soil to be manifested in the concentrations and ratios of N and P in streamwater in affected catchments, potentially affecting nutrient limitation in the stream and in downstream lakes. Moreover, as argued above, impacts of grassland degradation are likely to differentially affect the cycling of N and P. Study of such multi-element disruptions is facilitated by application of the theory of ecological stoichiometry, which involves the study of balance of multiple chemical elements in ecological systems (Sterner and Elser, 2002).

Organism growth can be limited by supplies of key nutrients while biota can reciprocally affect the availability of nutrients in the environment (Sterner and Elser, 2002). For example, microbial demand and use of resources are driven by the elemental stoichiometry of their own biomass and the efficiency of growth but are also regulated by the availability and stoichiometry of soil nutrients (Cleveland and Liptzin, 2007; Griffiths et al., 2012; Manzoni et al., 2012). A large proportion of soil microorganisms is heterotrophic and assumed to exhibit stronger homeostasis at the organismal level than photoautotrophic organisms (Sterner and Elser, 2002; Cleveland and Liptzin, 2007; Heuck et al., 2015; Fanin et al., 2017). Since different taxa of microbes can have different homeostatically regulated C:N:P ratios in their biomass as well as different abilities to acquire C, N, and P, changing soil environmental conditions likely shift microbial community composition and thus alter the stoichiometry of microbial biomass (Harris, 2009; Hall et al., 2011; Fanin et al., 2013; Tischer et al., 2014; Heuck et al., 2015). As argued above, impacts of grassland degradation are likely to differentially affect the cycling of N and P. With grassland degradation, changes in soil texture and depletion of soil organic matter and nutrients also affect microbial activities and communities (Abril and Bucher, 1999; Mekuria et al., 2007). For example, ammonifiers, nitrifiers, and cellulolytic groups all decline in abundance and activity with the intensity of grassland degradation (Abril and Bucher, 1999). Moreover, soil microorganisms control the fluxes of C, N, and P through respiration, mineralization, and immobilization, leading to feedbacks on their own biomass stoichiometry (van der Heijden et al., 2008; Mooshammer et al., 2012). Thus, in order to better understand biogeochemical cycles in terrestrial ecosystems in the face of landcover transformation and climate change, it is essential to understand the stoichiometric interactions between soil nutrients and soil microbes (Philippot et al., 2013; Handa et al., 2014).

Landcover Change Influences Stream Ecosystems

Inland waters are sentinels and integrators of terrestrial processes through the transport and storage of water, nutrients, and energy (Williamson et al., 2008). Small streams connect the terrestrial environment with larger rivers (Figueiredo et al., 2010) and are the primary receivers of nutrients and organic matter exported from terrestrial ecosystems (Vannote et al., 1980; Figueiredo et al., 2010; Deegan et al., 2011). On a larger scale, rivers play a critical role linking upland watersheds with downstream aquatic ecosystems, including lakes and coastal waters (Cronan, 2012). Both streams and rivers are strongly influenced by the landscapes through which they flow (Vannote et al., 1980; Allan, 2004) and thus are particularly sensitive to terrestrial change (Bring and Destouni, 2009; Holmes et al., 2012).

Aquatic impacts of changing landcover can often first be observed via their effects on stream biofilms, which include attached algae, bacteria, and fungi as well as detritus and extracellular materials. Biofilms play a vitally important role in stream ecosystems (Godwin and Carrick, 2008; Larned, 2010), acting as the dominant source of primary production in streams and thus contributing significantly to secondary production (Mayer and Likens, 1987; Hall et al., 2001). Moreover, biofilms are integral to biogeochemical cycling in stream ecosystems through nutrient uptake, transfer of nutrients to higher trophic levels, and remineralization (Schiller et al., 2007). The abundance and condition of biofilms are influenced by many environmental factors, such as nutrient availability, light intensity, water temperature, and flow velocity (Fanta et al., 2010). In streams flowing through a grassland watershed, nutrient availability might have a particularly important role for biofilm growth without the profound impacts of a forest canopy on light, temperature, and coarse organic matter inputs (Townsend and Riley, 1999; Riley et al., 2003). Stream biofilms also have a complex stoichiometry. In biofilm assemblages, various heterotrophic and autotrophic taxa are tightly linked to each other via trophic and competitive interactions (Fitter and Hillebrand, 2009) via the transfer of different types of nutrients associated with different biological processes (Peterson et al., 2001; Burgin and Hamilton, 2007; Ishida et al., 2008; Mulholland et al., 2008; Drake et al., 2012). The community composition, biomass, and stoichiometry of biofilms change significantly with available nutrients in streams (Fanta et al., 2010; Hill et al., 2011; Drake et al., 2012).

Moreover, bacteria and algae can compete with each other for inorganic nutrients, especially phosphorus (Danger et al., 2007) but may nevertheless show close positive correlations in biomass and productivity because bacteria also rely on algal activity and carbon exudates (Hepinstall and Fuller, 1994; Carr et al., 2005). C:N and C:P ratios of biofilms generally decrease with increasing nutrient availability (Frost et al., 2005; Kohler et al., 2011), while purely heterotrophic biofilms have been observed to have higher C:P ratios than autotrophic assemblages (Fitter and Hillebrand, 2009). However, elevated stream nutrient concentrations can decouple autotrophic and heterotrophic processes in biofilms due to decreased demand for recycled nutrients (Scott et al., 2008).

Stream biofilms are potentially linked to watershed conditions because landcover significantly controls the export of C, N, and P from catchments to aquatic ecosystems (Abell et al., 2011; Erol and Randhir, 2013; Umbanhowar et al., 2015), potentially shifting biofilm community structure. For example, previous research has demonstrated variation of stream diatom composition in relation to landcover change from forest to agriculture to urban catchments (Walker and Pan, 2006; Smucker et al., 2013; Teittinen et al., 2015), close relationships between longitudinal patterns of stream periphyton biomass and pasture degradation on the Qinghai-Tibet Plateau (Ren et al., 2013), and an influence of landcover conditions on biofilms stoichiometry (O'Brien and Wehr, 2010). Overall, these studies indicate that grassland degradation has strong potential to affect the quantity, composition, and stoichiometry of nutrients exported from the land into streams by changing soil nutrients and microbial communities. As the grassland degrades, soil nutrient contents decrease significantly (Wen et al., 2013; Wang et al., 2014), being lost to erosion and runoff. Small streams may be the most important component in regulating transport and transformation of these nutrients in large drainages because of their high proportion of channel length in the whole river network and their large surface area to-volume ratios, which favor rapid nutrient uptake and processing (Alexander et al., 2000; Peterson et al., 2001). Thus, changes in landcover in a watershed affect terrestrial biogeochemical cycles, amplifying the export of terrigenous nutrients to streams (Vannote et al., 1980; Allan, 2004).

Landcover Change Influences Lake Ecosystems

Aquatic ecosystems face increasing pressures and threats from disturbances of upland watersheds associated with landcover change (Li et al., 2009; Cronan, 2012). This is of interest because lakes have an intimate relationship with catchment characteristics through material transport by surface runoff (Zhang, 2011; Canham et al., 2012; Cronan, 2012; Sadro et al., 2012). Streams and rivers receive nutrients (such as nitrogen and phosphorus) and organic matter exported from terrestrial ecosystems (Vannote et al., 1980; Figueiredo et al., 2010; Deegan et al., 2011), and connect upland watershed with downstream lakes and coastal waters (Figueiredo et al., 2010; Cronan, 2012). Thus, catchment landcover substantially controls the export of carbon and nutrients to lake ecosystems (Abell et al., 2011; Erol and Randhir, 2013; Umbanhowar et al., 2015) and thus indirectly affects the biomass and community structure of phytoplankton and zooplankton (Dodson et al., 2005; Hoffmann and Dodson, 2005; Vanni et al., 2011), ultimately impacting the suitability of water resources for fisheries and water supplies.

In lakes, both nitrogen and phosphorus can be growth-limiting nutrients for many organisms, including bacteria, algae, and zooplankton (Elser et al., 2001; Sterner and Elser, 2002; Elser et al., 2007). Lake ecosystems respond rapidly to nutrient alterations, particularly oligotrophic lakes where a small change in absolute concentration can mean a large change in relative availability (Brahney et al., 2015). In lakes, phytoplankton form the base of food webs supporting higher trophic levels. The growth, reproduction, elemental composition, and even community structure of these primary producers strongly depends on the supplies of N and P (Sterner and Elser, 2002; Klausmeier et al., 2004). For example, relatively subtle changes in the supplies of N and P might shift phytoplankton between N- and P-limited growth (Elser et al., 2009) or primary producers might be synergistically limited by supplies of N and P (Elser et al., 2007; Harpole et al., 2011). As a result, N:P ratios are useful inferring potential nutrient limitation (N vs P) (Smith, 1983; Ptacnik et al., 2010). As argued above, grassland degradation is likely to differentially affect the N and P pools in soil, which potentially differentially affects N and P exported to a downstream lake, affecting lake trophic status depending on lake N/P limitation. Assessing nutrient limitation and stoichiometry facilitates understanding the responses of lake ecosystems to nutrient inputs and may help in mitigating the effects of changes of nutrient inputs.

Research Questions, Hypotheses, and Predictions

Overall, landcover changes not only alter terrestrial biogeochemical processes but also have strong potential to affect nutrient limitation and stoichiometry in aquatic ecosystems. Moreover, the microbial communities in terrestrial and aquatic ecosystems likely also experience strong impacts from grassland degradation, potentially influencing biogeochemical processes and microbial stoichiometry. Thus, a comprehensive study (Figure 1.3) of nutrient dynamics and stoichiometry, as well as microbial community composition and potential functions, that integrates data from terrestrial and aquatic ecosystems can provide more complete picture of the effects of grassland degradation.

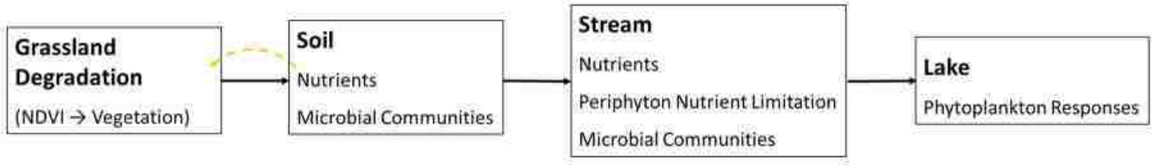

Figure 1.3 General framework of this study

Because this work was done with the help of many collaborators especially in the field sampling and laboratory analyses, I will use “we” instead of “I” in my thesis to reflect their contributions.

In **Chapter 2**, the research questions were: (1) do soil nutrient pools and stoichiometry respond to grassland degradation? By how much? In what direction? and (2) do C, N, and P concentrations and ratios in soil microbial biomass respond to grassland degradation? By how much? In what direction?

We hypothesized that (1) soil nutrient stocks and stoichiometry are affected by grassland degradation due to differential impacts on N vs. P processes, and (2) C, N, and P concentrations in soil microbial biomass and their ratios are affected by grassland degradation due to its differential impacts on N vs. P processes.

To test these hypotheses, we assessed the C, N, and P concentrations and stoichiometry in soil and soil microbial biomass in sample sites differing in grassland status (as indicated by normalized difference vegetation index, NDVI). If these hypotheses are correct, we predict that, when we assess soil nutrients and microbial stoichiometry across a gradient of grassland degradation in the Qinghai Lake basin: (1) Pools of available soil N and P will decline (Figure 1.4a) and N:P ratio will vary (increase or decrease; see different scenarios) with the degree of grassland degradation as indexed by NDVI (Figure 1.4). In Scenario 1, soil nitrogen declines more quickly than soil phosphorus with grassland degradation; as a result, soil N:P ratio will decrease with grassland degradation (Figure 1.4b). In Scenario 2, soil phosphorus is lost more quickly than soil nitrogen, as a result, soil N:P ratio will increase with grassland degradation (Figure 1.4c). (2) Soil microbial stoichiometry will vary with soil available nutrients due to impacts of grassland degradation. In Scenario 1, microbial biomass N:P will decrease with grassland degradation (Figure 1.4d). In Scenario 2, microbial biomass N:P will increase with grassland degradation (Figure 1.4e).

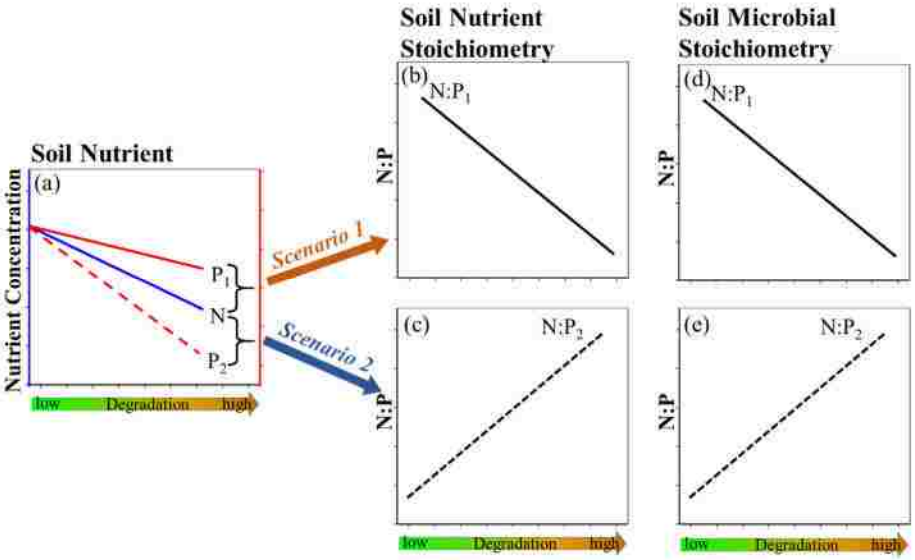

Figure 1.4 Predictions related to nutrient dynamics and stoichiometry of soil and soil microbes. (a) Nutrient concentration dynamics in soil. Scenario 1: soil nitrogen declines more severely than soil phosphorus with grassland degradation. Scenario 2: soil phosphorus declines more severely than soil nitrogen. (b-c) Responses of soil nutrient stoichiometry to grassland degradation in the two scenarios; (d-e) Responses of soil microbial stoichiometry to grassland degradation in the two scenarios.

In **Chapter 3**, our primary questions were (1) do streamwater nutrient concentrations and their stoichiometric ratios respond to grassland degradation? By how much? In what direction? and (2) do C, N, and P concentrations and stoichiometric ratios of stream biofilms respond to grassland degradation? By how much? In what direction?

We hypothesized that (1) streamwater nutrient concentrations and stoichiometric ratios are affected by grassland degradation due to differential inputs of N and P from degraded soil, (2) C, N, and P concentrations and stoichiometric ratios of stream biofilms are affected by grassland degradation due to differential changes of N and P in streamwater.

To test these hypotheses, we assessed C, N, and P concentrations and stoichiometric ratios of streamwater and stream biofilms in catchments differing in grassland status as indicated by NDVI. Based on hypothesized mechanisms described above, we predicted that, in sampling of stream and periphyton nutrients in subcatchments differing in grassland degradation, (1) in Scenario 1, streamwater N concentration will decrease more quickly than P concentration with grassland degradation (Figure 1.5a); as a result, streamwater N:P ratio will decrease with grassland degradation (Figure 1.5b). In Scenario 2, streamwater P concentrations will decrease more quickly than N concentrations (Figure 1.5a), leading to increases of N:P ratio (Figure 1.5c). (2) In Scenario 1, N:P of stream biofilms will decrease with grassland degradation (Figure 1.5d). In Scenario 2, N:P of stream biofilms will increase with grassland degradation (Figure 1.5e).

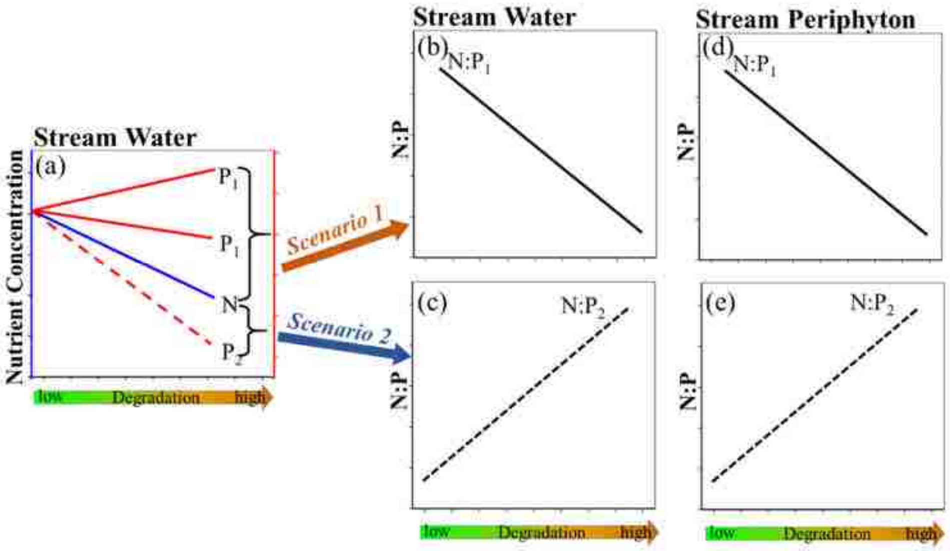

Figure 1.5 Predictions related to nutrient concentrations and stoichiometric ratios of streamwater and stream biofilms. (a) Scenario 1: Streamwater N concentration decreases more quickly than P concentration with grassland degradation. Scenario 2: Streamwater P concentration decreases more quickly than N concentration with grassland degradation. (b-c) Responses of streamwater nutrient stoichiometry to grassland degradation in the two scenarios; (d-e) Responses of stream biofilm nutrient stoichiometry to grassland degradation in the two scenarios.

In **Chapter 4**, our objectives were to address the following questions: (1) How do the concentrations, forms, and ratios of key nutrients (N, P) in streamwater respond to grassland degradation? (2) How do the impacts of grassland degradation on nutrient supply alter stream periphyton nutrient limitation? (3) How do lake phytoplankton respond to stream inflows as a function of grassland degradation? In order to clearly answer the third questions, a prerequisite question should be addressed: How do lake phytoplankton respond to different nutrient enrichments (N vs P)?

We hypothesized that: (1) Both total and dissolved inorganic nutrients are affected by grassland degradation but nitrogen and phosphorus are influenced differentially; (2) Nutrient limitation of stream periphyton is a function of degradation-associated variation in stream nutrients; (3) Lake phytoplankton are stimulated by stream inflows from different catchments as a function of their degradation status. For the prerequisite question, we hypothesized that lake phytoplankton is strongly limited by P due to very low P and relatively high N in lake water of Qinghai Lake.

To test these hypotheses, we coupled intensive analyses of different nutrient forms in streamwater with bioassay experiments for both stream periphyton and lake phytoplankton to assess the cascading influences of grassland status on nutrient dynamics and limitation of primary producers in the Qinghai Lake and its inflow streams. Based on sampling and experimentation across subcatchments in the Qinghai Lake basin that differ in their degradation status, based on these hypotheses our predictions were: (1) stream nutrient concentrations will differ systematically as a function of sub-catchment NDVI as we predicted in Chapter 3 (Figure 1.6a); (2) Periphyton will shift from P limitation to N limitation in Scenario 1 (Figure 1.6b) but will shift from N limitation to P limitation if Scenario 2 holds (Figure 1.6c); (3) Given that Qinghai Lake phytoplankton are P-limited (Figure 1.6d), we predict that, if Scenario 1 holds, lake phytoplankton will respond more strongly to streamwater from degraded catchments (Figure 1.6e). However, in Scenario 2, lake phytoplankton will respond more strongly to streamwater from relatively undegraded catchments (Figure 1.6f).

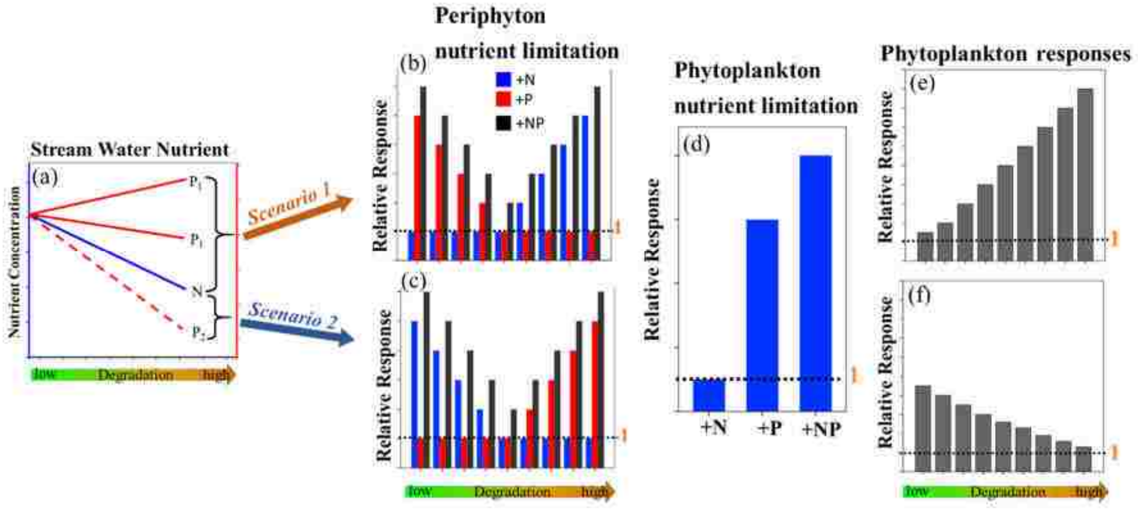

Figure 1.6 According to (a) streamwater nutrient dynamics, the predictions related to (b-c) stream periphyton nutrient limitation in Scenario 1 in which periphyton will shift from P limitation to N while, in Scenario 2, they are predicted to shift from N limitation to P

limitation. (d) Given that Qinghai Lake phytoplankton are P-limited, we predict that (e-f) phytoplankton will response addition of streamwater as a function of catchment grassland degradation depending on which of the two scenarios holds.

In **Chapter 5**, we examined the soil bacterial communities in a grassland watershed on the Qinghai-Tibet Plateau to test the hypothesis that grassland degradation affects soil microbial community structure. We assessed the bacterial communities using high-throughput 16S rRNA gene sequencing to address the following question: how do soil bacterial communities respond to grassland degradation? According to hypothesized mechanisms described above, we predicted that (1) bacterial diversity would decline with grassland degradation and concomitant environmental change, (2) different taxonomic groups would responded differentially to the concomitant environmental variations due to their evolutionary history and trophic strategy, and (3) bacterial community structure shifts would be associated with changes in soil properties.

In **Chapter 6**, we investigated the bacterial communities in stream biofilms in Qinghai Lake watershed on the northeast edge of QTP in sub-catchments that are experiencing different degrees of degradation. Our aim was to reveal the structural properties of bacterial communities in stream biofilms and to evaluate how bacterial diversity and community respond to grassland degradation. As in our studies of soil microbes, we predicted that grassland degradation and associated changes in environmental variables would be significantly associated with bacterial diversity and communities in stream biofilms in streams experiencing different levels of degradation.

Overall in this whole study, we hypothesized that grassland degradation and concomitant changes in environmental factors influence coupled terrestrial and aquatic ecosystems in terms of nutrient concentrations and stoichiometry, nutrient limitation, as well as microbial community structure. These integrated studies will provide insights into the influences of grassland degradation on terrestrial and aquatic ecosystems on the Qinghai-Tibet Plateau, and help to motivate and guide the protection and management of these fragile grassland ecosystems in a rapidly changing world.

References

Abell JM, Oezkundakci D, Hamilton DP, Miller SD. 2011. Relationships between land use and nitrogen and phosphorus in New Zealand lakes. Marine and Freshwater Research, 62: 162-175.

Abril A, Bucher EH. 1999. The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. Applied Soil Ecology, 12: 159-167.

Akiyama T, Kawamura K. 2007. Grassland degradation in China: methods of monitoring, management and restoration. Grassland Science, 53: 1-17.

Alexander RB, Smith RA, Schwarz GE. 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. Nature, 403: 758-761.

Allan JD. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics, 35: 257-284.

Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR. 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia, 54: 309-320.

Aponte C, Marañón T, García LV. 2010. Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry, 101: 77-92.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences, 110: 9374-9379.

Brahney J, Mahowald N, Ward DS, Ballantyne AP, Neff JC. 2015. Is atmospheric phosphorus pollution altering global alpine lake stoichiometry? Global Biogeochemical Cycles, 29: 1369-1383.

Bring A, Destouni G. 2009. Hydrological and hydrochemical observation status in the pan-Arctic drainage basin. Polar Research, 28: 327-338.

Burgin AJ, Hamilton SK. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment, 5: 89-96.

Canham CD, Pace ML, Weathers KC, McNeil EW, Bedford BL, Murphy L, Quinn S. 2012. Nitrogen deposition and lake nitrogen concentrations: a regional analysis of terrestrial controls and aquatic linkages. Ecosphere, 3: 1-16.

Carlson TN, Ripley DA. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. Remote Sensing of Environment, 62: 241-252.

Carr GM, Morin A, Chambers PA. 2005. Bacteria and algae in stream periphyton along a nutrient gradient. Freshwater Biology, 50: 1337-1350.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature, 397: 491-497.

Cleveland CC, Liptzin D. 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry, 85: 235-252.

Cronan CS. 2012. Biogeochemistry of the Penobscot River watershed, Maine, USA: nutrient export patterns for carbon, nitrogen, and phosphorus. Environmental Monitoring and Assessment, 184: 4279-4288.

Danger M, Leflaive J, Oumarou C, Ten-Hage L, Lacroix G. 2007. Control of phytoplankton-bacteria interactions by stoichiometric constraints. Oikos, 116: 1079-1086.

Deegan LA, Neill C, Haupert CL, Ballester MVR, Krusche AV, Victoria RL, Thomas SM, de Moor E. 2011. Amazon deforestation alters small stream structure, nitrogen biogeochemistry and connectivity to larger rivers. Biogeochemistry, 105: 53-74.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma, 235-236: 372-381.

Dodson SI, Lillie RA, Will-Wolf S. 2005. Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. Ecological Applications, 15: 1191-1198.

Dong SK, Wen L, Yi YY, Wang XX, Zhu L, Li XY. 2012. Soil-quality effects of land degradation and restoration on the Qinghai – Tibetan plateau. Soil Science Society of America Journal, 76: 2256-2264.

Drake WM, Scott JT, Evans-White M, Haggard B, Sharpley A, Rogers CW, Grantz EM. 2012. The effect of periphyton stoichiometry and light on biological phosphorus immobilization and release in streams. Limnology, 13: 97-106.

Dyksterhuis EJ. 1949. Condition and management of range land based on quantitative ecology. Journal of Range Management, 2: 104-115.

Eastwood JA, Yates MG, Thomson AG, Fuller RM. 1997. The reliability of vegetation indices for monitoring saltmarsh vegetation cover. International Journal of Remote Sensing, 18: 3901-3907.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters, 10: 1135-1142.

Elser JJ, Hayakawa K, Urabe J. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology, 82: 898-903.

Elser JJ, Kyle M, Steger L, Nydick KR, Baron JS. 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology, 90: 3062-3073.

Erol A, Randhir TO. 2013. Watershed ecosystem modeling of land-use impacts on water quality. Ecological Modelling, 270: 54-63.

Fanin N, Fromin N, Barantal S, Hattenschwiler S. 2017. Stoichiometric plasticity of microbial communities is similar between litter and soil in a tropical rainforest. Scientific Reports, 7: e12498.

Fanin N, Fromin N, Buatois B, Hättenschwiler S, Cleland E. 2013. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. Ecology Letters, 16: 764-772.

Fanta SE, Hill WR, Smith TB, Roberts BJ. 2010. Applying the light: nutrient hypothesis to stream periphyton. Freshwater Biology, 55: 931-940.

Figueiredo RO, Markewitz D, Davidson EA, Schuler AE, Dos S. Watrin O, de Souza Silva P. 2010. Land-use effects on the chemical attributes of low-order streams in the eastern Amazon. Journal of Geophysical Research, 115: 1-14.

Fitter A, Hillebrand H. 2009. Microbial food web structure affects bottom-up effects and elemental stoichiometry in periphyton assemblages. Limnology and Oceanography, 54: 2183-2200.

Frost PC, Cross WF, Benstead JP. 2005. Ecological stoichiometry in freshwater benthic ecosystems: an introduction. Freshwater Biology, 50: 1781-1785.

Gang C, Zhou W, Chen Y, Wang Z, Sun Z, Li J, Qi J, Odeh I. 2014. Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environmental Earth Sciences, 72: 4273-4282.

GB19377-2003. Parameters for degradation, sandification and salification of rangelands.

Gill RA, Burke DG, Milchunas DG, Lauenroth WK. 1999. Relationship between root biomass and soil organic matter pools in the short grass steppe of eastern Colorado. Ecosystems, 2: 226-236.

Godwin CM, Carrick HJ. 2008. Spatio-temporal variation of periphyton biomass and accumulation in a temperate spring-fed stream. Aquatic Ecology, 42: 583--595.

Griffiths BS, Spilles AA, Bonkowski M. 2012. C:N:P stoichiometry and nutrient limitation of the soil microbial biomass in a grazed grassland site under experimental P limitation or excess. Ecological Processes, 1: e6.

Hall EK, Maixner F, Franklin O, Daims H, Richter A, Battin T. 2011. Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. Ecosystems, 14: 261-273.

Hall RO, Likens GE, Malcom HM. 2001. Trophic basis of invertebrate production in 2 streams at the Hubbard Brook Experimental Forest. Journal of the North American Benthological Society, 20: 432-447.

Handa IT, Aerts R, Berendse F, Berg MP, Bruder A, Butenschoen O, Chauvet E, Gessner MO, Jabiol J, Makkonen M, McKie BG, Malmqvist B, Peeters ETHM, Scheu S, Schmid B, van Ruijven J, Vos VCA, Haettenschwiler S. 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature, 509: 218-221.

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters, 14: 852-862.

Harris J. 2009. Soil microbial communities and restoration ecology: facilitators or followers? Science, 325: 573-574.

Harris RB. 2010. Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. Journal of Arid Environments, 74: 1-12.

Hepinstall JA, Fuller RL. 1994. Periphyton reactions to different light and nutrient levels and the response of bacteria to these manipulations. Archiv für Hydrobiologie, 131: 161-173.

Heuck C, Weig A, Spohn M. 2015. Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. Soil Biology and Biochemistry, 85: 119-129.

Hilker T, Natsagdorj E, Waring RH, Lyapustin A, Wang Y. 2014. Satellite observed widespread decline in Mongolian grasslands largely due to overgrazing. Global Change Biology, 20: 418-428.

Hill WR, Rinchard J, Czesny S. 2011. Light, nutrients and the fatty acid composition of stream periphyton. Freshwater Biology, 56: 1825-1836.

Hiltbrunner D, Schulze S, Hagedorn F, Schmidt MWI, Zimmermann S. 2012. Cattle trampling alters soil properties and changes microbial communities in a Swiss subalpine pasture. Geoderma, 170: 369-377.

Hoffmann MD, Dodson SI. 2005. Land use, primary productivity, and lake area as descriptors of zooplankton diversity. Ecology, 86: 255-261.

Holmes RM, McClelland JW, Peterson BJ, Tank SE, Bulygina E, Eglinton TI, Gordeev VV, Gurtovaya TY, Raymond PA, Repeta DJ, Staples R, Striegl RG, Zhulidov AV, Zimov SA. 2012. Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic ocean and surrounding seas. Estuaries and Coasts, 35: 369-382.

Ishida CK, Arnon S, Peterson CG, Kelly JJ, Gray KA. 2008. Influence of algal community structure on denitrification rates in periphyton cultivated on artificial substrata. Microbial Ecology, 56: 140-152.

Jin LY, Ganopolski A, Chen FH, Claussen M, Wang HJ. 2005. Impacts of snow and glaciers over Tibetan Plateau on Holocene climate change: Sensitivity experiments with a coupled model of intermediate complexity. Geophysical Research Letters, 32: L1770917.

Klausmeier CA, Litchman E, Daufresne T, Levin SA. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature, 429: 171-174.

Kohler TJ, Murdock JN, Gido KB, Dodds WK. 2011. Nutrient loading and grazing by the minnow *Phoxinus erythrogaster* shift periphyton abundance and stoichiometry in mesocosms. Freshwater Biology, 56: 1133-1146.

Larned ST. 2010. A prospectus for periphyton: recent and future ecological research. Journal of the North American Benthological Society, 29: 182-206.

Li B. 1997. The rangeland degradation in North China and its preventive strategy. Scientia Agriculture Sinica, 30: 1-9.

Li P, Yang Y, Han W, Fang J. 2014. Global patterns of soil microbial nitrogen and phosphorus stoichiometry in forest ecosystems. Global Ecology and Biogeography, 23: 979-987.

Li S, Verburg PH, Lv S, Wu J, Li X. 2012a. Spatial analysis of the driving factors of grassland degradation under conditions of climate change and intensive use in Inner Mongolia, China. Regional Environmental Change, 12: 461-474.

Li XL, Gao J, Brierley G, Qiao YM, Zhang J, Yang YW. 2013. Rangeland degradation on the Qinghai‐Tibet plateau: Implications for rehabilitation. Land Degradation and Development, 24: 72-80.

Li XY, Ma YJ, Xu HY, Wang JH, Zhang DS. 2009. Impact of land use and land cover change on environmental degradation in Lake Qinghai watershed, northeast Qinghai-Tibet Plateau. Land Degradation and Development, 20: 69-83.

Li Y, Wu J, Liu S, Shen J, Huang D, Su Y, Wei W, Syers JK. 2012b. Is the C: N: P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? Global Biogeochemical Cycles, 26: 1-14.

Luo CF, Xu CJ, You HY, Jin SH. 2013. Analysis on grassland degradation in Qinghai Lake Basin during 2000-2010. Acta Ecologica Sinica, 33: 4450-4459 (In Chinese).

Manzoni S, Taylor P, Richter A, Porporato A, Agren GI. 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. New Phytologist, 196: 79-91.

Mayer MS, Likens GE. 1987. The importance of algae in a shaded headwater stream as food for an abundant caddisfly (Trichoptera). Journal of the North American Benthological Society, 6: 262-269.

Mchunu C, Chaplot V. 2012. Land degradation impact on soil carbon losses through water erosion and CO_2 emissions. Geoderma, 79: 177-178.

Mekuria W, Veldkamp E, Halle M, Nyssen J, Muys B, Gebrehiwota K. 2007. Effectiveness of exclosures to restore degraded soils as a result of overgrazing in Tigray, Ethiopia. Journal of Arid Environments, 69: 270-284.

Meurant G. 2012. The ecology of natural disturbance and patch dynamics. Academic press.

Mooshammer M, Wanek W, Schnecker J, Wild B, Leitner S, Hofhansl F, Bloechl A, Haemmerle I, Frank AH, Fuchslueger L, Keiblinger KM, Zechmeister-Boltenstern S, Richter A. 2012. Stoichiometric controls of nitrogen and phosphorus cycling in decomposing beech leaf litter. Ecology, 93: 770-782.

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature, 452: 202-205.

Ng EL, Patti AF, Rose MT, Schefe CR, Wilkinson K, Cavagnaro TR. 2014. Functional stoichiometry of soil microbial communities after amendment with stabilised organic matter. Soil Biology and Biochemistry, 76: 170-178.

O'Brien PJ, Wehr JD. 2010. Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. Hydrobiologia, 657: 89-105.

Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD. 2001. Control of nitrogen export from watersheds by headwater streams. Science, 292: 86-90.

Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones CM, Sarr A, Maron P. 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME Journal, 7: 1609-1619.

Pickett ST. 1989. Space-for-time substitution as an alternative to long-term studies. In Long-term studies in ecology. Springer, New York, NY.

Ptacnik R, Andersen T, Tamminen T. 2010. Performance of the Redfield ratio and a family of nutrient limitation indicators as thresholds for phytoplankton N vs. P limitation. Ecosystems, 13: 1201-1214.

Qiu J. 2008. China: The third pole. Nature, 454: 393-396.

Ren Z, Jiang ZY, Cai QH. 2013. Longitudinal patterns of periphyton biomass in Qinghai-Tibetan Plateau streams: An indicator of pasture degradation? Quaternary International, 313: 92-99.

Ren Z, Niu DC, Ma PP, Wang Y, Fu H, Elser JJ. 2019. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. Ecology, 100: e02755.

Riley RH, Townsend CR, Niyogi DK, Arbuckle CA, Peacock KA. 2003. Headwater stream response to grassland agricultural development in New Zealand. New Zealand Journal of Marine and Freshwater Research, 37: 389-403.

Ruiz-Sinoga JD, Romero Diaz A. 2010. Soil degradation factors along a Mediterranean pluviometric gradient in Southern Spain. Geomorphology, 118: 359-368.

Sadro S, Nelson CE, Melack JM. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. Ecosystems, 15: 363-386.

Schiller DV, Marti E, Riera JL, Sabater F. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. Freshwater Biology, 52: 891-906.

Schlesinger WH, Bernhardt ES. 2013. Biogeochemistry: an analysis of global change. Academic press, San Diego, California, USA.

Scott JT, Back JA, Taylo JM, King RS. 2008. Does nutrient enrichment decouple algal-bacterial production in periphyton? Journal of the North American Benthological Society, 27: 332-344.

Scurlock J, Hall DO. 1998. The global carbon sink: a grassland perspective. Global Change Biology, 4: 229-233.

Smith VH. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science, 221: 669-671.

Smucker NJ, Detenbeck NE, Morrison AC. 2013. Diatom responses to watershed development and potential moderating effects of near-stream forest and wetland cover. Freshwater Science, 32: 230-249.

Sousa WP. 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics, 15: 353-391.

Steffens M, Kölbl A, Totsche KU, Kögel-Knabner I. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). Geoderma, 143: 63-72.

Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton.

Su X, Wu Y, Dong S, Wen L, Li Y, Wang X. 2015. Effects of grassland degradation and re-vegetation on carbon and nitrogen storage in the soils of the Headwater Area Nature Reserve on the Qinghai-Tibetan Plateau, China. Journal of Mountain Science, 12: 582-591.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the World. No. 34. Food and Agriculture Organization of the United Nations. Rome, Italy.

Swift MJ, Izac AM, van Noordwijk M. 2004. Biodiversity and ecosystem services in agricultural landscapes - are we asking the right questions. Agriculture, Ecosystems and Environment, 104: 113-134.

Tan K, Ciais P, Piao S, Wu X, Tang Y, Vuichard N, Liang S, Fang J. 2010. Application of the ORCHIDEE global vegetation model to evaluate biomass and soil carbon stocks of Qinghai-Tibetan grasslands. Global Biogeochemical Cycles, 24: GB1013.

Teittinen A, Taka M, Ruth O, Soininen J. 2015. Variation in stream diatom communities in relation to water quality and catchment variables in a boreal, urbanized region. Science of the Total Environment, 530: 279-289.

Thiam AK. 2003. The causes and spatial pattern of land degradation risk in southern Mauritania using multitemporal AVHRR-NDVI imagery and field data. Land Degradation and Development, 14: 133-142.

Tischer A, Potthast K, Hamer U. 2014. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. Oecologia, 175: 375-393.

Townsend CR, Riley RH. 1999. Assessment of river health: accounting for perturbation pathways in physical and ecological space. Freshwater Biology, 41: 393-405.

Umbanhowar C, Camill P, Edlund M, Geiss C, Henneghan P, Passow K. 2015. Lake-landscape connections at the forest-tundra transition of northern Manitoba. Inland Waters, 5: 57-74.

van der Heijden MGA, Bardgett RD, van Straalen NM. 2008. The unseen majority: soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. Ecology Letters, 11: 296-310.

Vanni MJ, Renwick WH, Bowling AM, Horgan MJ, Christian AD. 2011. Nutrient stoichiometry of linked catchment-lake systems along a gradient of land use. Freshwater Biology, 56: 791-811.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences, 37: 130-137.

Viragh K, Toth T, Somodi I. 2011. Effect of slight vegetation degradation on soil properties in *Brachypodium pinnatum* grasslands. Plant and Soil, 345: 303-313.

Walker CE, Pan YD. 2006. Using diatom assemblages to assess urban stream conditions. Hydrobiologia, 561: 179-189.

Walker TW, Syers JK. 1976. The fate of phosphorus during pedogenesis. Geoderma, 15: 1-19.

Wang X, Dong S, Yang B, Li Y, Su X. 2014. The effects of grassland degradation on plant diversity, primary productivity, and soil fertility in the alpine region of Asia's headwaters. Environmental Monitoring and Assessment, 186: 6903-6917.

Wen L, Dong S, Li Y, Wang X, Li X, Shi J, Dong Q. 2013. The impact of land degradation on the C pools in alpine grasslands of the Qinghai-Tibet Plateau. Plant and Soil, 368: 329-340.

Williamson CE, Dodds W, Kratz TK, Palmer MA. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment, 6: 247-254.

Wu R, Tiessen H. 2002. Effect of land use on soil degradation in alpine grassland soil, China. Soil Science Society of America Journal, 66: 1648-1655.

Yao T, Thompson LG, Mosbrugger V, Zhang F, Ma Y, Luo T, Xu B, Yang X, Joswiak DR, Wang W, Joswiak ME, Devkota LP, Tayal S, Jilani R, Fayziev R. 2012. Third Pole Environment (TPE). Environmental Development, 3: 52-64.

Yengoh GT, Dent D, Olsson L, Tengberg A, Tucker C. 2014. The use of the Normalized Difference Vegetation Index (NDVI) to assess land degradation at multiple scales a review of the current status, future trends, and practical considerations. Lund University Center for Sustainability Studies (LUCSUS), and the Scientific and Technical Advisory Panel of the Global Environment Facility (STAP/GEF). Lund, Sweden.

Zhang T. 2011. Distance-decay patterns of nutrient loading at watershed scale: Regression modeling with a special spatial aggregation strategy. Journal of Hydrology, 402: 239-249.

Zhang ZS, Song XL, Lu XG, Xue ZS. 2013. Ecological stoichiometry of carbon, nitrogen, and phosphorus in estuarine wetland soils: influences of vegetation coverage, plant communities, geomorphology, and seawalls. Journal of Soils and Sediments, 13: 1043-1051.

Zhao F, Sun J, Ren C, Kang D, Deng J, Han X, Yang G, Feng Y, Ren G. 2015. Land use change influences soil C, N, and P stoichiometry under 'Grain-to-Green Program' in China. Scientific Reports, 5: e10195.

Zhao W, Yang P, Li H, Hu G, Liu X. 2011. Characteristics of soil erosion, nitrogen and phosphorus losses under three grassland use patterns in Hulun Lake watershed. Transactions of the Chinese Society of Agricultural Engineering, 27: 220-225.

Zhao YZ, Zou XY, Cheng H, Jia HK, Wu YQ, Wang GY, Zhang CL, Gao SY. 2006. Assessing the ecological security of the Tibetan plateau: methodology and a case study for Lhaze County. Journal of Environmental Management, 80: 120-131.

Zhu L. 2011. Study on the variation of reproduction and soil nutrient of cultivated and degraded alpine grassland in the headwater areas of Qinghai-Tibetan Plateau, China. Masters' thesis. Beijing Normal University, Beijing, China.

Chapter 2 Effects of grassland degradation on ecological stoichiometry of soil ecosystems on the Qinghai-Tibet Plateau

Abstract

Grasslands across the world are being degraded due to impacts of overgrazing and climate change. However, the influences of grassland degradation on carbon (C), nitrogen (N), and phosphorus (P) dynamics and stoichiometry in soil ecosystems are not well studied, especially at high elevations where ongoing climate change is most pronounced. This study sought to assess the responses of these soil elements to grassland degradation in the Qinghai Lake watershed on the Qinghai-Tibet Plateau. Substituting space for time, we quantified normalized difference vegetation index (NDVI) to gauge grassland degradation. C, N, and P concentrations and their molar ratios in soil and in soil microbial biomass were also measured. The results showed that grassland degradation decreased the concentrations of C and N, as well as the ratios of C:P and N:P, in soil. The average soil C:N:P ratio was 144:10:1. Moreover, C, N, and P concentrations in soil microbial biomass decreased with increased grassland degradation. C:N:P ratios of soil microbial biomass were highly constrained with an average value of 49:8:1, suggesting that soil microorganisms exhibited strong homeostatic behavior while variations of microbial biomass C:N:P ratios suggest changes in microbial activities and community structure. Overall, the soil became relatively more P rich and thus N limitation is anticipated to be more apparent with grassland degradation. This study provides insights from a stoichiometric perspective into microbial and biogeochemical responses of grassland ecosystems as they undergo degradation on the Qinghai-Tibet Plateau.

Keywords: microbial biomass, homeostasis, grassland degradation, stoichiometry, NDVI

Introduction

Grasslands are one of the largest ecosystems in the world, covering ~40% of Earth's land surface excluding Antarctica and Greenland (Suttie et al., 2005). However, grasslands are under pressure of degradation throughout the world (Gang et al., 2014). Grassland degradation involves a process of retrogressive succession (Dyksterhuis, 1949; Li, 1997) that reduces the ability of grasslands to carry out their key functions in providing ecosystem goods and services (Daily, 1995; Wick et al., 2016). As grassland degradation proceeds, aboveground vegetation coverage and biomass decline, leading to simultaneous impairment of soil physical, chemical, and biological properties (Abril and Bucher, 1999; Viragh et al., 2011; Li et al., 2012a; Dlamini et al., 2014). Consequently, the biogeochemical cycles of carbon (C), nitrogen (N), and phosphorus (P) are impacted, altering the structure and function of the grassland (Schlesinger and Bernhardt, 2013). On the Qinghai-Tibet Plateau, grassland is the dominant landcover (Tan et al., 2010) and is experiencing serious degradation due to its sensitivity and vulnerability to external disturbances such as livestock grazing (Harris, 2010).

In grassland soil, large proportions of organic carbon and nutrients stocks are concentrated in the upper layers (Gill et al., 1999) but the sources of those elements differ. Plants are the major source of organic C and N-fixing microbes are the major source of N (but see Houlton et al., 2018) while weathering of primary rock minerals provides the dominant source of P (Walker and Syers, 1976; Chadwick et al., 1999). Thus, vegetation cover, plant community composition, geomorphology, as well as soil texture and geology, potentially affect carbon and nutrient pools in soil (Aponte et al., 2010; Li et al., 2012b). Previous studies have consistently demonstrated an overall reduction in stocks of soil organic matter and nitrogen in degraded grasslands (Wu and Tiessen, 2002; Steffens et al., 2008). Due to the different biogeochemical processes associated with different elements (C, N, and P; Walker and Syers, 1976; Sterner and Elser, 2002), we can expect that grassland degradation would differentially affect rates of accumulation or loss of C, N and P. Given these multi-element impacts, a framework is required to help understand the relative changes of these elements in soil and soil microbial biomass as well as their interactions under the threat of grassland degradation. The conceptual framework of ecological stoichiometry provides such an approach as it deals explicitly with the interactions of multiple chemical elements in ecological interactions and processes (Elser et al., 2000; Sterner and Elser, 2002).

However, the influences of grassland degradation on element dynamics and stoichiometry of soil ecosystems are not well studied, especially on the Qinghai-Tibet Plateau.

Grassland degradation also drives changes of soil texture and depletion of soil organic matter and nutrients that can affect microbial activities and communities (Abril and Bucher, 1999; Mekuria et al., 2007). Microbial demand and use of resources are driven by the elemental stoichiometry of their own biomass and the efficiency of their growth but can also be affected by the relative supplies of available resources (Sterner and Elser, 2002; Elser et al., 2003). Organism growth is often limited by supplies of key elements while biota can themselves reciprocally affect the availability of nutrients in the environment (Sterner and Elser, 2002). Previous study has shown that C, N, and P concentrations of soil microbial biomass can vary by several orders of magnitude but their ratios are relatively constrained (Sterner and Elser, 2002; Cleveland and Liptzin, 2007), likely because most soil microorganisms are heterotrophic and assumed to exhibit stronger homeostatic regulation (relative to photoautotrophic organisms) at the organismal level (Sterner and Elser, 2002). Thus, in order to better understand biogeochemical cycles in grassland ecosystems on the Qinghai-Tibet Plateau, it is essential to document and understand the effects of grassland degradation on the C:N:P stoichiometry of soil microbes themselves, as well as on the stoichiometric relationships between soil environments and microbes.

In this study, we aim to address the following questions: (1) do soil nutrient pools and stoichiometry respond to grassland degradation? By how much? In what direction? and (2) consequently, do C, N, and P concentrations and ratios in soil microbial biomass respond to grassland degradation? By how much? In what direction? Based on the mechanisms discussed above, we hypothesized that (1) soil nutrient stocks and stoichiometry are affected by grassland degradation due to its differential impacts on N vs. P processes, and (2) C, N, and P concentrations in soil microbial biomass and their ratios are affected by grassland degradation due to differential impacts on N vs. P processes. To test these hypotheses, we assessed the C, N, and P concentrations and stoichiometry in soil and soil microbial biomass in sample sites differing in grassland status as indicated by normalized difference vegetation index (NDVI). Based on these hypotheses, we predicted that, in sampling soils across a strong gradient of grassland degradation (as indexed by NDVI): (1)

Pools of available soil N and P will be reduced and N:P ratio will vary (increase or decrease; see different scenarios) with the degree of grassland degradation as indexed by NDVI. In one scenario (Scenario 1, hereafter), soil nitrogen declines more strongly than soil phosphorus with grassland degradation (because degradation strongly impairs the ability of microbes and vegetation to fix and hold N); as a result, soil N:P ratio will decrease with grassland degradation. In the second scenario (Scenario 2, hereafter), soil phosphorus is lost more readily than soil nitrogen (because degradation strongly amplifies erosive loss of particle-bound P), as a result, soil N:P ratio will increase with grassland degradation. (2) Soil microbial stoichiometry will vary with soil available nutrients due to impacts of grassland degradation according to which scenario holds. In Scenario 1, microbial biomass N:P will decrease with grassland degradation. In Scenario 2, microbial biomass N:P will increase with grassland degradation.

Methods

Study area

This study was conducted in the Qinghai Lake watershed (36°15′ - 38°20′ N and 97°50′ - 101°20′ E, Figure 2.1). Qinghai Lake is the largest lake in China located on northeast of the Qinghai-Tibet Plateau with a surface altitude of 3,194 m above sea level, an average depth of 21 m, a surface area of 4,260 km^2, and a catchment area of 29,660 km^2 (Li et al., 2007). In the Qinghai Lake watershed, the air temperature ranges from -31°C in January to 28°C in July, with the mean annual temperature of -0.7°C (Yi et al., 2010). The mean annual precipitation is 362.5 mm and the annual evapotranspiration is 1,300-2,000 mm (Yi et al., 2010). Grassland covers 75% of land area in the watershed while around half of the grassland is degraded due to overgrazing and climate change (Luo et al., 2013).

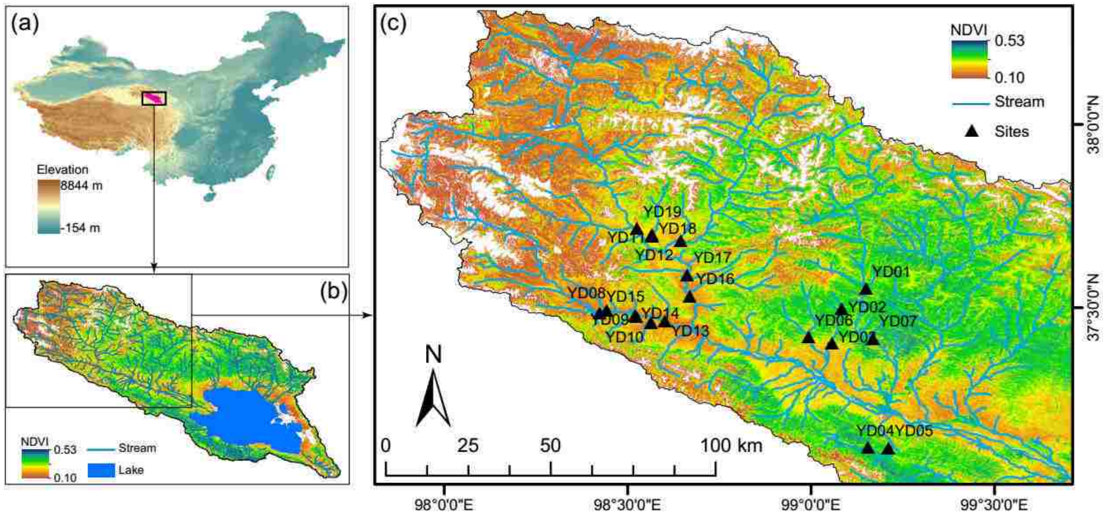

Figure 2.1 Maps of study area and sample sites. (a) Location of the Qinghai Lake watershed (the map was colored by elevation). (b) The distribution of the normalized difference vegetation index (NDVI) across the watershed (the map was colored by NDVI). (c) The distribution of the 19 sampling sites (the map was colored by NDVI). The maps were created using ArcGIS 14.0. NDVI value was calculated using Landsat images download from USGS (https://earthexplorer.usgs.gov/)

Field sampling and chemical analyses

Based on grassland phenology, field sampling was conducted in three seasons: peak growing season in mid-July 2017, late growing season in mid-September 2017, and early growing season in late-April 2018. In each season, soil samples were collected from 19 sites (Figure 2.1). In each soil site, three plots (1 m × 1 m) were established with 15-m intervals. In each plot, topsoil (0-10 cm in depth) was collected from five points (four vertices and one center) by drill (10 cm in diameter) and pooled into one composite sample. The drill was washed with sterile water and air dried before each sampling. The composite sample was sieved through a 2-mm sieve to remove all visible roots, residues, and stones. The sieved sample was stored at 4°C for the measurement of C, N, and P concentrations in soil and soil microbial biomass, as well as other physicochemical properties. Soil pH was measured in 1:2.5 (mass to volume) aqueous extract in distilled water using a pH meter. Soil moisture was gravimetrically measured with 10 g fresh soil dried at 105±1°C for 48 h. Soil organic carbon (SOC) was measured by oxidizing with potassium dichromate (HJ615-

2011). Soil total nitrogen (TN) was determined by the modified Kjeldahl method (HJ717-2014). Soil total phosphorus (TP) was determined by the spectrophotometric ammonium molybdate-ascorbic acid method. Microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and microbial biomass phosphorus (MBP) were determined using the chloroform fumigation extraction method (Brookes et al., 1985; Vance et al., 1987).

Normalized difference vegetation index and "space for time" substitution

The normalized difference vegetation index (NDVI) generally exhibits close relationships with coverage and biomass of above-ground vegetation (Carlson and Ripley, 1997; Eastwood et al., 1997) and is widely used to estimate regional vegetation status and to indicate grassland degradation (Thiam, 2003; Akiyama and Kawamura, 2007; Li et al., 2013; Hilker et al., 2014). Vegetation status can be quantified using NDVI by measuring the difference between the reflectance of near-infrared light (NIR) and red light (RED): NDVI=(NIR-RED)/(NIR+RED) (Rouse et al., 1973). NDVI value of 0~0.1 represents bare areas such as soil, rock, and sand. Negative NDVI represents water bodies. In this study, Landsat images acquired in July 2017 were downloaded from USGS (https://earthexplorer.usgs.gov/) and used to calculate and visualize NDVI using ArcGIS 14.0. The NDVI value for each soil sample site is the the average NDVI in a 300-m radius buffer. NDVI values of July 2017 were used to represent grassland status in 2017 (Figure 2.1b). High values of NDVI indicate healthy vegetation while low NDVI is indicative of degraded conditions.

Indeed, in our study area, NDVI had strong relationships with *in situ* measured aboveground biomass, fractional vegetation cover, and grass species richness (Ren et al., 2019). Moreover, grassland vegetation factors (including aboveground biomass, fractional vegetation cover, grass species richness, and NDVI) were not closely associated with elevation (Ren et al., 2019). In addition, NDVI also exhibited large spatial variation across our study region. The relationship between NDVI difference and geographic distance of paired sample sites was statistically significant (P=0.002) but relatively weak (R^2=0.039), suggesting that spatial non-independence should not interfere strongly in our space-for-time approach. This spatial variation permits use of "space-for-time" substitution (Figure

2.2) to study potential impacts of grassland degradation by comparing sampling sites differing in NDVI. “Space-for-time” substitution is an alternative approach to study long-term phenomena in ecology (Pickett, 1989; Blois et al., 2013). It has been used widely and successfully, especially in systems that experience strong successional dynamics (Pickett, 1989). In this approach, temporal processes (past or future trajectories) of ecosystems are studied and inferred via contemporary spatial patterns (Blois et al., 2013) (Figure 2.2). A good example for the application of “space-for-time” substitution is in study of the impact of natural disturbance on ecosystems (Pickett, 1989), an inherently long-term process. However, systematic long-term studies are usually absent (Sousa, 1984). Thus, spatial distribution of disturbances has been widely used to construct disturbance temporal regimes (Pickett, 1989). A variety of studies using the “space-for-time” substitution approach indicate that the approach provides consistent estimates of realistic long-term variations (Meurant, 2012; Blois et al., 2013). However, intensive and systematic long-term studies of grassland degradation are lacking. Thus, following the “space-for-time” substitution approach, spatial patterns of grassland status were used to study the impacts of grassland degradation in Qinghai Lake watershed in this study (Figure 2.2).

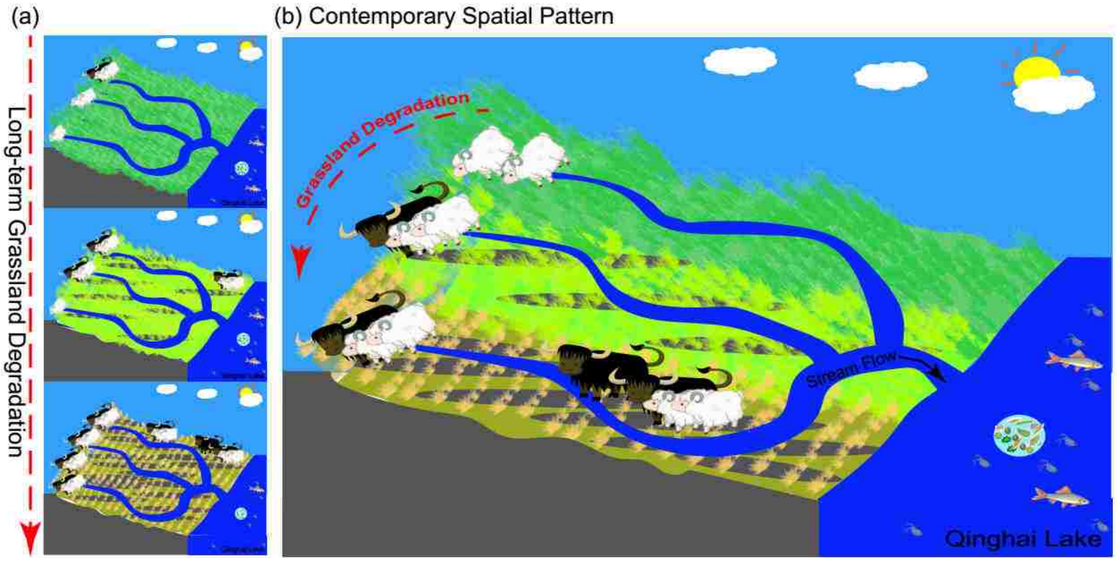

Figure 2.2 “Space-for-Time” substitution of grassland degradation in Qinghai Lake watershed. (a) Long-term grassland degradation process. (b) Contemporary spatial pattern of grassland status.

Analyses

To test these hypotheses and predictions, stoichiometric ratios were first calculated as molar ratios from data on C, N, and P contents or concentrations. To test the hypothesis that soil nutrient stocks and stoichiometry are affected by grassland degradation (as indicated by NDVI), standard major axis (SMA) regression and curve estimation were used to test for statistical associations of NDVI with SOC, TN, and TP concentrations and their ratios in soil for each season (sampling event). We predicted that, in Scenario 1, soil nitrogen declines more quickly than soil phosphorus with grassland degradation, as a result, soil N:P ratio will decrease with grassland degradation. In Scenario 2, soil phosphorus is lost more quickly than soil, as a result, soil N:P ratio will increase with grassland degradation. If the regressions are significantly positive, then Scenario 1 holds, and the hypothesis is supported. If the regressions are significantly negative, then Scenario 2 holds, and the hypothesis is also supported. If the regressions are non-significant, then the hypothesis is not supported, indicating that grassland degradation does not affect soil nutrient pools or stoichiometry. To test the hypothesis that C, N, and P concentrations in soil microbial biomass and their ratios are affected by grassland degradation, standard major axis (SMA) regression and curve estimation were used to test associations of NDVI with C, N, and P concentrations and their ratios in soil microbial biomass for each season (sampling event). We predicted that in Scenario 1, microbial biomass N:P will decrease with grassland degradation. In Scenario 2, microbial biomass N:P will increase with grassland degradation. If the regressions are significantly positive, then Scenario 1 holds, and the hypothesis is supported. If the regressions are significantly negative, then Scenario 2 holds, and the hypothesis is also supported. If the regressions are non-significant, then the hypothesis is not supported, indicating that grassland degradation does not affect microbial stoichiometry. In addition, standard major axis (SMA) regression and curve estimation were used to test the relationships between NDVI versus soil moisture and pH, as well as between microbial biomass carbon versus soil moisture and pH to assess potential influences of grassland degradation on soil moisture and pH and their influences on soil microbial biomass. The Akaike information criterion (AIC) was used for selecting the best regression model. The lower AIC (less information loss) of a model, the higher quality of that model. P-values were adjusted using FDR method (Benjamini and Hochberg, 1995). Moreover, to determine the relationships among C, N, and P concentrations in soil and soil microbial biomass, standard major axis (SMA) regression was conducted according to the log-log function: $\log_{10}(y) = a \times \log_{10}(x) + b$ (Warton et al., 2006), which is commonly used for bivariate line-fitting in biological studies. The slope was compared to 1. If the slope does not significantly differ from 1, then y and x have an isometric relationship; otherwise, y and x have a non-isometric relationship. Standard major axis regression was conducted using the R package SMART 3 (Warton et al., 2012). All the analyses were carried out in R 3.4.1 (R Core Team, 2017).

Results

Summary of C, N, and P concentrations and stoichiometric ratios in soil and soil microbial biomass

Soil C, N, and P concentrations varied substantially across space in the basin. For soil samples across all sites, SOC ranged from 809 to 13,708 mmol/kg, TN ranged from 40 to 942 mmol/kg, and TP ranged from 14 to 37 mmol/kg (Table 2.1). SOC and TN had high variability (coefficient of variation, CV: 73.6% and 71.0%, respectively, Table 2.1) while TP had a low CV (17.91%, Table 2.1). The ratios of SOC:TN, SOC:TP, and TN:TP varied from 12 to 21, 41 to 396, and 2 to 27, respectively (Table 2.1). On average, the SOC:TN:TP ratio was 144:10:1 for the soils in our study (Table 2.1). The variation in SOC:TN (CV=15.3%) was much lower than that of SOC:TP (CV=57.0%) and TN:TP (CV=55.0%) (Table 2.1).

C, N, and P concentrations of soil microbial biomass also showed substantial spatial variation. For soil microbial biomass across all sites, MBC ranged from 4.08 to 151 mmol/kg, MBN ranged from 1.55 to 18.4 mmol/kg, and MBP ranged from 0.12 to 3.77 mmol/kg (Table 2.1). MBC, MBN, and MBP had high variabilities (CV: 70.3%, 54.6%, and 73.4%, respectively, Table 2.1). The ratios of MBC:MBN, MBC:MBP, and MBN:MBP varied from 2 to 14, 16 to 174, and 2 to 10, respectively (Table 2.1). The average stoichiometric ratio of MBC:MBN:MBP was 49:8:1 (Table 2.1).MBC:MBP had higher variabilities (CV=58.9%) than MBN:MBP (CV=41.8%) and MBC:MBN (CV=37.8%).

Table 2.1 Basic physicochemical parameters of soil samples. The stoichiometric ratios were calculated on a molar basis.

	Minimum	Maximum	Mean	Std. Deviation	CV%
SOC (mmol/kg)	808.6	13708	3675	2704	73.57
TN (mmol/kg)	40.23	942.1	248.4	176.3	70.98
TP (mmol/kg)	13.61	37.19	24.52	4.39	17.91
SOC:TN	11.52	20.80	14.94	2.28	15.26
SOC:TP	41.28	396.4	143.5	81.81	56.99
TN:TP	2.05	27.24	9.67	5.31	54.96
MBC (mmol/kg)	4.08	151.4	40.93	28.78	70.32
MBN (mmol/kg)	1.55	18.41	6.70	3.66	54.63
MBP (mmol/kg)	0.12	3.77	0.98	0.72	73.45
MBC:MBN	1.71	14.27	6.13	2.31	37.75
MBC:MBP	16.37	174.4	49.12	28.91	58.86
MBN:MBP	2.95	20.13	8.17	3.42	41.78
Moisture (%)	2.60	99.13	24.70	20.44	82.75
pH	6.58	9.08	8.14	0.45	5.56

Hypothesis test: grassland degradation affects C, N, and P concentrations and stoichiometry in soil

If the grassland degradation hypothesis is correct, then we predict that the concentrations of soil C, N, and P would be associated with NDVI across the study sites. The results showed that SOC and TN significantly decreased with decreased NDVI (except TN in September, Table 2.2). However, TP did not significantly change with NDVI (Table 2.2). These results are generally consistent with the hypothesis under Scenario 1.

If the grassland degradation hypothesis is correct, then we predict that C:N:P ratios would be associated with NDVI across the study sites. The results showed significant declines in SOC:TP and TN:TP with decreasing NDVI (except TN:TP in September, Table 2.2). SOC:TN also significantly decreased with decreasing NDVI in July (Table 2.2). These results are also consistent with the hypothesis under Scenario 1.

Table 2.2 Summary of regression models of the relationships between NDVI (independent variable) versus soil nutrient concentrations and their ratios (dependent variables). P-values were adjusted using FDR method. Statistically significant results are indicated by bold face.

Dependent Variable	Season	Function	R^2	P	AIC
SOC	April	$y = 31260x - 4729$	0.243	**0.032**	279
		$y = -2394x^{-1} + 12804$	0.238	**0.034**	358
	July	$y = 29857x - 4853$	0.273	**0.022**	274
		$y = -2284x^{-1} + 11881$	0.267	**0.024**	353
	September	$y = 26017x - 3781$	0.227	**0.039**	274
		$y = -2032x^{-1} + 10957$	0.232	**0.037**	352
TN	April	$y = 1947x - 270$	0.199	0.055	178
		$y = -148x^{-1} + 816$	0.191	**0.008**	257
	July	$y = 1795x - 265$	0.249	**0.030**	170
		$y = -137x^{-1} + 737$	0.241	**0.033**	249
	September	$y = 1386x - 156$	0.167	0.082	170
		$y = -108x^{-1} + 627$	0.169	0.081	248
TP	April	$y = 25.5x + 15.5$	0.069	0.277	36.7
		$y = -1.67x^{-1} + 28.8$	0.050	0.358	115
	July	$y = 17.4x + 21.3$	0.056	0.330	26.5
		$y = -1.09x^{-1} + 30.2$	0.037	0.433	105
	September	$y = 16.1x + 20.2$	0.028	0.497	37.5
		$y = -0.917x^{-1} + 28.1$	0.015	0.618	116
SOC:TN	April	$y = 17.9x + 9.53$	0.307	**0.017**	-8.99
		$y = -1.45x^{-1} + 19.9$	0.338	**0.011**	63.9
	July	$y = 7.82x + 12.8$	0.019	0.577	17.7
		$y = -0.672x^{-1} + 17.5$	0.023	0.535	95.9
	September	$y = 17.8x + 10.3$	0.111	0.163	13.1
		$y = -1.50x^{-1} + 20.7$	0.132	0.126	91.0
SOC:TP	April	$y = 992x - 111$	0.299	**0.015**	143
		$y = -77.9x^{-1} + 452$	0.308	**0.014**	221
	July	$y = 963.45x - 141$	0.307	**0.014**	141
		$y = -74.7x^{-1} + 403$	0.309	**0.013**	219
	September	$y = 850x - 103$	0.262	**0.025**	140
		$y = -68.3x^{-1} + 386$	0.282	**0.019**	218
TN:TP	April	$y = 60.6x - 5.66$	0.233	**0.037**	42.9
		$y = -4.71x^{-1} + 28.6$	0.234	**0.036**	121
	July	$y = 57x - 7.24$	0.276	**0.021**	36.1
		$y = -4.41x^{-1} + 24.9$	0.277	**0.021**	115
	September	$y = 44.4x - 3.46$	0.190	0.062	36.0
		$y = -3.56x^{-1} + 22.1$	0.204	0.052	114

Hypothesis test: grassland degradation affects C, N, and P concentrations and stoichiometry in soil microbial biomass

If the grassland degradation hypothesis is correct, then we predict that the concentrations of C, N, and P in soil microbial biomass would be associated with NDVI across the study sites. The results showed that MBC (except in July), MBN (except in September), and MBP (except in September) significantly declined with decreasing NDVI (Table 2.3). These results are generally consistent with the hypothesis under Scenario 1.

If the grassland degradation hypothesis is correct, then we predict that MBC:MBN:MBP ratios would be associated with NDVI across the study sites. The results showed that only MBC:MBN in September decreased significantly with decreasing NDVI (Table 2.3) while MBC:MBP and MBN:MBP did not significantly change with NDVI (Table 2.3). These results did not match the predictions.

Table 2.3 Summary of regression models of the relationships between NDVI (independent variable) versus nutrient concentrations in soil microbial biomass and their ratios (dependent variables). P-values were adjusted using FDR method. Statistically significant results are indicated by bold face.

Dependent Variable	Season	Function	R^2	P	AIC
MBC	April	$y = 304x - 45.3$	0.181	0.070	110
		$y = -22.4x^{-1} + 122$	0.163	**0.016**	189
	July	$y = 128x + 7.39$	0.051	0.355	104
		$y = -9.82x^{-1} + 79.2$	0.050	0.358	183
	September	$y = 232x - 25.0$	0.161	**0.013**	103
		$y = -17.22x^{-1} + 103$	0.148	0.104	181
MBN	April	$y = 36.6x - 3.49$	0.142	0.111	35.2
		$y = -2.66x^{-1} + 16.5$	0.126	**0.018**	114
	July	$y = 23.7x + 1.02$	0.105	0.176	25.2
		$y = -1.87x^{-1} + 14.5$	0.109	**0.008**	104
	September	$y = 3.00x + 4.86$	0.003	0.831	17.9
		$y = -0.184x^{-1} + 6.37$	0.002	0.866	96.4
MBP	April	$y = 4.86x - 0.405$	0.108	0.169	-35.6
		$y = -0.366x^{-1} + 2.30$	0.102	**0.031**	42.9
	July	$y = 4.67x - 0.284$	0.148	0.104	-43.8
		$y = -0.346x^{-1} + 2.29$	0.135	**0.010**	34.8
	September	$y = 5.25x - 0.500$	0.075	0.258	-24.9

		$y = -0.424x^{-1} + 2.53$	0.081	0.237	53.3
MBC:MBN	April	$y = 12.2x + 2.23$	0.126	0.136	-4.06
		$y = -0.916x^{-1} + 8.99$	0.120	0.147	74.5
	July	$y = -2.51x + 6.25$	0.008	0.723	-8.18
		$y = 0.244x^{-1} + 4.65$	0.012	0.656	70.1
	September	$y = 36.0x - 2.87$	0.274	**0.021**	18.9
		$y = -2.66x^{-1} + 16.9$	0.250	**0.029**	97.9
MBC:MBP	April	$y = 125x + 6.65$	0.140	0.115	82.3
		$y = -8.55x^{-1} + 72.9$	0.109	0.168	161
	July	$y = -48.2x + 56.8$	0.030	0.475	77.3
		$y = 3.17x^{-1} + 31.7$	0.022	0.544	155
	September	$y = 209x + 3.85$	0.050	0.355	123
		$y = -14.9x^{-1} + 117$	0.043	0.395	202
MBN:MBP	April	$y = 2.67x + 6.92$	0.002	0.846	16.9
		$y = -0.059x^{-1} + 7.88$	0.000	0.955	95.4
	July	$y = -4.77x + 9.25$	0.015	0.621	3.47
		$y = 0.193x^{-1} + 7.20$	0.004	0.796	82.1
	September	$y = -22.6x + 15.3$	0.045	0.385	40.9
		$y = 1.79x^{-1} + 2.36$	0.047	0.372	119

Discussion

Influences of grassland degradation on C, N, and P stoichiometry in soil

Our results indicate that grassland degradation strongly influences SOC and TN concentrations and SOC:TP and TN:TP stoichiometric ratios in soil (Table 2.2). As grassland degradation increased (indicated by decreased NDVI), SOC and TN decreased significantly but TP did not, leading to decreases in SOC:TP and TN:TP ratios (Table 2.2). Overall, these results are consistent with the hypothesis under Scenario 1. In soil, organic C and N pools are mainly derived from free-living and legume-associated bacteria. At the global scale, the concentrations of soil C and N vary by orders of magnitude (Cleveland and Liptzin, 2007) and large proportions of organic carbon and nutrient stocks are concentrated in the top soil layers (Gill et al., 1999). In our study, SOC and TN in soil were highly variable. The decreased vegetation biomass due to grassland degradation is likely responsible for decreased C and N concentrations in soil. On the other hand, soil erosion is highly related to vegetation properties such as density and coverage. Degraded grasslands have higher soil erosion than non-degraded grasslands (Zhao et al., 2011; Su et al., 2015), influencing the dynamic nature of the plant–soil interactions through modifications of the water cycle and the fluxes of other elements, potentiating depletion of SOC and TN (Podwojewski et al., 2011; Mchunu and Chaplot, 2012; Dlamini et al., 2014). Moreover, grasslands are an important component of the global C cycle with a storage of approximately 10% of the global soil carbon stock (Suttie et al., 2005). Indeed, the QTP grasslands account for approximately 2.5% of the global soil carbon pool (Wang et al., 2002). Our results indicate that grassland degradation causes losses of soil C pools by reducing C input from vegetation and increasing C loss in erosion. In contrast to soil C and N, P levels in soil are less strongly related to vegetation-driven processes but instead are connected to weathering of primary rock minerals (Walker and Syers, 1976). Due to differences in geological background and ecosystem succession, soil P also varies by orders of magnitude at the global scale (Cleveland and Liptzin, 2007). In our study, however, the relatively small catchment area has a relatively uniform geological background and successional stage. Thus, soil P had little variation and did not vary with grassland status as indicated by NDVI.

Although concentrations of SOC and TN were highly variable, SOC:TN:TP ratios were relatively constrained, especially for SOC:TN (Table 2.1 and Table S2.1). SMA regression analyses showed that SOC, TN, and TP across all sites had close relationships (Table S2.1). The relationship between SOC and TN (R^2=0.94, P<0.001) was stronger than the relationships between SOC and TP (R^2=0.33, P<0.001) and between TN and TP (R^2=0.39, P<0.001). SOC and TN had an isometric relationship, while SOC and TP as well as TN and TP had a non-isometric relationship (Table S2.1). The strong association between soil C and N may be explained by the fact that inputs of C and N are linked to the biomolecules of primary producers that have relatively constrained element ratios (Reich and Oleksyn, 2004) and to their residues entering the soil (Kögel-Knabner, 2002). Microbe-derived residues are another important component of soil C and N, further contributing to the strong relationship between soil C and N (Miltner et al., 2012). At the global scale, soil C:N:P ratios are highly constrained with an average value in grassland soils of 166:12:1 (Cleveland and Liptzin, 2007). In our study, the mean soil C:N:P ratio was 144:10:1, somewhat more P rich than the global value. Moreover, the differential relationships between NDVI and soil C, N, and P indicated that, as grassland degradation increases, significant decreases in SOC and TN were observed but soil TP did not vary, resulting in increased SOC:TP and TN:TP with decreasing NDVI. It has been demonstrated that shifts in nutrient availability and stoichiometry can be associated with community-level shifts of aboveground vegetation (Elser et al., 2007; Niu et al., 2019) and microorganisms (Fanin et al., 2013; Leff et al., 2015). Furthermore, downstream aquatic ecosystems could also be affected by the shifts in terrestrial nutrient storage and cycling (Ren et al., 2019).

Influences of grassland degradation on C, N, and P stoichiometry in soil microbial biomass

Reflecting observed declines in SOC, TN, SOC:TP, and TN:TP in soil with grassland degradation, C, N, and P in soil microbial biomass also significantly decreased with grassland degradation (Table 2.3). Soil microbial biomass spanned several orders of magnitude and had strong relationships with soil C, N, and P concentrations (Table 2.1 and Table S2.1). Since total soil nutrients include microbial biomass (Horwath, 2003) and soil nutrient availabilities have strong influences on microbial nutrient constraints (Tischer et al., 2014; Nottingham et al., 2015), it is not surprising to observe the close relationships between C, N, and P concentrations in soil and soil microbial biomass (Table S2.1). However, the results suggested that grassland degradation may decrease concentrations of microbial biomass C and N due to several potential mechanisms. One likely cause is that decreased SOC and TN in soil caused by grassland degradation leads to decreases of microbial biomass C and N. On the other hand, grassland degradation also results in declines of soil moisture and increases in soil pH (Table S2.2). Thus, the development of microbial communities could be limited by high pH and low moisture (Abril and Bucher, 1999; Frouz and Nováková, 2005; Viragh et al, 2011), also leading to decreases of soil microbial biomass (Table S2.3).

Despite the large variation of overall microbial C, N, and P concentrations in soil, soil microbial C:N:P ratios were highly constrained (Table S2.1) and were not affected by grassland degradation. In our study, the average value of soil microbial C:N:P was 49:8:1, close to estimated soil microbial C:N:P ratio (60:7:1) at the global scale (Cleveland and

Liptzin, 2007). It has been widely argued that a large proportion of soil microorganisms are heterotrophic and thus assumed to exhibit stronger homeostatic behavior at the organismal level than autotrophic organisms (Sterner and Elser, 2002; Cleveland and Liptzin, 2007). This generalization was supported in our data by the close relationships among soil microbial C, N, and P contents (Table S2.1), suggesting that the stoichiometry of soil microbial biomass is highly constrained. Specifically, despite considerable variation in C:N:P ratios of soil pools, MBC:MBN:MBP did not vary with decreasing NDVI (except MBC:MBN in September, Table 2.3). This further supported a view that the degree of stoichiometric flexibility of the microbial community present is limited.

The homeostatic nature of soil microbial biomass could also provide insights into influences of grassland degradation on microbial nutrient limitation, community structure, and activities. Since different taxa of microbes likely have different homeostatically regulated C:N:P ratios in their biomass (Harris, 2009; Hall et al., 2011; Tischer et al., 2014; Heuck et al., 2015) as well as different biochemical potential and substrate preferences (Six et al., 2006), changing soil environmental conditions may have nevertheless shifted microbial community composition and activities and contributing to the NDVI-associated shift in microbial C:N ratio that we observed. It has been demonstrated that fungal-to-bacterial ratios of soil microbial communities decrease in response to high pH and drought. Fungi prefer to high C:N ratio substrates while bacteria prefer to low C:N ratio substrates (Eiland et al., 2001; Rousk and Bååth, 2009). Moreover, fungal biomass generally has relatively higher C:N ratio than bacterial biomass (Paul, 2014). Thus, as grassland degradation proceeds, decreased moisture and soil C:N ratio as well as increased pH may increase the relative abundance of bacteria compared to fungi. In addition, the activities of most eco-enzymes increase under high soil pH and drought, indicating increased nitrogen requirement and allocation for protein synthesis (Sterner and Elser, 2002). Consequently, C:N ratios in soil microbial biomass decreased with decreased NDVI. Soil microbial communities are often P limited because of the relatively low C:P and N:P ratio in soil microbial biomass and relatively low P concentrations in soil (Gallardo and Schlesinger, 1994; Cleveland et al., 2002). Thus, our data suggest that, as grassland degrades, N limitation is anticipated to be more apparent for soil microbial communities. Moreover, organic carbon, nitrogen, and phosphorus are presented in different forms in soil, such as detritus and exudates, which may affect the utilization of microbes due to their different C:N:P ratios (Sistla and Schimel, 2012; Mooshammer et al., 2014). Deeper analyses of soil resource forms and nutrient species can provide additional insight into the influences of grassland degradation on ecological stoichiometry of soil ecosystems.

Conclusions

Grassland degradation is globally pervasive and impacts a number of ecosystem components. From the perspective of ecological stoichiometry, our study provides a picture of the differential influences of grassland degradation on C, N, and P in soil ecosystems in a grassland watershed on the Qinghai-Tibet Plateau. The results showed that grassland degradation decreased C and N concentrations, as well as C:P and N:P ratios in soil. Moreover, grassland degradation decreased C, N, and P concentrations in soil microbial biomass. These results suggest potential shifts in microbial nutrient limitation, community structure, and activities caused by grassland degradation. These shifts require experimental testing. Overall, by assessing the adverse influences of grassland degradation on C, N, and P concentrations and stoichiometry in soil ecosystems in the Qinghai Lake watershed, this study adds to our knowledge of biogeochemical responses of terrestrial ecosystems to ongoing grassland degradation on the Qinghai-Tibet Plateau.

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References

Abril A, Bucher EH. 1999. The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. Applied Soil Ecology, 12: 159-167.
Akiyama T, Kawamura K. 2007. Grassland degradation in China: methods of monitoring, management and restoration. Grassland Science, 53: 1-17.

Aponte C, Marañón T, García LV. 2010. Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry, 101: 77-92.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57: 289-300.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences, 110: 9374-9379.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry, 17: 837-842.

Carlson TN, Ripley DA. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. Remote Sensing of Environment, 62: 241-252.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature, 397: 491-497.

Cleveland CC, Liptzin D. 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry, 85: 235-252.

Cleveland CC, Townsend AR, Schmidt SK. 2002. Phosphorus limitation of microbial processes in moist tropical forests: evidence from short-term laboratory incubations and field studies. Ecosystems, 57: 680-691.

Daily GC. 1995. Restoring value to the world's degraded lands. Science, 269: 350-354.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma, 235-236: 372-381.

Dyksterhuis EJ. 1949. Condition and management of range land based on quantitative ecology. Journal of Range Management, 2: 104-115.

Eastwood JA, Yates MG, Thomson AG, Fuller RM. 1997. The reliability of vegetation indices for monitoring saltmarsh vegetation cover. International Journal of Remote Sensing, 18: 3901-3907.

Eiland F, Klamer M, Lind AM, Leth M, Bååth E. 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. Microbial Ecology, 41: 272-280.

Elser JJ, Acharya K, Kyle M, Cotner J, Makino W, Markow T, Watts T, Hobbie S, Fagan W, Schade J, Hood J, Sterner RW. 2003. Growth rate-stoichiometry couplings in diverse biota. Ecology Letters, 6: 936-943.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters, 10: 1135-1142.

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann EH, Sterner RW. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature, 408: 578-580.

Fanin N, Fromin N, Buatois B, Hättenschwiler S, Cleland E. 2013. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. Ecology Letters, 16: 764-772.

Gallardo A, Schlesinger WH. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. Soil Biology and Biochemistry, 26: 1409-1415.

Gang C, Zhou W, Chen Y, Wang Z, Sun Z, Li J, Qi J, Odeh I. 2014. Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environmental Earth Sciences, 72: 4273-4282.

Gill RA, Burke DG, Milchunas DG, Lauenroth WK. 1999. Relationship between root biomass and soil organic matter pools in the short grass steppe of eastern Colorado. Ecosystems, 2: 226-236.

Hall EK, Maixner F, Franklin O, Daims H, Richter A, Battin T. 2011. Linking microbial and ecosystem ecology using ecological stoichiometry: a synthesis of conceptual and empirical approaches. Ecosystems, 14: 261-273.

Harris J. 2009. Soil microbial communities and restoration ecology: facilitators or followers? Science, 325: 573-574.

Harris RB. 2010. Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. Journal of Arid Environments, 74: 1-12.

Heuck C, Weig A, Spohn M. 2015. Soil microbial biomass C:N:P stoichiometry and microbial use of organic phosphorus. Soil Biology and Biochemistry, 85: 119-129.

Hilker T, Natsagdorj E, Waring RH, Lyapustin A, Wang Y. 2014. Satellite observed widespread decline in Mongolian grasslands largely due to overgrazing. Global Change Biology, 20: 418-428.

Horwath WR. 2003. Biomass: Soil Microbial Biomass. In Encyclopedia of Environmental Microbiology. Wiley, New York.

Houlton BZ, Morford SL, Dahlgren RA. 2018. Convergent evidence for widespread rock nitrogen sources in Earth's surface environment. Science, 360: 58-62.

Kögel-Knabner I. 2002. The macromolecular organic composition of plant and microbial residues as inputs to soil organic matter. Soil Biology and Biochemistry, 34: 139-162.

Leff JW, Jones SE, Prober SM, Barberan A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schuetz M, Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences, 112: 10967-10972.

Li B. 1997. The rangeland degradation in North China and its preventive strategy. Scientia Agriculture Sinica, 30: 1-9.

Li S, Verburg PH, Lv S, Wu J, Li X. 2012a. Spatial analysis of the driving factors of grassland degradation under conditions of climate change and intensive use in Inner Mongolia, China. Regional Environmental Change, 12: 461-474.

Li XL, Gao J, Brierley G, Qiao YM, Zhang J, Yang YW. 2013. Rangeland degradation on the Qinghai - Tibet plateau: Implications for rehabilitation. Land Degradation and Development, 24: 72-80.

Li XY, Xu HY, Sun YL, Zhang DS, Yang ZP. 2007. Lake-level change and water balance analysis at Lake Qinghai, west China during recent decades. Water Resources Management, 21: 1505-1516.

Li Y, Wu J, Liu S, Shen J, Huang D, Su Y, Wei W, Syers JK. 2012b. Is the C: N: P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? Global Biogeochemical Cycles, 26: 1-14.

Luo CF, Xu CJ, You HY, Jin SH. 2013. Analysis on grassland degradation in Qinghai Lake Basin during 2000-2010. Acta Ecologica Sinica, 33: 4450-4459 (In Chinese).

Mchunu C, Chaplot V. 2012. Land degradation impact on soil carbon losses through water erosion and CO_2 emissions. Geoderma, 79: 177-178.

Mekuria W, Veldkamp E, Halle M, Nyssen J, Muys B, Gebrehiwota K. 2007. Effectiveness of exclosures to restore degraded soils as a result of overgrazing in Tigray, Ethiopia. Journal of Arid Environments, 69: 270-284.

Meurant G. 2012. The ecology of natural disturbance and patch dynamics. Academic press.

Miltner A, Bombach P, Schmidt-Brücken B, Kästner M. 2012. SOM genesis: microbial biomass as a significant source. Biogeochemistry, 111: 41-55.

Mooshammer M, Wanek W, Zechmeister-Boltenstern S, Richter A. 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology, 5: e0022.

Niu D, Zhang C, Ma P, Fu H, Elser JJ. 2019. Responses of leaf C:N:P stoichiometry to water supply in the desert shrub *Zygophyllum xanthoxylum*. Plant and Soil, 21: 82-88.

Nottingham AT, Turner BL, Whitaker J, Ostle NJ, McNamara NP, Bardgett RD, Salinas N, Meir P. 2015. Soil microbial nutrient constraints along a tropical forest elevation gradient: a belowground test of a biogeochemical paradigm. Biogeosciences, 12: 6071-6083.

Paul EA. 2014. Soil microbiology, ecology and biochemistry. Academic press.

Pickett ST. 1989. Space-for-time substitution as an alternative to long-term studies. In Long-term studies in ecology. Springer, New York, NY.

Podwojewski P, Janeau JL, Grellier S, Valentin C, Lorentz S, Chaplot V. 2011. Influence of grass soil cover on water runoff and soil detachment under rainfall simulation in a sub - humid South African degraded rangeland. Earth Surface Processes and Landforms, 36: 911-922.

R Core Team. 2017. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences India Section A-Physical Sciences, 101: 11001-11006.

Ren Z, Niu DC, Ma PP, Wang Y, Fu H, Elser JJ. 2019. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. Ecology, 100: e02755.

Rouse JW, Haas RH, Schell JA, Deering DW. 1973. Monitoring vegetation systems in the Great Plains with ERTS. Third Earth Resources Technology Satelite-1 Symposium. 309-317.

Rousk J, Bååth E. 2009. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiology Ecology, 62: 258-267.

Schlesinger WH, Bernhardt ES. 2013. Biogeochemistry: an analysis of global change. Academic press, San Diego, California, USA.

Sistla SA, Schimel JP. 2012. Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytologist, 196: 68-78.

Six J, Frey SD, Thiet RK, Batten KM. 2006. Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Science Society of America Journal, 70: 555-569.

Sousa WP. 1984. The role of disturbance in natural communities. Annual Review of Ecology and Systematics, 15: 353-391.

Steffens M, Kölbl A, Totsche KU, Kögel-Knabner I. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). Geoderma, 143: 63-72.

Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton.

Su X, Wu Y, Dong S, Wen L, Li Y, Wang X. 2015. Effects of grassland degradation and re-vegetation on carbon and nitrogen storage in the soils of the Headwater Area Nature Reserve on the Qinghai-Tibetan Plateau, China. Journal of Mountain Science, 12: 582-591.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the World. No. 34. Food and Agriculture Organization of the United Nations. Rome, Italy.

Tan K, Ciais P, Piao S, Wu X, Tang Y, Vuichard N, Liang S, Fang J. 2010. Application of the ORCHIDEE global vegetation model to evaluate biomass and soil carbon stocks of Qinghai-Tibetan grasslands. Global Biogeochemical Cycles, 24: GB1013.

Thiam AK. 2003. The causes and spatial pattern of land degradation risk in southern Mauritania using multitemporal AVHRR-NDVI imagery and field data. Land Degradation and Development, 14: 133-142.

Tischer A, Potthast K, Hamer U. 2014. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. Oecologia, 175: 375-393.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19: 703-707.

Viragh K, Toth T, Somodi I. 2011. Effect of slight vegetation degradation on soil properties in *Brachypodium pinnatum* grasslands. Plant and Soil, 345: 303-313.

Walker TW, Syers JK. 1976. The fate of phosphorus during pedogenesis. Geoderma, 15: 1-19.

Wang GX, Ju Q, Cheng GD, Lai YM. 2002. Soil organic carbon pool of grassland soils on the Qinghai-Tibetan Plateau and its global implication. Science of the Total Environment, 291: 207-217.

Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. SMART 3 - an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution, 3: 257-259.

Warton DI, Wright IJ, Falster DS, Westoby M. 2006. Bivariate line-fitting methods for allometry. Biological Reviews, 81: 259-291.

Wick AF, Geaumont BA, Sedivec K, Hendrickson J. 2016. Grassland degradation. Biological and Environmental Hazards, Risks and Disasters, 8: 257-276.

Wu RG, Tiessen H. 2002. Effect of land use on soil degradation in alpine grassland soil, China. Soil Science Society of America Journal, 66: 1648-1655.

Yi WJ, Li XY, Cui BL, Ma YJ. 2010. Climate change and impact on water level of the Qinghai Lake watershed. Journal of Arid Meteorology, 28: 375-383.

Zhao W, Yang P, Li H, Hu G, Liu X. 2011. Characteristics of soil erosion, nitrogen and phosphorus losses under three grassland use patterns in Hulun Lake watershed. Transactions of the Chinese Society of Agricultural Engineering, 27: 220-225.

Supplementary Information

Table S2.1 Isometric relationships among C, N, and P concentrations in soil and soil microbial biomass according to the function $\log_{10}(y) = a \times \log_{10}(x) + b$. Slope was tested comparing to 1. All the variables have the same unit, mmol/kg. P-values were adjusted using FDR method

y	x	Regression				Slope Test (slope=1)	
		R^2	P	Intercept (b)	Slope (a)	r	P
TN	SOC	0.942	<0.000	-1.404	1.066	-0.257	0.054
TP	SOC	0.330	<0.000	0.354	0.296	0.883	**<0.000**
TP	TN	0.392	<0.000	0.744	0.277	0.905	**<0.000**
MBN	MBC	0.710	<0.000	-0.536	0.853	0.285	**0.032**
MBP	MBC	0.619	<0.000	-1.820	1.120	-0.181	0.179
MBP	MBN	0.735	<0.000	-1.116	1.313	-0.472	**<0.000**
MBC	SOC	0.614	<0.000	2.096	0.909	0.152	0.261
MBN	TN	0.711	<0.000	1.440	1.137	-0.233	0.081
MBP	TP	0.395	<0.000	1.411	0.240	0.930	**<0.000**

Table S2.2 Summary of regression models of the relationships between NDVI (independent variable) versus soil moisture and pH (dependent variables). P-values were adjusted using FDR method. Statistically significant results are indicated by bold face.

Dependent Variable	Season	Function	R^2	P	AIC
Moisture	April	$y = 242.7x - 46.9$	0.358	**0.007**	84.0
		$y = -18.0x^{-1} + 87.1$	0.329	**0.010**	163
	July	$y = 272x - 53.7$	0.289	**0.018**	94.4
		$y = -19.5x^{-1} + 94.2$	0.248	**0.030**	174
	September	$y = 215x - 29.6$	0.318	**0.012**	82.8
		$y = -16.3x^{-1} + 89.7$	0.301	**0.015**	162
pH	April	$y = -4.08x + 9.22$	0.215	**0.046**	-57.7
		$y = 0.321x^{-1} + 6.89$	0.222	**0.042**	20.5
	July	$y = -4.67x + 9.56$	0.234	**0.036**	-54.7
		$y = 0.348x^{-1} + 6.97$	0.218	**0.044**	24.1
	September	$y = -5.297x + 9.57$	0.256	**0.027**	-52.1
		$y = 0.415x^{-1} + 6.56$	0.263	**0.025**	26.1

Table S2.3 Summary of regression models of the relationships between soil microbial biomass carbon (dependent variables) versus soil moisture and pH (independent variable). P-values were adjusted using FDR method. Statistically significant results are indicated by bold face.

Independent Variable	Season	Function	R^2	P	AIC
Moisture	April	$y = 1.53x + 7.50$	0.757	0.027	315
		$y = -206x^{-1} + 58.5$	0.256	0.000	187
	July	$y = 0.840x + 24.2$	0.562	0.020	326
		$y = -152x^{-1} + 58.3$	0.279	0.000	177
	September	$y = 1.34x - 0.825$	0.773	0.002	304
		$y = -1065x^{-1} + 82.9$	0.455	0.000	173
pH	April	$y = -42.0x + 379$	0.268	0.026	191
		$y = 2604x^{-1} - 284$	0.260	0.023	187
	July	$y = -32.9x + 315$	0.314	0.016	184
		$y = 2083x^{-1} - 210$	0.295	0.013	177
	September	$y = -38.7x + 353$	0.491	0.002	182
		$y = 2178x^{-1} - 230$	0.449	0.001	173

Chapter 3 C:N:P stoichiometry and nutrient limitation of stream biofilms impacted by grassland degradation on the Qinghai-Tibet Plateau

Abstract

Grassland degradation is an extensive global threat, especially on the Qinghai-Tibet Plateau. Both terrestrial and aquatic habitats are affected by grassland degradation in a watershed. However, the influences of grassland degradation on carbon (C) : nitrogen (N) : phosphorus (P) ratios of streamwater and biofilms, as well as their implications for nutrient limitation, are not well studied. Using a "space for time" substitution, contemporary grassland status quantified by landscape-level normalized difference vegetation index (NDVI) were used to study potential impacts of grassland degradation on nutrient cycling in streams feeding Qinghai Lake (China). C, N, and P concentrations and ratios of streamwater and benthic biofilms were assessed. The results showed that grassland degradation was associated with lower DOC, TN, DOC:TP, and TN:TP of streamwater, especially in September. DOC:TN:TP ratios of streamwater demonstrated relatively high variability with an average of 387:169:1 (molar). C, N, P biomass per unit area of stream biofilm also demonstrated considerable variability but their ratios were relatively constrained, with an average of 471:32:1 (molar). Moreover, biofilm C, N, and P concentrations and ratios were not closely associated with streamwater nutrients or grassland degradation. The complex chemical and biological composition of stream biofilms may make their stoichiometry difficult to interpret. However, the non-isometric relationships between biofilm C and P, as well as the large imbalance between C:N:P ratios of streamwater and biofilms, suggest that stream biofilms in this catchment are sensitive to P variation and potentially limited by DOC and P. By differentially impacting DOC and nutrient supplies, grassland degradation may alleviate P limitation but aggravate carbon limitation in stream biofilms. This study helps to improve our understanding the influences of grassland degradation on stream ecosystems on the Qinghai-Tibet Plateau.

Keywords: stream biofilms, grassland degradation, stoichiometry, dissolved organic matter, nutrient limitation

Introduction

Grassland degradation involves retrogressive succession (Dyksterhuis, 1949; Li, 1997) that reduces its capability to provide important ecological functions, such as biodiversity conservation, livestock production, regulation of nutrient cycles and hydrological processes, and prevention of soil erosion (Daily, 1995; Conant et al., 2001; Zhang et al., 2011; Dlamini et al., 2014; Su et al., 2015). Understanding the consequences of this degradation is important because grassland is one of the world's largest terrestrial biomes (Suttie et al., 2005). However, due to intensification of human activity, increased grazing, and climate change, around half of the world's grassland ecosystems have been degraded (Gang et al., 2014). On the Qinghai-Tibet Plateau (QTP) in particular, grassland ecosystems are very sensitive and vulnerable to external disturbance (Wang et al., 2008) and have experienced serious degradation in recent decades (Harris, 2010). Anthropogenic impacts on grasslands not only influence nutrient status in the terrestrial realm (Ren et al., in preparation, Chapter 2), but also affect aquatic systems (Ren et al., 2019) because terrestrial and aquatic ecosystems are highly linked in watersheds (Williamson et al., 2008). However, our understanding of the influences of grassland degradation on stream ecosystems is still very limited, especially from the perspective of ecological stoichiometry, the study of the balance of multiple chemical elements in living systems (Sterner and Elser, 2002).

In a watershed, streams are the primary receivers of nutrients and organic matter exported from terrestrial ecosystems (Vannote et al., 1980; Figueiredo et al., 2010; Deegan et al., 2011). As sentinels and integrators of terrestrial processes, streams are strongly influenced by changes in the terrestrial landscapes through which they flow (Vannote et al., 1980; Allan, 2004). Landcover changes lead to modifications of the structure, function, and elemental balances of terrestrial ecosystems (Tischer et al., 2014), potentially shifting the bulk carbon (C), nitrogen (N), and phosphorus (P) stoichiometry in soil (Zhao et al., 2015) and, as a consequence, altering aquatic biogeochemistry (Figueiredo et al., 2010; Nielsen et al., 2012). In a grassland watershed, soil C and N are primarily affected by processes driven by vegetation status while soil P is originally provided by rock weathering (Walker and Syers, 1976; Chadwick et al., 1999). Due to different fates in ecological processes, these elements may be affected differentially by grassland degradation, resulting in decreased C and N concentrations and C:P and N:P ratios in soil (Ren et al., in preparation, Chapter 2). Our previous study showed that grassland degradation has the potential to differentially increase P but decrease N and N:P ratios in streamwater (Ren et al., 2019). In addition to nitrogen and phosphorus, dissolved organic carbon is also an important factor affecting heterotrophic components of stream biofilms (Currie, 1999; Bernhardt and Likens, 2002; Prairie, 2008; Tank et al., 2010). However, the influences of grassland degradation on dissolved organic carbon (DOC) and C:N:P stoichiometry of streamwater are not well studied.

In stream ecosystems, benthic biofilms are commonly the dominant source of primary production, contributing significantly to secondary production (Battin et al., 2016). Comprising varying amounts of bacteria, fungi, algae, and their exudation products, as well as detritus, stream biofilms strongly mediate the metabolism and biogeochemical cycles in stream ecosystems (Schiller et al., 2007) and are influenced by many environmental factors, such as organic carbon, nutrient availability, light intensity, water temperature, and flow velocity (Fanta et al., 2010). Carbon source and nutrient availability have particularly important roles in streams flowing through grasslands, which lack a canopy. Stream biofilms usually have considerable variations in C:N:P ratios (Stelzer and Lamberti, 2001; Bowman et al., 2005), which can be associated with changes of community composition and nutrient supply (Hillebrand and Kahlert, 2001; Cross et al., 2005). Because landcover significantly controls the export of C, N, and P from catchments to aquatic ecosystems, stream biofilm stoichiometric ratios are linked to watershed conditions (O'Brien and Wehr, 2010). Indeed, influences of landcover on stoichiometry of stream biofilms have been demonstrated previously in studies mainly focusing on the different influences between distinct land-use types, such as agriculture, forest, and urban (Godwin et al., 2009; Johnson et al., 2009; O'Brien and Wehr, 2010). However, stoichiometric responses of stream biofilms to grassland degradation as well the implications for nutrient limitation are not well-studied.

The impacts of grassland degradation on stream C;N:P stoichiometry will depend on if and how degradation differentially impacts losses of C, N, and P. In one scenario (Scenario 1), degradation impacts C and N losses more strongly than P because soil C and N are more strongly dependent on biological processes in grassland vegetation and soil that are impaired during degradation while geochemical processes that supply P are less impacted (Ren et al, in prep). In a second scenario (Scenario 2 hereafter), degradation differentially impacts P losses by amplifying erosive processes that mobilize P into runoff (Ren et al, 2019). In this study, our primary questions were: (1) Do streamwater nutrient concentrations and their stoichiometric ratios respond to grassland degradation? By how much? In what direction? (2) Do C, N, and P concentrations and stoichiometric ratios of stream biofilms respond to grassland degradation? By how much? In what direction? (3) What are the implications of grassland degradation for nutrient limitation of stream biofilms? Based on mechanisms described above, we hypothesized that: (1) Streamwater nutrient concentrations and stoichiometric ratios are affected by grassland degradation due to differential inputs of N and P from degraded soil, and (2) C, N, and P concentrations and stoichiometric ratios of stream biofilms are affected by grassland degradation due to differential changes of N and P in streamwater. To test these hypotheses, we assessed C, N, and P concentrations and stoichiometric ratios of streamwater and stream biofilms in catchments differing in grassland status as indicated by normalized difference vegetation index (NDVI). We predicted that in sampling of stream and periphyton nutrients in subcatchments differing in grassland degradation, (1) in Scenario 1, streamwater N concentration will decrease more quickly than P concentration with grassland degradation; as a result, streamwater N:P ratio will decrease with grassland degradation. In Scenario 2, streamwater P concentrations will decrease more quickly than N concentrations, leading to increases of N:P ratio. (2) In Scenario 1, N:P of stream biofilms will decrease with grassland degradation. In Scenario 2, N:P of stream biofilms will increase with grassland degradation.

Methods

Study area

The Qinghai Lake watershed (Figure 3.1) is an endorheic saline lake located on the northeast edge of the Qinghai-Tibet Plateau. The catchment area is 29,660 km^2 and the altitude ranges from 3194 m (lake surface) to 5174 m. Qinghai Lake is the largest lake in China with a surface area of 4260 km^2 and an average depth of 21 m (Li et al., 2007). Qinghai Lake has more than 40 tributaries, most of which are seasonal. The Buha River is the largest tributary and contributes approximately 50% of the total surface runoff flowing into the Qinghai Lake (Cui and Li, 2015). In the watershed, the average annual precipitation is 362.5 mm and the annual evaporation is 4 times greater than precipitation (Yi et al., 2010). The air temperature ranges from -31 °C in January to 28 °C in July (Yi et al., 2010). Grassland is the major landcover, covering 75% of the watershed and experiencing serious degradation (Luo et al., 2013).

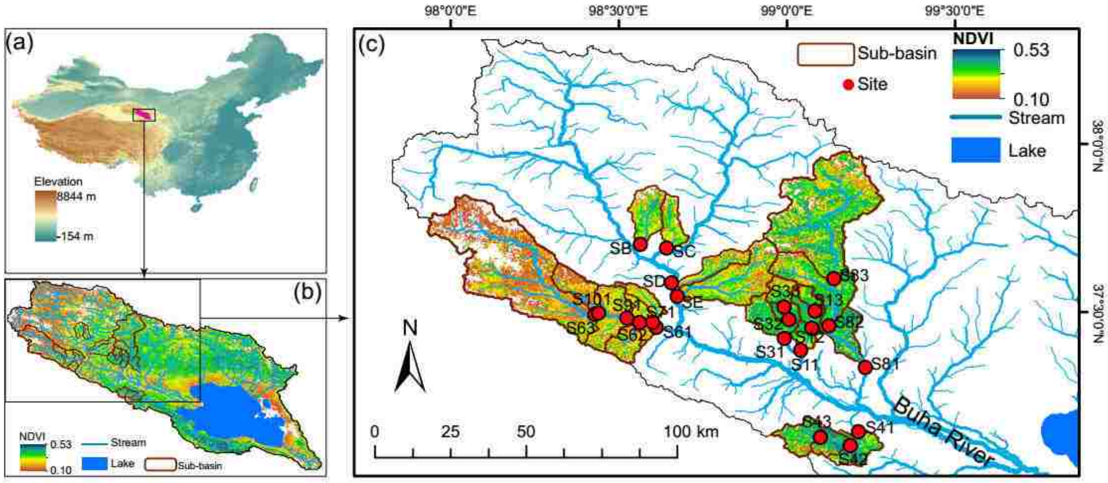

Figure 3.1 Study area and sample sites. (a) Qinghai Lake watershed is located on the northeast of the Qinghai-Tibet Plateau (the color shows elevation). (b) The location of studied sub-basins (the color shows the normalized difference vegetation index (NDVI) across the watershed). (c) The distribution of the 22 sampling sites (the color shows NDVI). The map was modified from Ren et al. (2019).

Field sampling and chemical analyses

This study was conducted in the Buha River basin. According to grassland phenology, samples were collected in mid-July 2017 (peak growing season), mid-September 2017 (late growing season), and late-April 2018 (early growing season). Samples were collected from 22 sites in each season (Figure 3.1). At each sampling site, we randomly chose 6 to 9 submerged rocks along the stream cross section. Benthic biofilms were removed from the upper surface (an area of 4.5-cm-diameter) of each rock by rigorously brushing with a sterilized nylon brush. The slurry was rinsed with 500 mL distilled water. 20 mL of mixed slurry was then filtered on a pre-combusted glass fiber filter (25-mm-diameter, Whatman, UK) with six replicates. Three filters were used to analyze biofilm carbon (BFC) and nitrogen (BFN) using an elemental analyzer (LECO 628, USA). Three filters were used to analyze biofilm phosphorus (BFP) using the ascorbate acid colorimetric method after digestion with potassium persulfate (EPA 365.3). Water samples were also collected for chemical analyses. At each stream sample site, pH, and conductivity (Cond) were measured *in situ* using a YSI handheld meter (model 80; YSI, Yellow Springs, Ohio). Elevation was measured using a GPS unit (Triton 500, Magellan, Santa Clara, California). Total nitrogen (TN) in the water samples was quantified by ion chromatography after persulfate oxidation (EPA 300.0). Total phosphorus (TP) was analyzed using the ascorbate acid colorimetric method after oxidation (EPA 365.3). DOC was analyzed on filtered streamwater (pre-combusted GF/F filters) using a Shimadzu TOC Analyzer (TOC-VCPH, Shimadzu Scientific Instruments, Columbia, Maryland).

Normalized difference vegetation index and grassland degradation

The normalized difference vegetation index (NDVI) is commonly used to measure landcover status and monitor land degradation (Thiam, 2003; Yengoh et al., 2014). In this study, grassland status was measured by NDVI which was calculated using Landsat images acquired in 2017 (https://earthexplorer.usgs.gov/). For each sample site, the average NDVI of the sub-basin above the site was calculated as the mean NDVI of each pixel (30 m × 30 m) in this sub-basin (Ren et al., 2019). NDVI was calculated and visualized in ArcGIS 14.0.

Our previous study has demonstrated that the NDVI in our study area has strong linear relationships with *in situ* measured vegetation status, including aboveground biomass, fractional vegetation cover, and grass species richness (Ren et al., 2019). Thus, NDVI can be used to indicate grassland status in this study. Moreover, both NDVI and the *in situ* measured vegetation indexes did not show clear relationships with elevation (Ren et al., 2019) while NDVI had large variations across the study area. As shown in Chapter 1, the relationship between NDVI difference and geographic distance of paired sample sites was statistically significant (P=0.002) but the weak relationship strongly (R^2=0.039) indicates that spatial non-independence should not interfere with the space-for-time approach. Thus, according to "space-for-time" substitution (Pickett, 1989; Blois et al., 2013), the contemporary spatial pattern of grassland status (indicated by spatial variation of NDVI) could be used to represent the long-term grassland degradation progress, allowing us to study the influences of grassland degradation on stream ecosystems, relatively unconfounded by other variables such as elevation.

Analyses

In this study, stoichiometric ratios are reported as molar ratios. To test the hypothesis that streamwater nutrient concentrations and stoichiometric ratios are affected by grassland degradation, standard major axis (SMA) regression and curve estimation were used to test associations of NDVI with DOC, TN, and TP concentrations and ratios of streamwater for each season (sampling event). We predicted that in Scenario 1, streamwater N concentration will decrease more quickly than P concentration with grassland degradation; as a result, streamwater N:P ratio will decrease with grassland degradation. In Scenario 2, streamwater P concentrations will decrease more quickly than N concentrations, leading to increases of N:P ratio. If the regressions are significantly positive, then Scenario 1 holds, and the hypothesis is supported. If the regressions are significantly negative, then Scenario 2 holds, and the hypothesis is also supported. If the regressions are non-significant, then the hypothesis is not supported, indicating the grassland degradation does not affect stream nutrients and/or their stoichiometry. To test the hypothesis that C, N, and P concentrations and stoichiometric ratios of stream biofilms are affected by grassland degradation, standard major axis (SMA) regression and curve estimation were used to test associations of NDVI with C, N, and P concentrations and their ratios in soil microbial biomass for each season (sampling event). We predicted that in Scenario 1, N:P of stream biofilms will decrease with grassland degradation. In Scenario 2, N:P of stream biofilms will increase with grassland degradation. If the regressions are significantly positive, then Scenario 1 holds, and the hypothesis is supported. If the regressions are significantly negative, then Scenario 2 holds, and the hypothesis is also supported. If the regressions are non-significant, then the hypothesis is not supported, indicating that grassland degradation does not affect the N:P stoichiometry of stream biofilms. The Akaike information criterion (AIC) was used to select the best regression results and the P-values were adjusted using the false discovery rate (FDR) correction method of Benjamini and Hochberg (1995). AIC estimates the relative quality of different models for a same set of data by estimating the relative amount of information lost by each model. The lower AIC (less information lost) of a model, the higher quality of that model. In addition, to assess the isometric (proportional) relationships between C, N, and P concentration of stream biofilms, the log-log function $\log_{10}(y) = a \times \log_{10}(x) + b$ was used in SMA regression, and the slope (a) was tested in comparison to 1. R package SMART 3 (Warton et al., 2012) was used for standard major axis regression. R 3.4.1 (R Core Team, 2017) was used to carry out all the analyses and results visualizations.

Results

Summary of nutrient concentrations and ratios of streamwater and stream bioiflms

For streamwater across all sites, DOC ranged from 61 to 559 µmol C/L, TN ranged from 39 to 202 µmol N/L, and TP ranged from 0.2 to 3.0 µmol P/L (Table 3.1). DOC and TN had lower variability (coefficient of variation, CV: 42.2% and 39.2%, respectively, Table 3.1) than TP (CV: 69.3%, Table 3.1). The ratios of DOC:TN, DOC:TP, and TN:TP varied from 0.5 to 8.6 (CV=66.1%), 36 to 1150 (CV=69.1%), and 40 to 437 (CV=66.4%), respectively (Table 3.1). On average, the DOC:TN:TP ratio was 387:169:1 in streamwater (Table 3.1).

For stream biofilms across all sites, BFC (biofilm carbon) ranged from 80 to 2360 nmol C/mm^2, BFN (biofilm nitrogen) ranged from 3 to 148 nmol N/mm^2, and BFP (biofilm phosphorus) ranged from 0.08 to 4.94 nmol P/mm^2. BFC, BFN, and BFP varied considerably (CV: 69.9%, 67.0%, and 68.8%, respectively, Table 3.1). BFC:BFN, BFC:BFP, and BFN:BFP varied from 6 to 39, 173 to 1224, and 11 to 93, respectively (Table 3.1). The average C:N:P stoichiometry of stream biofilms was 471:32:1 (Table 3.1).

Table 3.1 Nutrient concentrations and stoichiometric ratios of streamwater and biofilms. Stoichiometric ratios are given on a molar basis.

	Minimum	Maximum	Mean	Std. Deviation	CV%
DOC (μmol/L)	60.83	558.8	251.0	105.8	42.16
TN (μmol/L)	38.76	202.5	113.8	44.63	39.23
TP (μmol/L)	0.215	2.945	0.932	0.646	69.30
DOC:TN	0.532	8.637	2.581	1.706	66.13
DOC:TP	36.00	1150	386.7	267.1	69.07
TN:TP	39.96	437.1	169.1	112.3	66.40
BFC (nmol/mm^2)	80.12	2359	755.1	527.7	69.89
BFN (nmol/mm^2)	2.706	148.2	52.60	35.26	67.03
BFP (nmol/mm^2)	0.081	4.943	1.891	1.301	68.81
BFC:BFN	6.031	38.71	15.48	5.747	37.13
BFC:BFP	173.6	1224	470.7	232.1	49.30
BFN:BFP	10.70	92.52	31.93	15.21	47.64

Hypothesis test: DOC and nutrient concentrations and ratios of streamwater are affected by grassland degradation

If the grassland degradation hypothesis is correct, then we predict that DOC, TN, and TP concentrations and their ratios of streamwater will be associated with NDVI across the study sub-basins. Furthermore, the results will help assess which of the two scenarios about the stoichiometric impact of these changes holds. Regression analyses showed that DOC and TN decreased with decreasing NDVI (increasing degradation) in the sub-basin in April and September (Table 3.2). However, TP increased with decreasing NDVI in September (Table 3.2). Moreover, DOC:TP and TN:TP increased with decreasing NDVI in September (Table 3.2). These results partially match the predictions derived from the hypothesis under Scenario 1.

Table 3.2 Summary of regression models of the relationships between NDVI (independent variable) versus stream nutrient concentrations and their ratios (dependent variables). The results of TN, TP, and TN:TP are cited from Ren et al., 2019. P-values were adjusted using FDR method. Statistically significant results are indicated by bold face.

Dependent Variable	Season	Function	R^2	p	AIC
DOC	April	$y = 1571x - 206$	0.303	**0.010**	57.31
		$y = -54.6x^{-1} + 414$	0.349	**0.005**	239.5
	July	$y = 2062x - 251$	0.035	0.404	181.5
		$y = -27.7x^{-1} + 379$	0.052	0.306	269.8
	September	$y = 2072x - 217$	0.310	**0.007**	174.3
		$y = -68.6x^{-1} + 586$	0.318	**0.006**	262.8
TN	April	$y = 959x - 125$	0.207	**0.038**	139.3
		$y = -23.3x^{-1} + 211$	0.170	**0.063**	223.8
	July	$y = 798x - 84$	0.143	**0.082**	137.1
		$y = -15.5x^{-1} + 179$	0.109	0.134	226.7
	September	$y = 954x - 126$	0.252	**0.017**	142.0
		$y = -25.9x^{-1} + 219$	0.213	**0.031**	231.8
TP	April	$y = 17x - 3$	0.014	0.609	-24.29
		$y = -0.111x^{-1} + 1.77$	0.012	0.643	59.37
	July	$y = 6x - 1$	0.098	0.156	-79.58
		$y = -0.098x^{-1} + 0.906$	0.088	0.181	9.418
	September	$y = 6.16x +2.55$	0.122	0.112	-76.33
		$y = 0.161x^{-1} + 0.334$	0.199	**0.038**	10.40
DOC:TN	April	$y = -23x + 8$	0.002	0.837	-11.69
		$y = -0.035x^{-1} + 2.10$	0.001	0.914	71.95
	July	$y = -33x + 11$	0.014	0.609	1.483
		$y = 0.167x^{-1} + 1.78$	0.007	0.712	85.83
	September	$y = -40x + 13$	0.060	0.272	6.898
		$y = 0.474x^{-1} + 1.30$	0.042	0.362	96.07
DOC:TP	April	$y = -5490x + 1633$	0.013	0.617	217.2
		$y = 19.9x^{-1} + 182$	0.004	0.791	301.0
	July	$y = -5241x + 1871$	0.011	0.652	213.7
		$y = 11.7x^{-1} + 524$	0.001	0.870	298.3
	September	$y = 2941x - 409$	0.296	**0.009**	190.2
		$y = -102 x^{-1} + 750$	0.350	**0.004**	277.2
TN:TP	April	$y = 2034x - 373$	0.009	0.676	175.5
		$y = -11.3x^{-1} + 182$	0.009	0.683	259.2
	July	$y = -2307x + 830$	0.000	0.930	179.5
		$y = 5.79x^{-1} + 234$	0.002	0.854	263.8
	September	$y = 1109x - 160$	0.384	**0.002**	144.4
		$y = -40.2x^{-1} + 284$	0.382	**0.002**	233.2

Hypothesis test: grassland degradation affects C, N, and P stoichiometry of stream biofilms

If the grassland degradation hypothesis is correct, then we predict that concentrations and ratios of C, N, and P of stream biofilms would be associated with NDVI across the study sub-basins. Furthermore, the results will help delineate which of the two scenarios about the influences of grassland degradation on biofilm stoichiometry holds. The results showed that BFC, BFN, and BFP varied considerably but did not vary with NDVI (Figure S3.1) nor nutrient concentrations of streamwater (Figure S3.2). Despite the large variations in C, N, and P concentrations of stream biofilms per unit area (nmol/mm^2), C:N:P ratios of stream biofilms were relatively constrained (Table S3.1). Nutrient ratios of stream biofilms did not have significant relationships with NDVI (Figure S3.1) nor with nutrient ratios of streamwater (Figure S3.2). These results did not match the predictions, suggesting the stream biofilm nutrient concentrations and stoichiometry are not impacted by grassland degradation.

Discussion

Grassland degradation influences nutrient and nutrient ratios of streamwater

Our data for stream DOC and nutrient concentrations and ratios indicate that grassland degradation decreased DOC and TN (significant in April and September) but increased TP (significant in September) concentrations in these QTP streams, resulting in lower DOC:TP and TN:TP ratios (significant in September) in streams draining highly degraded catchments (Table 3.2). These results are consistent with Scenario 1, in which grassland degradation differentially impacts C and N losses relative to P because biological processes in vegetation and soil are essential in providing C and N to soil but these are strongly impacted during the process of grassland degradation. The landscape through which a stream flows influences organic C, N, and P concentrations of streamwater (Williams et al., 2012). Especially in headwater streams, N, P, and organic C are mainly terrestrially derived (Howarth and Fisher, 1976; Meyer et al., 1981; Saunders et al., 2006; Tank et al., 2010). Thus, to understand these changes it is important to consider how grassland degradation affects nutrient pools in soil and in vegetation. In terrestrial ecosystems, organic C and N in soil are mainly provided by plants (Reich and Oleksyn, 2004). Grassland degradation reduces aboveground vegetation biomass, leading to the decline of soil nitrogen and organic C concentrations (Ren et al., in preparation, Chapter 2; Aponte et al., 2010; Li et al., 2012). The decline of DOC and TN concentrations of streamwater with increased grassland degradation of the sub-basins that we document likely is a cumulative effect of the sustained decreases of soil C and N associated with chronic grassland degradation. Even though initial stages of soil erosion may enhance the loss of soil organic matter and nutrients into streams (Wu and Tiessen, 2002; Steffens et al., 2008), over time there is less and less vegetation-derived organic matter and N in soils and thus less to be lost as degradation proceeds, eventually leading to lower DOC and TN concentrations in streamwater. In contrast, soil P is determined by primary rock weathering processes (Walker and Syers, 1976) that are less impacted by grassland degradation (Ren et al., in preparation, Chapter 2). Grassland degradation aggravates soil erosion, enhancing P loss from soil to adjacent streams (Zhao et al., 2011; Su et al., 2015) and leading to increased P concentrations in streamwater. Thus, the differential influences of grassland degradation on DOC, N, and P in stream result in decreased DOC:TN and TN:TP (significant in September). These inferences are supported by assessments of soil stoichiometry that were assessed in the same area - soil C:N and N:P ratios decreased with grassland degradation (Ren et al., in preparation, Chapter 2). The seasonal shifts in the relationships between nutrients and NDVI may reflect seasonal dynamics of aboveground and belowground interactions in the surrounding vegetation during vegetation accrual and decline. This requires further study. Overall, these results provide a broad view that the influences of grassland degradation are transferred from land to water, changing C:N:P stoichiometry both in soil and in streamwater and potentially shifting biological communities and nutrient limitation patterns in these ecosystems.

Nutrient stoichiometric ratios of stream biofilms

In the Qinghai Plateau streams that we sampled, changes in DOC and nutrient concentrations and stoichiometric ratios were associated with grassland degradation status to some extent but C, N, and P concentrations of stream biofilms were not (Figure S3.1). Moreover, wide-ranging DOC, TN, and TP concentrations and ratios in streamwater had no relationships with C, N, and P concentration and ratios of stream biofilms (Figure S3.2). The results are not without implications. Biofilm C, N, and P concentrations had close relationships with each other, suggesting biological constraints on biofilm C:N:P ratios (Table S3.1). In stream biofilm assemblages, various heterotrophic and photoautotrophic taxa are tightly linked to each other via trophic and competitive interactions (Fitter and Hillebrand, 2009) and are involved in the transfer of nutrients via a variety of biological processes (Peterson et al., 2001; Burgin and Hamilton, 2007; Ishida et al., 2008; Mulholland et al., 2008; Drake et al., 2012). Variations in availabilities of key nutrients have been shown to decouple autotrophic and heterotrophic processes (Scott et al., 2008) as well as shift community structure and biomass in stream biofilms (Van Horn et al., 2011). Moreover, biofilm community composition can be strongly affected by landcover change (Qu et al., 2017; Ren et al., 2017). For example, previous research has demonstrated variation of stream diatom composition in relation to changes in landcover type from forest to agriculture to urban (Walker and Pan, 2006; Smucker et al., 2013; Teittinen et al., 2015). It has been suggested that bacterial communities as a whole can display variable stoichiometry while particular bacterial taxa show homeostatic properties (Makino et al., 2003; Danger et al., 2008). Thus, the stoichiometric variations of stream biofilms that we observed may be related to shifts in the relative abundance of different microbial taxa as well as physiological plasticity of some autotrophic members in the community (Fanin et al., 2013). Besides the living organisms, stream biofilms also contain non-living components, such as detritus of algae and terrestrial plants (Hamilton et al., 2005), which can further cause considerable variation of C:N:P ratios of stream biofilms (Stelzer and Lamberti, 2001; Bowman et al., 2005). The complex chemical and biological composition of stream biofilms makes their stoichiometry difficult to interpret and predict.

Implications for element limitation of stream biofilms

Although biofilm C, N, and P as well as their stoichiometric ratios were not associated with NDVI and streamwater nutrients (Figure S3.1), the data still provide insight regarding potential nutrient limitation of stream biofilms in this basin and possible relationship to grassland degradation. In streamwater and biofilms, average C:P ratios were 387:1 (DOC:TP) and 471:1 (BFC:BFP), respectively. The relatively low DOC:TP in streamwater (compared to BFC:BFP in this study and to other forest streams such as in Dillon and Molot (1997) in which DOC:TP ratios ranged from 1292 to 1845:1) may suggest potential carbon limitation of heterotrophs in these stream biofilms. As a dominant form of organic carbon in streams (Tank et al., 2010), DOC is also a primary source of metabolic substrates for heterotrophic microorganisms and is extensively processed by stream biofilms (Romani et al., 2004; Schiller et al., 2017), fueling the stream microbial food web (Prairie, 2008; Risse-Buhl et al., 2012). Furthermore, stream metabolism and nitrogen transformations are powered by oxidizable carbon (Cook et al., 2015; Hall et al., 2016; Bernhardt et al., 2018). Carbon limitation of bacteria and fungi has been found in many ecosystems, and consequently influences biogeochemical cycles of N and P, such as repressing denitrification in streams (Bernhardt and Likens, 2002). Our data indicate that grassland degradation appears to lower soil organic carbon and ultimately reduce DOC concentrations in streamwater. This could aggravate carbon limitation of stream heterotrophs in biofilms and slow biogeochemical processes. On the other hand, the large imbalance of N:P ratios between streamwater (average TN:TP=169:1) and biofilms (average BFN:BFP=32:1) does suggest that at least some components of stream biofilms are P-limited. In addition, the non-isometric relationship between BFC and BFP (disproportionate change of BFP relative to BFC) and the isometric relationship between BFC and BFN (proportionate changes) also suggest that biofilms in these QTP streams are likely more sensitive to P variation than to N variation (Table S3.1), indicating that that biofilms are more likely limited by P. Low P availability can strongly limit biofilm biomass, activity, and ecosystem processes in stream ecosystems (Elser et al., 2007). Indeed, effects of grassland degradation on nutrient limitation of biofilms in these streams were documented using nutrient diffusing substrata bioassay experiments (Ren et al., 2019). Taken together, our findings indicate that grassland degradation increases P concentrations and decreases N:P ratios in streamwater and alleviates P limitation of stream biofilms (Ren et al., 2019). However, as discussed above, the stream biofilm is composed by living and non-living components and even the living components are complex in composition (Stelzer and Lamberti, 2001; Bowman et al., 2005; Hamilton et al., 2005), making the predictions of their nutrient limitation more difficult. Moreover, benthic consumers may selectively ingest or assimilate certain components of stream biofilms (Lodge, 1986;

Raikow and Hamilton, 2001; Clapcott and Bunn, 2003). Thus, potential consequences of grassland degradation for stream nutrient limitation seem apparent even though its impacts on C:N:P stoichiometry are difficult to discern given the complexities provided by the diverse contributions and chemical compositions of the components in stream biofilms (Cross et al., 2005; Hamilton et al., 2005; Small et al., 2011).

Conclusions

Grassland degradation is a global threat for both terrestrial and aquatic components in grassland watersheds. By documenting the responses of C, N, and P concentrations and ratios of streamwater and biofilms, our study provides a picture of potentially differential influences of grassland degradation on DOC, TN, and TP concentrations and ratios in streamwater. Nevertheless, C, N, and P concentrations and ratios of stream biofilms were not clearly affected by grassland degradation. However, streamwater DOC:TN ratios were lower than C:N ratios of stream biofilms while streamwater had higher N:P ratios than biofilms. These imbalances of C:N:P stoichiometry between streamwater and biofilms suggested that these stream biofilms were limited by P and organic carbon. The non-isometric relationships between biofilm C and P and the isometric relationship between biofilm C and N further suggested that stream biofilms were more sensitive to P variation. Thus, grassland degradation could potentially alleviate P limitation and aggravate C limitation in these catchments by differentially changing DOC and nutrient supplies. This study adds to our understanding of how stream ecosystems are likely to respond to further grassland degradation on the Qinghai-Tibet Plateau.

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References

Allan JD. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics, 35: 257-284.

Aponte C, Marañón T, García LV. 2010. Microbial C, N and P in soils of Mediterranean oak forests: influence of season, canopy cover and soil depth. Biogeochemistry, 101: 77-92.

Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology, 14: 251-263.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57: 289-300.

Bernhardt ES, Heffernan JB, Grimm NB, Stanley EH, Harvey JW, Arroita M, Appling AP, Cohen MJ, McDowell WH, Hall RO, Read JS, Roberts BJ, Stets EG, Yackulic CB. 2018. The metabolic regimes of flowing waters. Limnology and Oceanography, 631: 99-118.

Bernhardt ES, Likens GE. 2002. Dissolved organic carbon enrichment alters nitrogen dynamics in a forest stream. Ecology, 83: 1689-1700.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences, 110: 9374-9379.

Bowman MF, Chambers PA, Schindler DW. 2005. Changes in stoichiometric constraints on epilithon and benthic macroinvertebrates in response to slight nutrient enrichment of mountain rivers. Freshwater Biology, 50: 1836-1852.

Burgin AJ, Hamilton SK. 2007. Have we overemphasized the role of denitrification in aquatic ecosystems? A review of nitrate removal pathways. Frontiers in Ecology and the Environment, 5: 89-96.

Chadwick OA, Derry LA, Vitousek PM, Huebert BJ, Hedin LO. 1999. Changing sources of nutrients during four million years of ecosystem development. Nature, 397: 491-497.

Clapcott JE, Bunn SE. 2003. Can C_4 plants contribute to the aquatic food webs of subtropical streams. Freshwater Biology, 48: 1105-1116.

Conant RT, Paustian K, Elliott ET. 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecological Applications, 11: 343-355.

Cook RA, Gawne B, Petrie R, Baldwin DS, Rees GN, Nielsen DL, Ning NSP. 2015. River metabolism and carbon dynamics in response to flooding in a lowland river. Marine and Freshwater Research, 66: 919-927.

Cross WF, Benstead JP, Frost PC, Thomas SA. 2005. Ecological stoichiometry in freshwater benthic systems: recent progress and perspectives. Freshwater Biology, 50: 1895-1912.

Cui B, Li X. 2015. Runoff processes in the Qinghai Lake Basin, Northeast Qinghai-Tibet Plateau, China: insights from stable isotope and hydrochemistry. Quaternary International, 380-381: 123-132.

Currie WS. 1999. The responsive C and N biogeochemistry of the temperate forest floor. Trends in Ecology and Evolution, 14: 316-320.

Daily GC. 1995. Restoring value to the world's degraded lands. Science, 269: 350-354.

Danger M, Daufresne T, Lucas F, Pissard S, Lacroix G. 2008. Does Liebig's law of the minimum scale up from species to communities? Oikos, 117: 1741-1751.

Deegan LA, Neill C, Haupert CL, Ballester MVR, Krusche AV, Victoria RL, Thomas SM, de Moor E. 2011. Amazon deforestation alters small stream structure, nitrogen biogeochemistry and connectivity to larger rivers. Biogeochemistry, 105: 53-74.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma, 235-236: 372-381.

Drake WM, Scott JT, Evans-White M, Haggard B, Sharpley A, Rogers CW, Grantz EM. 2012. The effect of periphyton stoichiometry and light on biological phosphorus immobilization and release in streams. Limnology, 13: 97-106.

Dyksterhuis EJ. 1949. Condition and management of range land based on quantitative ecology. Journal of Range Management, 2: 104-115.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters, 10: 1135-1142.

Fanin N, Fromin N, Buatois B, Hättenschwiler S, Cleland E. 2013. An experimental test of the hypothesis of non-homeostatic consumer stoichiometry in a plant litter-microbe system. Ecology Letters, 16: 764-772.

Fanta SE, Hill WR, Smith TB, Roberts BJ. 2010. Applying the light: nutrient hypothesis to stream periphyton. Freshwater Biology, 55: 931-940.

Figueiredo RO, Markewitz D, Davidson EA, Schuler AE, Dos S. Watrin O, de Souza Silva P. 2010. Land-use effects on the chemical attributes of low-order streams in the eastern Amazon. Journal of Geophysical Research, 115: 1-14.

Fitter A, Hillebrand H. 2009. Microbial food web structure affects bottom-up effects and elemental stoichiometry in periphyton assemblages. Limnology and Oceanography, 54: 2183-2200.

Gang C, Zhou W, Chen Y, Wang Z, Sun Z, Li J, Qi J, Odeh I. 2014. Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environmental Earth Sciences, 72: 4273-4282.

Godwin CM, Arthur MA, Carrick HJ. 2009. Periphyton nutrient status in a temperate stream with mixed land-uses: implications for watershed nitrogen storage. Hydrobiologia, 623: 141-152.

Hall RO, Tank JL, Baker MA, Rosi-Marshall EJ, Hotchkiss ER. 2016. Metabolism, gas exchange, and carbon spiraling in rivers. Ecosystems, 19: 73-86.

Hamilton SK, Sippel SJ, Bunn SE. 2005. Separation of algae from detritus for stable isotope or ecological stoichiometry studies using density fractionation in colloidal silica. Limnology and Oceanography: Methods, 3: 149-157.

Harris RB. 2010. Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. Journal of Arid Environments, 74: 1-12.

Hillebrand H, Kahlert M. 2001. Effect of grazing and nutrient supply on periphyton biomass and nutrient stoichiometry in habitats of different productivity. Limnology and Oceanography, 46: 1881-1898.

Howarth RW, Fisher SG. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. Freshwater Biology, 6: 221-228.

Ishida CK, Arnon S, Peterson CG, Kelly JJ, Gray KA. 2008. Influence of algal community structure on denitrification rates in periphyton cultivated on artificial substrata. Microbial Ecology, 56: 140-152.

Johnson LT, Tank JL, Dodds WK. 2009. The influence of land use on stream biofilm nutrient limitation across eight North American ecoregions. Canadian Journal of Fisheries and Aquatic Sciences, 66: 1081-1094.

Li B. 1997. The rangeland degradation in North China and its preventive strategy. Scientia Agriculture Sinica, 30: 1-9.

Li XY, Xu HY, Sun YL, Zhang DS, Yang ZP. 2007. Lake-level change and water balance analysis at Lake Qinghai, west China during recent decades. Water Resources Management, 21: 1505-1516.

Li Y, Wu J, Liu S, Shen J, Huang D, Su Y, Wei W, Syers JK. 2012. Is the C: N: P stoichiometry in soil and soil microbial biomass related to the landscape and land use in southern subtropical China? Global Biogeochemical Cycles, 26: 1-14.

Lodge DM. 1986. Selective grazing on periphyton: a deter-minant of freshwater gastropod microdistributions. Freshwater Biology, 16: 831-841.

Luo CF, Xu CJ, You HY, Jin SH. 2013. Analysis on grassland degradation in Qinghai Lake Basin during 2000-2010. Acta Ecologica Sinica, 33: 4450-4459 (In Chinese).

Makino W, Cotner JB, Sterner RW, Elser JJ. 2003. Are bacteria more like plants or animals? Growth rate and resource dependence of bacterial C:N:P stoichiometry. Functional Ecology, 17: 121-130.

Meyer JL, Likens GE, Sloane J. 1981. Phosphorus, nitrogen and organic carbon flux in a headwater stream. Archiv fur Hydrobiologie, 99: 28-44.

Mulholland PJ, Helton AM, Poole GC, Hall RO, Hamilton SK, Peterson BJ, Tank JL, Ashkenas LR, Cooper LW, Dahm CN, Dodds WK, Findlay SEG, Gregory SV, Grimm NB, Johnson SL, McDowell WH, Meyer JL, Valett HM, Webster JR, Arango CP, Beaulieu JJ, Bernot MJ, Burgin AJ, Crenshaw CL, Johnson LT, Niederlehner BR, O Brien JM, Potter JD, Sheibley RW, Sobota DJ, Thomas SM. 2008. Stream denitrification across biomes and its response to anthropogenic nitrate loading. Nature, 452: 202-205.

Nielsen A, Trolle D, Sondergaard M, Lauridsen TL, Bjerring R, Olesen JE, Jeppesen E. 2012. Watershed land use effects on lake water quality in Denmark. Ecological Applications, 22: 1187-1200.

O'Brien PJ, Wehr JD. 2010. Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. Hydrobiologia, 657: 89-105.

Peterson BJ, Wollheim WM, Mulholland PJ, Webster JR, Meyer JL, Tank JL, Marti E, Bowden WB, Valett HM, Hershey AE, McDowell WH, Dodds WK, Hamilton SK, Gregory S, Morrall DD. 2001. Control of nitrogen export from watersheds by headwater streams. Science, 292: 86-90.

Pickett ST. 1989. Space-for-time substitution as an alternative to long-term studies. In Long-term studies in ecology. Springer, New York, NY.

Prairie YT. 2008. Carbocentric limnology: looking back, looking forward. Canadian Journal of Fisheries and Aquatic Sciences, 65: 543-548.

Qu XD, Ren Z, Zhang HP, Zhang M, Zhang YH, Liu XB, Peng WQ. 2017. Influences of anthropogenic land use on microbial community structure and functional potentials of stream benthic biofilms. Scientific Reports, 7: e15117.

R Core Team. 2017. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Raikow DF, Hamilton SK. 2001. Bivalve diets in a midwestern U.S. stream: A stable isotope enrichment study. Limnology and Oceanography, 46: 514-522.

Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences India Section A-Physical Sciences, 101: 11001-11006.

Ren Z, Gao HK, Elser JJ. 2017. Longitudinal variation of microbial communities in benthic biofilms and association with hydrological and physicochemical conditions in glacier-fed streams. Freshwater Science, 36: 479-490.

Ren Z, Niu DC, Ma PP, Wang Y, Fu H, Elser JJ. 2019. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. Ecology, 100: e02755.

Ren Z, Wang Y, Ma PP, Wang ZM, Niu DC, Fu H, Elser JJ. in preparation, Chapter 2. Effects of grassland degradation on ecological stoichiometry of soil ecosystems on the Qinghai-Tibet Plateau.

Risse-Buhl U, Trefzger N, Seifert A, Schoenborn W, Gleixner G, Kuesel K. 2012. Tracking the autochthonous carbon transfer in stream biofilm food webs. FEMS Microbiology Ecology, 79: 118-131.

Romani AM, Guasch H, Munoz I, Ruana J, Vilalta E, Schwartz T, Emtiazi F, Sabater S. 2004. Biofilm structure and function and possible implications for riverine DOC dynamics. Microbial Ecology, 47: 316-328.

Saunders TJ, McClain ME, Llerena CA. 2006. The biogeochemistry of dissolved nitrogen, phosphorus, and organic carbon along terrestrial-aquatic flowpaths of a montane headwater catchment in the Peruvian Amazon. Hydrological Processes, 20: 2549-2562.

Schiller DV, Acuna V, Aristi I, Arroita M, Basaguren A, Bellin A, Boyero L, Butturini A, Ginebreda A, Kalogianni E, Larranaga A, Majone B, Martinez A, Monroy S, Munoz I, Paunovic M, Pereda O, Petrovic M, Pozo J, Rodriguez-Mozaz S, Rivas D, Sabater S, Sabater F, Skoulikidis N, Solagaistua L, Vardakas L, Elosegi A. 2017. River ecosystem processes: A synthesis of approaches, criteria of use and sensitivity to environmental stressors. Science of the Total Environment, 596: 465-480.

Schiller DV, Marti E, Riera JL, Sabater F. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. Freshwater Biology, 52: 891-906.

Scott JT, Back JA, Taylo JM, King RS. 2008. Does nutrient enrichment decouple algal-bacterial production in periphyton? Journal of the North American Benthological Society, 27: 332-344.

Small GE, Bixby RJ, Kazanci C, Pringle CM. 2011. Partitioning stoichiometric components of epilithic biofilm using mixing models. Limnology and Oceanography: Methods, 9: 185-193.

Smucker NJ, Detenbeck NE, Morrison AC. 2013. Diatom responses to watershed development and potential moderating effects of near-stream forest and wetland cover. Freshwater Science, 32: 230-249.

Steffens M, Kölbl A, Totsche KU, Kögel-Knabner I. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). Geoderma, 143: 63-72.

Stelzer RS, Lamberti GA. 2001. Effects of N:P ratio and total nutrient concentration on stream periphyton community structure, biomass, and elemental composition. Limnology and Oceanography, 46: 356-367.

Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton.

Su X, Wu Y, Dong S, Wen L, Li Y, Wang X. 2015. Effects of grassland degradation and re-vegetation on carbon and nitrogen storage in the soils of the Headwater Area Nature Reserve on the Qinghai-Tibetan Plateau, China. Journal of Mountain Science, 12: 582-591.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the World. No. 34. Food and Agriculture Organization of the United Nations. Rome, Italy.

Tank JL, Rosi-Marshall EJ, Griffiths NA, Entrekin SA, Stephen ML. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. Journal of the North American Benthological Society, 29: 118-146.

Teittinen A, Taka M, Ruth O, Soininen J. 2015. Variation in stream diatom communities in relation to water quality and catchment variables in a boreal, urbanized region. Science of the Total Environment, 530: 279-289.

Thiam AK. 2003. The causes and spatial pattern of land degradation risk in southern Mauritania using multitemporal AVHRR-NDVI imagery and field data. Land Degradation and Development, 14: 133-142.

Tischer A, Potthast K, Hamer U. 2014. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. Oecologia, 175: 375-393.

Van Horn DJ, Sinsabaugh RL, Takacs-Vesbach CD, Mitchell KR, Dahm CN. 2011. Response of heterotrophic stream biofilm communities to a gradient of resources. Aquatic Microbial Ecology, 64: 149-161.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences, 37: 130-137.

Walker CE, Pan YD. 2006. Using diatom assemblages to assess urban stream conditions. Hydrobiologia, 561: 179-189.

Walker TW, Syers JK. 1976. The fate of phosphorus during pedogenesis. Geoderma, 15: 1-19.

Wang XD, Zhong XH, Liu SZ, Liu JG, Wang ZY, Li MH. 2008. Regional assessment of environmental vulnerability in the Tibetan Plateau: Development and application of a new method. Journal of Arid Environments, 72: 1929-1939.

Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. SMART 3 - an R package for estimation and inference about allometric lines. Methods in Ecology and Evolution, 3: 257-259.

Williams CJ, Scott AB, Wilson HF, Xenopoulos MA. 2012. Effects of land use on water column bacterial activity and enzyme stoichiometry in stream ecosystems. Aquatic Sciences, 74: 483-494.

Williamson CE, Dodds W, Kratz TK, Palmer MA. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment, 6: 247-254.

Wu RG, Tiessen H. 2002. Effect of land use on soil degradation in alpine grassland soil, China. Soil Science Society of America Journal, 66: 1648-1655.

Yengoh GT, Dent D, Olsson L, Tengberg A, Tucker C. 2014. The use of the Normalized Difference Vegetation Index (NDVI) to assess land degradation at multiple scales a review of the current status, future trends, and practical considerations. Lund University Center for Sustainability Studies (LUCSUS), and the Scientific and Technical Advisory Panel of the Global Environment Facility (STAP/GEF). Lund, Sweden.

Yi WJ, Li XY, Cui BL, Ma YJ. 2010. Climate change and impact on water level of the Qinghai Lake watershed. Journal of Arid Meteorology, 28: 375-383.

Zhang S, Hua D, Meng X, Zhang Y. 2011. Climate change and its driving effect on the runoff in the "Three-River Headwaters" region. Journal of Geographical Sciences, 21: 963-978.

Zhao F, Sun J, Ren C, Kang D, Deng J, Han X, Yang G, Feng Y, Ren G. 2015. Land use change influences soil C, N, and P stoichiometry under 'Grain-to-Green Program' in China. Scientific Reports, 5: e10195.

Zhao W, Yang P, Li H, Hu G, Liu X. 2011. Characteristics of soil erosion, nitrogen and phosphorus losses under three grassland use patterns in Hulun Lake watershed. Transactions of the Chinese Society of Agricultural Engineering, 27: 220-225.

Supplementary Information

Figure S3.1 Scatter plots showing relationships between NDVI and (a) C, N, and P concentration and (b) C:N:P ratios of stream biofilms. No significant relationships were found.

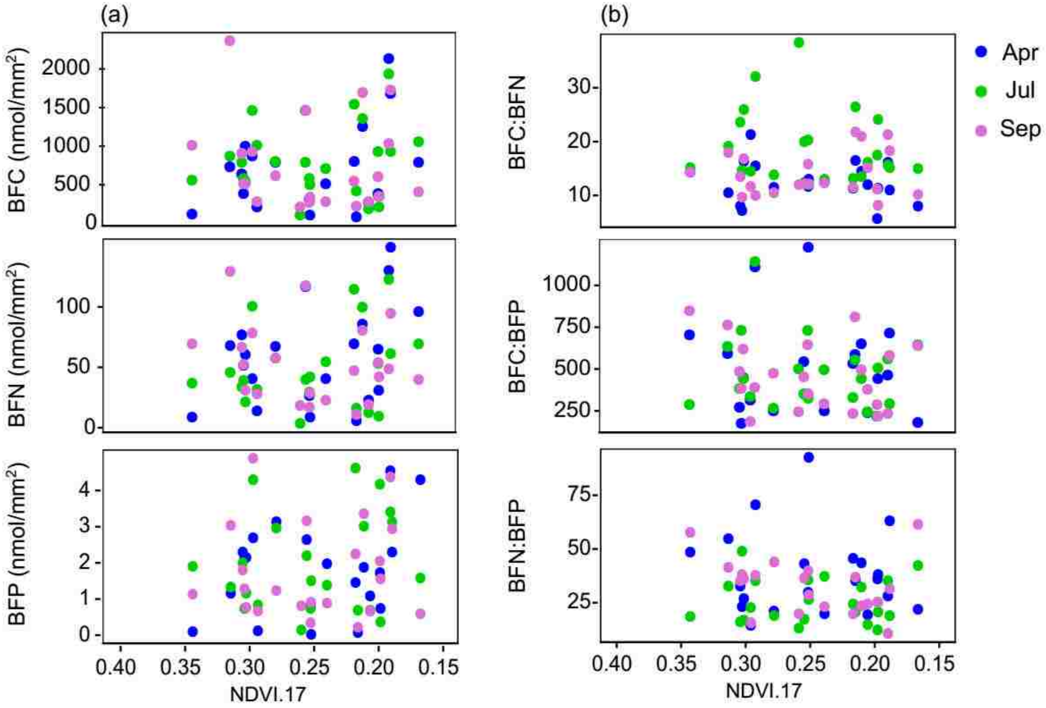

Figure S3.2 Regression plots showing relationships (a) between element concentrations of streamwater and stream biofilms and (b) between stoichiometric ratios of streamwater and stream biofilms. No significant relationships were found.

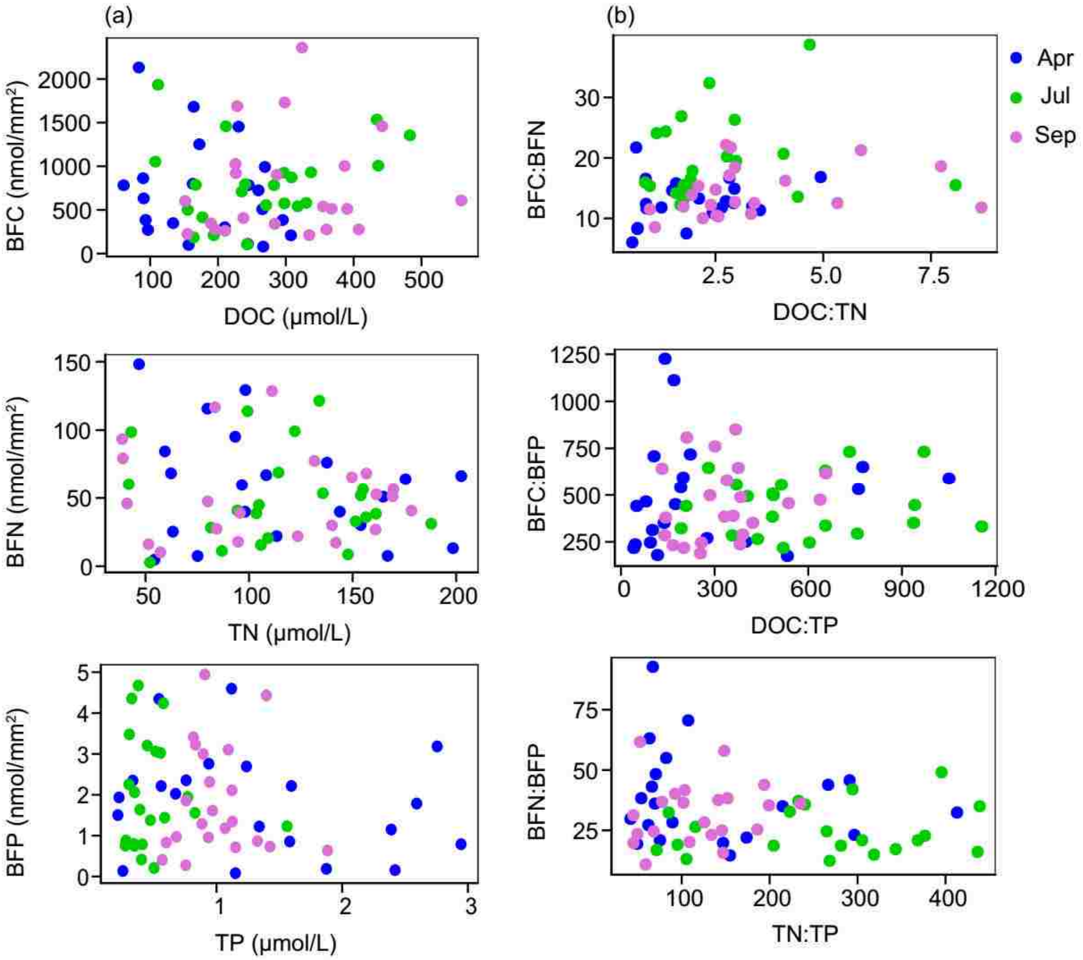

Table S3.1 Standard major axis regression between nutrient concentrations of stream biofilms according to the function $\log_{10}(y) = a \times \log_{10}(x) + b$. Slope was tested comparing to 1. The variables have the same unit ($nmol/mm^2$)

y	x	Regression				Slope Test (slope=1)	
		R^2	P	Intercept (b)	Slope (a)	r	P
BFN	BFC	0.841	<0.000	-1.393	1.083	0.196	0.118
BFP	BFC	0.739	<0.000	-3.120	1.179	0.308	**0.013**
BFP	BFN	0.765	<0.000	-1.603	1.089	0.174	0.167

Chapter 4 Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams

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Abstract

Nitrogen (N) and phosphorus (P) are key growth-limiting nutrients for organisms and their absolute and relative supplies regulate the structure and function of ecosystems. Landcover changes lead to modifications of terrestrial biogeochemistry, consequently influencing aquatic nutrient conditions. This study sought to evaluate the potential impacts of grassland degradation on nutrient availability and nutrient limitation in the Qinghai Lake (China) and its inflow streams. We sampled nutrient concentrations and tested stream nutrient limitation by conducting nutrient diffusing substrata (NDS) bioassays in streams flowing through sub-basins with different grassland status. To test nutrient limitation and the responses of lake phytoplankton to stream inflows, bioassays were conducted by adding different nutrients (N, P, and joint NP) as well as water from different streams to lake water with phytoplankton, respectively. In general, N concentrations as well as N:P ratios decreased while P concentrations increased with decreased normalized difference vegetation index (NDVI, an index of vegetation status), especially in September, suggesting that grassland degradation (low NDVI) has the potential to differentially decrease N availability and increase P availability in streams. Consistent with this, relative responses (RR) of stream periphyton to P and combined NP enrichments in the NDS bioassays decreased with stream P concentrations while increased with streamwater N:P ratios. Lake phytoplankton responded strongly to P and combined NP addition indicating strong P-limitation of lake phytoplankton. RR of lake phytoplankton to streamwater decreased with nitrate concentration and N:P ratios in streamwater and increased with the concentrations of ammonium, total phosphorus, and soluble reactive phosphorus, indicating that streamwater with higher P but lower N and N:P from degraded sub-catchments is associated with increased impact on P-limited Lake phytoplankton. Overall, this study suggests that grassland degradation has the potential to differentially influence the nutrients delivered to streams with substantial increases in P but decreases in N and N:P, alleviating P limitation of stream periphyton and, ultimately, stimulating P-limited phytoplankton growth in the lake.

Keywords degradation, ecological stoichiometry, Qinghai-Tibet Plateau, periphyton, phytoplankton, NDVI

Introduction

Grasslands account for 40.5% of world's land surface (excluding Greenland and Antarctica) and are among the largest terrestrial biomes in the world (Cross et al., 2005; Hamilton et al., 2005; Small et al., 2011). On the Qinghai-Tibet Plateau (QTP), grassland is the dominant landcover accounting for 44% of China's grasslands and 6% of the world's grasslands (Scurlock and Hall, 1998; Tan et al., 2010). As the "Third Pole" of the Earth with an average elevation of >4000m, QTP plays a significant role in maintaining global climate and ecological security and is extremely sensitive and vulnerable to climate change and human activities (Jin et al., 2005; Zhao et al., 2006; Qiu, 2008; Yao et al., 2012). Nutrient processing and soil erosion prevention are among the most important ecological functions of grassland ecosystem (Daily, 1995; Conant et al., 2001; Zhang et al., 2011b; Dlamini et al., 2014; Su et al., 2015). In recent decades, however, the QTP grassland has experienced serious degradation due to numerous driving forces, such as climate change and overgrazing (Harris, 2010). In the Qinghai Lake watershed, half of the grassland was classified as degraded in 2010 (Luo et al., 2013). Changes in landcover significantly affect the structure, function, and element balances of terrestrial ecosystems (Tischer et al., 2014). As degradation increases, aboveground vegetation coverage and biomass decline, leading to simultaneous impairment of soil physical, chemical, and biological properties, such as declines in soil moisture, soil nutrients, perturbation of microbial communities, as well as increases in soil erosion (Abril and Bucher, 1999; Viragh et al., 2011; Li et al., 2012; Dlamini et al., 2014).

The impacts of landcover are important for streams and lakes because aquatic and terrestrial ecosystems are highly connected (Williamson et al., 2008). Landcover changes lead to modifications of terrestrial biogeochemistry and, as a consequence, alter aquatic biogeochemistry (Figueiredo et al., 2010; Nielsen et al., 2012). As the primary receiver of nutrients and organic matter inputs from terrestrial ecosystems and the main vector linking upland watersheds with downstream aquatic ecosystems (Vannote et al., 1980; Cronan, 2012), streams play a critical role in biogeochemical processes (Figueiredo et al., 2010; Deegan et al., 2011) and are particularly sensitive to terrestrial change (Williamson et al., 2008; Bring and Destouni, 2009; Holmes et al., 2012). Aquatic impacts of changing landcover can often first be observed via their effects on stream periphyton, the biofilm including attached algae, bacteria, and fungi. In a previous study, periphyton biomass and stoichiometry in streams were strongly affected by a gradient of land-use change from urban to rural (O'Brien and Wehr, 2010). Understanding such impacts is important because periphyton plays a vital role in stream ecosystems (Godwin and Carrick, 2008; Larned, 2010), contributing substantially to primary production and thus to benthic consumers as a major food resource (Mayer and Likens, 1987; Hall et al., 2001). Moreover, periphyton is integral in biogeochemical cycling in stream ecosystems through nutrient uptake, transfer of nutrients to higher trophic levels, and remineralization (Schiller et al., 2007). Periphyton community structure and primary production are influenced by many hydrological and physicochemical factors, such as nutrient availability, light intensity, water temperature, and flow velocity (Schiller et al., 2007; Fanta et al., 2010; Battin et al., 2016), all of which can be impacted by land use change. For example, in degraded grassland systems, losses of nutrients can increase due to erosion (Wen et al., 2013; Wang et al., 2014). In streams flowing through grassland without a forest canopy, nutrient availability has a particularly important role in periphyton growth and thus understanding impacts of land use on nutrient flows is important for such systems.

Streams and rivers receive nutrients (such as nitrogen and phosphorus) and organic matter exported from terrestrial ecosystems and are strongly influenced by the landscapes through which they flow (Bring and Destouni, 2009; Holmes et al., 2012). As a result, lakes have an intimate relationship with catchment characteristics through materials transported by surface runoff (Zhang, 2011a; Canham et al., 2012; Cronan, 2012; Sadro et al., 2012). Thus, changes in catchment landcover can alter the export of carbon and nutrients from catchments to lake ecosystems (Abell et al., 2011; Erol and Randhir, 2013; Umbanhowar et al., 2015) and can indirectly shift nutrient status and limitation of lake ecosystems (Dodson et al., 2005; Hoffmann and Dodson, 2005; Vanni et al., 2011). For example, in a set of catchment-lake systems in three contrasting catchments (forested, agricultural, and mixed), land use practices strongly influenced rates and ratios of exported nutrients and thus influenced nutrient stoichiometry and limitation (Vanni et al., 2011).

In ecosystems across the globe, both nitrogen (N) and phosphorus (P) are growth-limiting nutrients for many organisms (Elser et al., 2001; Sterner and Elser, 2002; Elser et al., 2007) and shape the structure and function of both aquatic and terrestrial ecosystems (Schlesinger and Bernhardt, 2013). Landcover changes will not only alter terrestrial biogeochemical processes but also have strong potential influences on nutrient limitation and stoichiometry in aquatic ecosystems. Ongoing climate change and expansion of human activities such as livestock grazing have significantly affected grassland ecosystems across the world (Suttie et al., 2005; Wiesmair et al., 2016). Indeed, the Qinghai Lake watershed is facing serious grassland degradation (Luo et al., 2013). However, how grassland degradation influences linked stream-lake ecosystems is poorly known. In this study, we coupled intensive nutrient sampling with bioassay experiments for both stream periphyton and lake phytoplankton to assess the cascading influences of grassland status on nutrient dynamics and limitation of primary producers in the Qinghai Lake and its inflow streams. Our objectives were to address the following questions: (1) How do stream nutrients respond to grassland degradation? (2) How do grassland degradation impact on nutrient supply alter stream periphyton nutrient limitation? (3) How do lake phytoplankton respond to stream inflows as a function of grassland degradation? Based on the hypothesis that grassland degradation differentially alters inputs of N and P to streams, we predicted that, across a set of streams in sub-catchments differing the degree of grassland degradation: (1) concentrations of total and dissolved inorganic nitrogen and phosphorus will be differentially associated with sub-catchments grassland degradation status and thus N:P ratios of nutrient pools will change with grassland degradation; (2) nutrient limitation of stream periphyton will be a function of degradation-associated variation in stream nutrients; (3) lake phytoplankton will be differently stimulated by stream inflows from different sub-catchment as a function of sub-catchment grassland degradation status.

Methods

Study area

This study was conducted in the Qinghai Lake watershed, which is located on the northeast edge of the Qinghai-Tibet Plateau (QTP; Figure 4.1). Qinghai Lake is the largest lake in China, with a surface area of 4,260 km^2, a catchment area of 29,660 km^2, and an average depth of 21 m (Li et al., 2007). The elevation of the lake surface is 3,192 m. Qinghai Lake is a remote endorheic saline (salinity=11.8‰) lake with more than 40 tributaries, most of which are seasonal with an average salinity of 0.21‰ (Meng et al., 2014). The lake is frozen from late December to mid-March. In the Qinghai Lake watershed, the mean annual precipitation is 362.5 mm but annual evapo-transpiration is 1,300-2,000 mm (Yi et al., 2010). The precipitation in summer (June to August) accounts 65% of the annual precipitation (Cao et al., 2013). Air temperatures range from -31 °C in January to 28 °C in July on average. Mean annual temperature is -0.7°C (Yi et al., 2010). June to mid-September is the growing season for terrestrial vegetation while late September to May is the plant-withering season (Liu and Jiang, 2004). Based on previous studies (An et al., 2006; Hao, 2008; Li et al., 2009), terrestrial and aquatic ecosystems in the Qinghai Lake area are sensitive to global climate change and other anthropogenic impacts. Grassland is the main landcover type in the Qinghai Lake watershed, accounting for 75% of land area. This grassland is deteriorating due to intensive livestock grazing (yak and sheep) and climate change (increasing temperature and evapotranspiration), with degraded grassland accounting for 37% of the whole watershed area in 2010 (Luo et al., 2013). Grassland degradation refers to retrograde succession of structure and function of a grassland, in comparison to its original quasi-equilibrium state with specific manifestations such as declines of biodiversity, loss of stability and resilience, and declines of above-ground vegetation productivity. It has been demonstrated that the climate warming rate on the QTP has been three times the global rate over the last 50 years (Piao et al., 2011). This climate warming has caused desiccation and the loss of permafrost and seasonally-frozen soil, substantially contributing, along with intensive livestock grazing, to deterioration of degradation on the QTP (Harris, 2010).

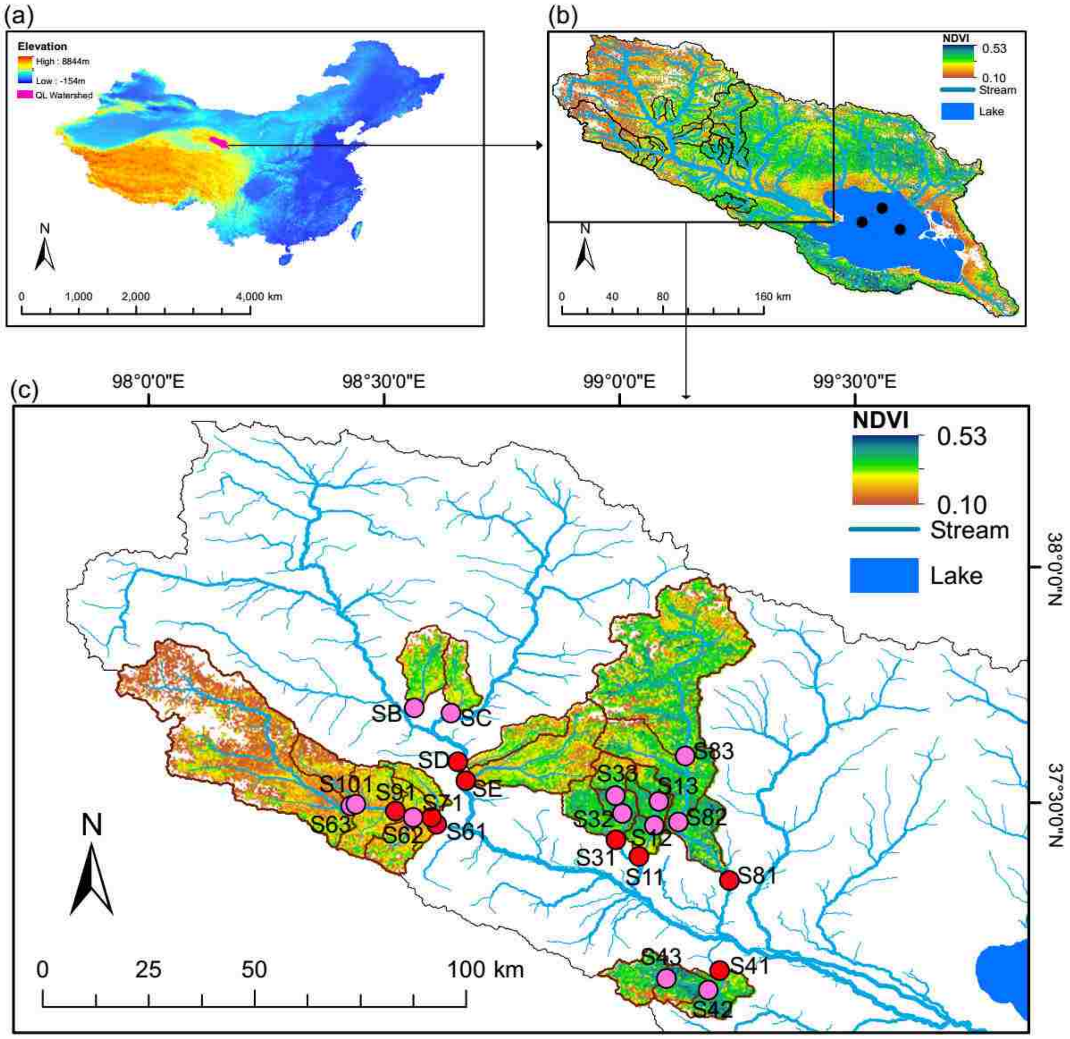

Figure 4.1 The location of the study area. (a) Qinghai Lake watershed is located on the northeast edge of the Qinghai-Tibet Plateau. The map is colored by elevation. Cool colors represent low elevation and warm colors represent higher elevation (b) Lake samples were collected at 3 sites (black circles) in the lake. The map is colored by NDVI. Cool colors represent low degradation and warm colors represent higher degradation. (3) Stream samples were collected at 22 sites (pink and red circles) with different grassland status in their sub-basins with nutrient diffusing substrata (NDS) bioassays conducted at 9 sites (red circles) to determine the potential nutrient limitation of periphyton. Streamwater samples

were collected for the streamwater bioassay at the same 9 sites to determine lake phytoplankton responses to stream inflows. The maps were created in ArcGIS 14.0 (http://desktop.arcgis.com/en/arcmap/) using ASTER GDEM images for the elevation and Landsat images for NDVI value. All the images were download from USGS (https://earthexplorer.usgs.gov/)

Field sampling and sample analyses

We collected samples from 22 stream sites and 3 lake sites in July 2017, September 2017, and April 2018 (Figure 4.1) for a total of 66 stream samples and 9 lake samples. The basic information of the studied lake and streams is summaried in Table S4.1 and Table S4.2. Streamwater samples were collected at the depth of 10 cm and lake water samples were collected at the depth of 0.5 m. Total nitrogen (TN) was quantified by ion chromatography after persulfate oxidation (EPA 300.0). Total phosphorus (TP) was analyzed using the ascorbate acid colorimetric method after oxidation (EPA 365.3). Water samples used for analyzing nitrate (NO_3^--N), ammonium (NH_4^+-N), and soluble reactive phosphorus (SRP) were filtered using glass fiber (GF/F) filters. NO_3^--N was determined by ion chromatography (EPA 300.0). NH_4^+-N was determined using the indophenol colorimetric method (EPA 350.1). Soluble reactive phosphorus (SRP) was quantified using the ascorbate acid colorimetric method (EPA 365.3).

Normalized difference vegetation index

To address objective 1, we compared stream nutrients to normalized difference vegetation index (NDVI), a measure of landscape degradation, to assess how stream nutrients are influenced by grassland degradation. Normalized difference vegetation index (NDVI) is the most commonly used vegetation index and is proposed as a measure of landcover status and an inverse measure of land degradation (Thiam, 2003; Yengoh et al., 2014). High values of NDVI indicate healthy vegetation while low NDVI is indicative of degraded conditions.

In the Qinghai Lake watershed, grassland status was measured by NDVI using Landsat images downloaded from USGS (https://earthexplorer.usgs.gov/). Landsat images acquired in July from 1986 to 2017 were used to assess the dynamics of grassland status in the past decades. For each sub-basin, the average NDVI was calculated as the mean NDVI of each pixel (30 m×30 m) in the sub-basin. The average NDVI for 2017 (NDVI.17, Table S4.1) was used to represent the current grassland status of the study area and was used in the following analyses.

To verify the relationships between NDVI and vegetation status, *in situ* grass species richness, aboveground biomass, and fractional vegetation coverage were measured in July 2017. In total, 19 vegetation samples (1 m × 1 m plot with three replicates) were collected close to the stream sites. Correspondingly, the NDVI of 2017 was calculated for each of these vegetation sample sites. The relationship between NDVI difference and geographic distance of paired sample sites was statistically significant ($P=0.002$) but explained little variance ($R^2=0.039$), indicating that potential spatial non-independence of the observations should not strongly complicate the space-for-time approach.

Nutrient diffusing substrata bioassay

To address objective 2, we used nutrient diffusing substrata (NDS) bioassays to assess potential nutrient limitation of periphyton in a subset of 9 streams spanning a gradient of grassland degradation in July 2017, September 2017, and April 2018. NDS are widely used to test potential nutrient limitation of biofilms in streams (Gibeau and Miller, 1989; Tank and Dodds, 2003; Scott et al., 2009; Hauer and Lamberti, 2011). NDS were constructed based on the design of Gibeau and Miller (1989), using 50-mL centrifuge tubes filled with 20 g/L agar solution (2% agar by weight) for four treatments: untreated controls (Control), nitrogen addition (+N), phosphorus addition (+P), and combined nitrogen and phosphorus addition (+NP) (Figure S4.1). For the +N treatment, a concentration of 50.6 g/L KNO_3 was added to the pre-autoclaved base agar solution resulting in 0.5 mol/L N. For +P treatment, a concentration of 68 g/L KH_2PO_4 was added resulting in 0.5 mol/L P. For +NP, 50.6 g/L KNO_3 and 68 g/L KH_2PO_4 were added for 0.5 mol/L N and P (Tank and Dodds, 2003; Hauer and Lamberti, 2011). We cut 20-mm diameter holes in the caps of 50-mL centrifuge tubes and placed a GF⁄F glass fiber filter (25-mm diameter) (Tank and Dodds, 2003) across the top of the tubes to cover the agar completely and serve as a growth substrate for periphyton colonization that enabled nutrients to diffuse from the agar medium through the filter. NDS tubes were placed on a rack and fixed on the streambed with the tube caps at a depth of 10 cm.

The NDS bioassay was conducted in 9 streams during the field sampling in July and September in 2017 and April in 2018 (Figure 4.1). The sub-basins of these 9 streams have different grassland status according to NDVI. In each stream, a full nitrogen x phosphorus factorial experiment (with five replicates) was established and incubated for one week. Laboratory assays have previously shown that the rate of nutrient diffusion from the 2% agar cups is constant through 17 days and then declines only slightly until day 21 (Tank and Dodds, 2003). At the end of the incubation period, filters were collected and frozen until analysis for chlorophyll-*a* (Chl-*a*). Chl-*a* was extracted into 10 mL 90% buffered acetone and measured using a spectrophotometer (EPA 445.0). To facilitate comparison of bioassay results across sites and sample dates, results were summarized by calculating the relative responses of primary producer biomass (Chl-*a*) to the addition of N (RR-N), P (RR-P), and NP (RR-NP) by normalizing Chl-a of each treatment (Chl-X) to the control (Chl-C): RR-X = Chl-X/Chl-C, where X is N, P, or NP. An RR value of 1 indicates no response to nutrient supplementation.

Nutrient limitation bioassay for lake phytoplankton

In order to clearly understand the responses of lake phytoplankton to stream inflows as a function of grassland degradation, we need to identify the primary limiting nutrient (N vs P) for lake phytoplankton growth. Thus, nutrient limitation bioassays for lake phytoplankton were conducted in July 2017, September 2017, and April 2018 (Figure S4.2a). Clean plastic bottles were filled with 500 mL of 62-µm screened lake water. Four treatments in triplicates were established: untreated controls (Control), nitrogen addition (+N), phosphorus addition (+P), and combined nitrogen and phosphorus addition (+NP). Nutrients were added to the bottles to increase N-concentrations by 100 µg/L (7.2 µmol/L) and/or P-concentrations by 10 µg/L (0.3 µmol/L) (Tank and Dodds, 2003). N was added as KNO_3 and P as KH_2PO_4. Bottles were incubated as described before. After incubation, the water in each bottle was filtered onto a GF/F filter and then frozen for Chl-a analysis. The relative response of each treatment (RR-X) was calculated as RR-X = Chl-X/Chl-C, where X is N, P, or NP.

Streamwater bioassay for lake phytoplankton

To address objective 3, streamwater bioassays (Figure S4.2b) were conducted in July 2017, September 2017, and April 2018 to compare the relative impacts of water from various streams in stimulating lake phytoplankton production. Lake water collected from the three lake sampling locations was pooled together in equal proportions to be used in the following bioassay. First, clean plastic bottles were filled with 400 mL of 62-µm screened lake water (62-µm LW). Then, 100 mL of 0.2-µm filtered lake water (0.2-µm LW) was added as the control and 100 mL aliquots of 0.2-µm filtered streamwater (0.2-µm SW_n) from 9 streams (S11, S31, S41, S61, S71, S81, S91, SD, and SE in Figure 4.1) were added separately as different treatments (Treatment-n). Each treatment was performed in triplicate and incubated in a light-temperature board held at temperatures similar to the lake environment at 0.5-m depth where the lake water samples were collected. Bottles were shaken twice daily. After 72 h incubation, the water in each bottle was filtered onto a GF/F filter and then frozen for later Chl-a analysis. Chl-*a* was extracted into 10 mL 90% buffered acetone and measured using a spectrophotometer (EPA 445.0). To compare across treatments the relative response of each treatment (RR-SW_n) was calculated as RR-SW_n = Chl-SW_n/Chl-C, where Chl-SW_n was the Chl-a concentration of each treatment and Chl-C was the Chl-a concentration of the control.

Statistical analyses

Linear regression was used to verify the relationships between NDVI and *in situ* vegetation status, as well as to assess the relationships between elevation and vegetation factors. Standard major axis (SMA) regression and curve estimation (inverse function) were used to assess the relationships between NDVI and streamwater nutrient concentrations and relative responses of stream periphyton, as well as between streamwater nutrient concentrations and relative responses of stream periphyton and lake phytoplankton. The regressions were selected based on Akaike Information Criterion (AIC). AIC is used to estimate the relative quality of models fit for a same set of data by estimating the relative amount of information lost by each model. The less information lost by a model (lower AIC), the higher quality of that model. Regression results with P-values between 0.05 to 0.10 were presented for cases where the relationship was concordant with other regressions for which $P<0.05$. In doing this, we follow to the recommendations of the American Statistical association (Wasserstein and Lazar, 2016) to avoid blind adherence to "mechanical 'bright-line' rules (such as $P<0.05$)". However, most regressions had $P<0.05$, providing the primary basis for our interpretations. T-tests were conducted for all experiments to assess treatment differences from the control in stream NDS experiments and in the lake bioassays. ANOVA analyses with *post hoc* pairwise comparison and P-value correction (Benjamini-Hochberg method; Benjamini and Hochberg, 1995) were conducted to test whether the relative responses of stream periphyton to each nutrient treatment differed between different seasons and to compare the effects of enrichment on stream periphyton and lake phytoplankton. All analyses were conducted using R 3.5.1 (R Core Team, 2017).

Results

Spatial and temporal variation of grassland status quantified using NDVI

NDVI and *in situ* vegetation status had strong linear relationships (Figure S4.3), supporting the use of NDVI to indicate grassland status in this study. Moreover, vegetation did not have clear relationships with elevation (Figure S4.4), also supporting the use of NDVI to indicate grassland gradation across the study area. The grassland status of each sub-basin was quantified using average NDVI of the sub-basin from 1986 to 2017 (Figure 4.2a). In these sub-basins, NDVI decreased 39.5% on average from 2009 to 2017 (Figure 4.2a), suggesting substantial grassland degradation. Across the study area, NDVI values in 2017 (NDVI.17) indicate that current grassland status of the study sub-basins had large spatial variation (Figure 4.2b). Sub-basin S32 had the highest NDVI.17 (0.344) while sub-basin S63 had the lowest (0.167).

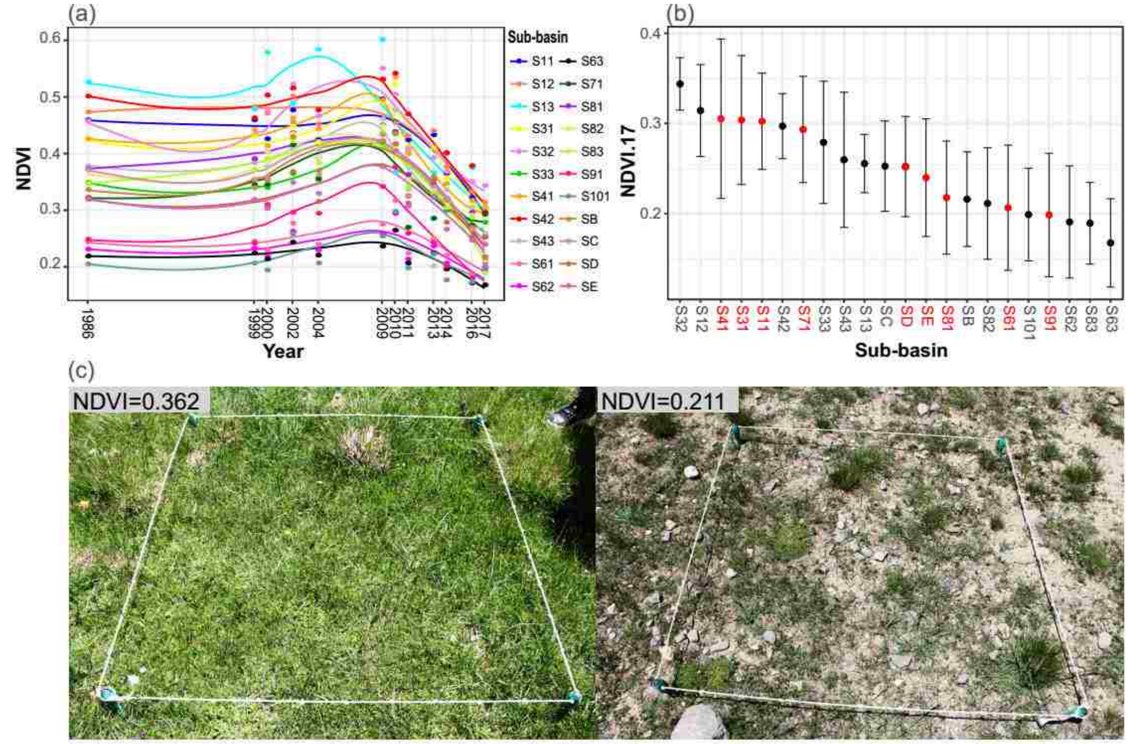

Figure 4.2 Temporal and spatial variation of grassland status quantified using normalized difference vegetation index (NDVI), an inverse proxy for grassland degradation (low NDVI indicates degraded conditions). (a) NDVI of each sub-basin from 1986 to 2017. (b) NDVI value in 2017 indicating current grassland status of the study sub-basins. The dot represents the mean value and the whisker represents standard deviation. Sub-basins are ranked by NDVI.17 value from high to low and the red represents the sub-basins targeted for nutrient diffusing substrata (NDS) bioassays to determine the potential nutrient limitation of periphyton and for streamwater bioassays to determine lake phytoplankton responses to stream inflows. (c) Field pictures showing i*n situ* grassland status and corresponding NDVI value.

Influences of grassland status on streamwater nutrients

If the grassland degradation hypothesis is correct, then we predict that nutrient concentrations and ratios of streamwater would be associated with NDVI across the study sub-basins. In streamwater, NO_3^--N was the major nitrogen component, accounting for 73% of TN on average. Regression analyses showed that nitrogen concentrations (especially TN and NO_3^--N) in streamwater decreased with decreased NDVI.17, although NH_4^+-N increased with decreased NDVI.17 in July but decreased in September (Figure 4.3 and Table S4.3). In contrast, SRP only accounted for 29% of TP on average. Concentrations of both TP and SRP increased with decreased NDVI.17 in September (Figure 4.3 and Table S4.3). These results indicate that grassland degradation (decreasing NDVI) tended to increase streamwater P (as seen in September) but to consistently reduce N in streamwater. Consistent with these differential shifts in N and P, stoichiometric ratios of TN:TP decreased with decreasing NDVI.17 in September ($P<0.05$) and DIN:SRP decreased in July ($P = 0.09$) and September ($P=0.001$) (Figure 4.3 and Table S4.3). Overall, these data indicate that grassland degradation (as indicated by decreasing NDVI) was associated with higher P concentrations (in September), strongly lower N concentrations, and lower N:P ratios (especially DIN:SRP) in streamwater. These results partially match the predictions.

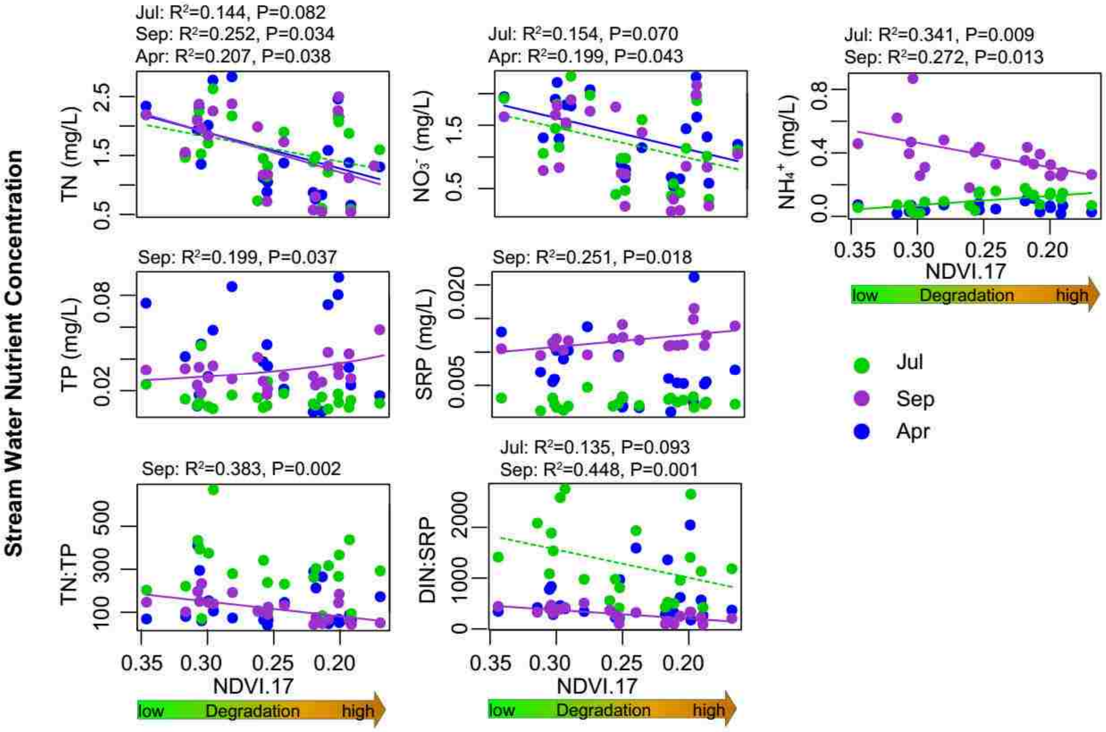

Figure 4.3 Regression analyses showing the relationships between grassland conditions (NDVI.17) and nutrient concentrations and stoichiometric ratios of streamwater in the sub-basins in July 2017, September 2017, and April 2018. Each point represents one sample with three replicates. The solid lines represent relationships with $p<0.05$ while dashed lines indicate relationships with $0.05<p<0.10$.

Nutrient limitation of stream periphyton and its responses to streamwater nutrients

Seasonality of nutrient limitation

In NDS experiments, stream periphyton had stronger relative responses to P and NP enrichment in July than in September and April (Figure S4.5 and Table S4.4). Moreover, P and NP enrichment produced stronger responses than N enrichment in July. In September and April, however, NP enrichment produced larger responses than single nutrient enrichment (Figure S4.5 and Table S4.5). Stream periphyton shifted from predominant P limitation in July to N-P colimitation in September and April (Figure S4.5). Regression analyses showed that there were no relationships ($P>0.10$) between the relative responses of stream periphyton to nutrient additions (RR-X) and sub-catchment average NDVI, suggesting that nutrient limitation of stream periphyton was not tightly associated with grassland status (but see below).

Influences of streamwater nutrients on nutrient limitation of stream periphyton

If the grassland degradation hypothesis is correct, then we predict that nutrient limitation of stream periphyton would be associated with NDVI across the study sub-basins. While stream periphyton responses to nutrient enrichment were not closely associated with NDVI itself, stream periphyton responses to P and NP enrichments (RR-P and RR-NP, respectively) declined with increasing streamwater concentrations of TP and SRP (Figure 4.4 and Table S4.6), which themselves increased consistently with declining NDVI (Figure 4.3). Stream periphyton also showed decreased responses to P and NP enrichments with decreasing streamwater TN:TP and DIN:SRP (Figure 4.4 and Table S4.6). Overall, these data indicate that increasing TP and SRP concentrations and declining TN:TP and DIN:SRP ratios in streamwater in degraded sub-catchments (Figure 4.3) were associated with reduced responses of stream periphyton to P addition as well as to combined NP addition. This suggests that grassland degradation potentially alleviated P limitation and N-P colimitation of periphyton in these Qinghai basin streams. These results tentatively match the predictions.

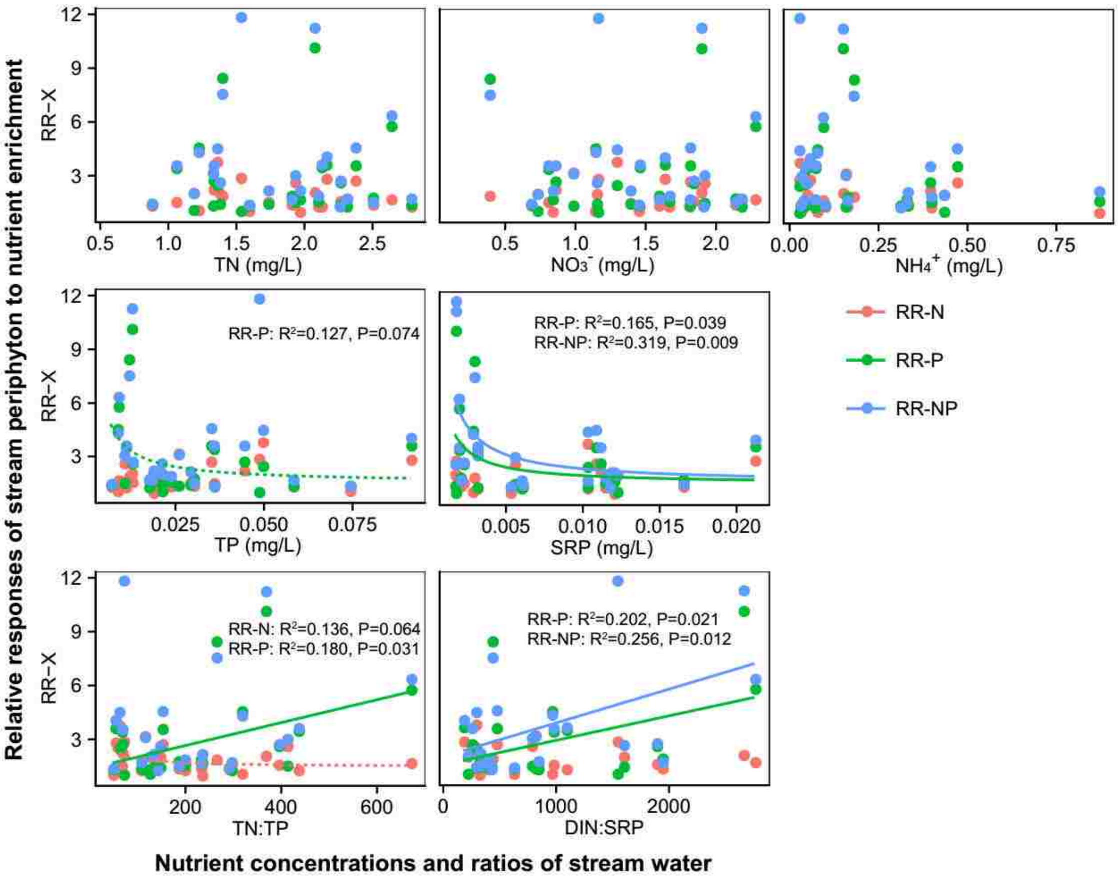

Figure 4.4 Regression analyses between the relative responses of stream periphyton to nutrient enrichment (RR-N, RR-P, RR-NP) and ambient stream nutrient concentrations in nine streams spanning a grassland degradation gradient. Data represent results from nutrient diffusing substrata bioassay experiments (NDS) and ambient streamwater nutrient concentrations in July 2017, September 2017 and April 2018. Each point represents the mean value of 5 replicates. X-axis represents the nutrient concentrations and ratios of streamwater. Y-axis represents the relative responses (RR-X) of stream periphyton to nutrient enrichment. The solid lines represent relationships with $p<0.05$. The dashed lines represent relationships with $0.05<p<0.10$.

Nutrient limitation of lake phytoplankton and its responses to stream inflows

If the grassland degradation hypothesis is correct, then we predict that the responses of lake phytoplankton to stream inflows would be associated with NDVI across the study sub-basins. Lake phytoplankton responded more strongly to phosphorus addition (RR-P) than to nitrogen addition (RR-N) and the response to combined phosphorus and nitrogen addition (RR-NP) was close to RR-P, suggesting that the phytoplankton growth in Qinghai Lake was strongly P limited (Figure 4.5h and Table S4.7). These results help in interpreting the streamwater bioassays as they indicate that lake phytoplankton should respond most strongly to streamwater with higher concentrations of P and having low N:P ratios. Regression analyses showed that the relative responses of lake phytoplankton to streamwater (RR-SW) from different catchments were related to nutrients in that streamwater, decreasing with its NO_3^--N, increasing with its concentrations of NH_4^+-N, TP, and SRP, and decreasing with its TN:TP and DIN:SRP ratios (Figure 4.5 and Table S4.6). Overall, these results indicate that low TN:TP and DIN:SRP streamwater from low-NDVI (degraded) sub-catchments (Figure 4.3) more strongly stimulated growth of P-limited Qinghai Lake phytoplankton. These results tentatively match the predictions.

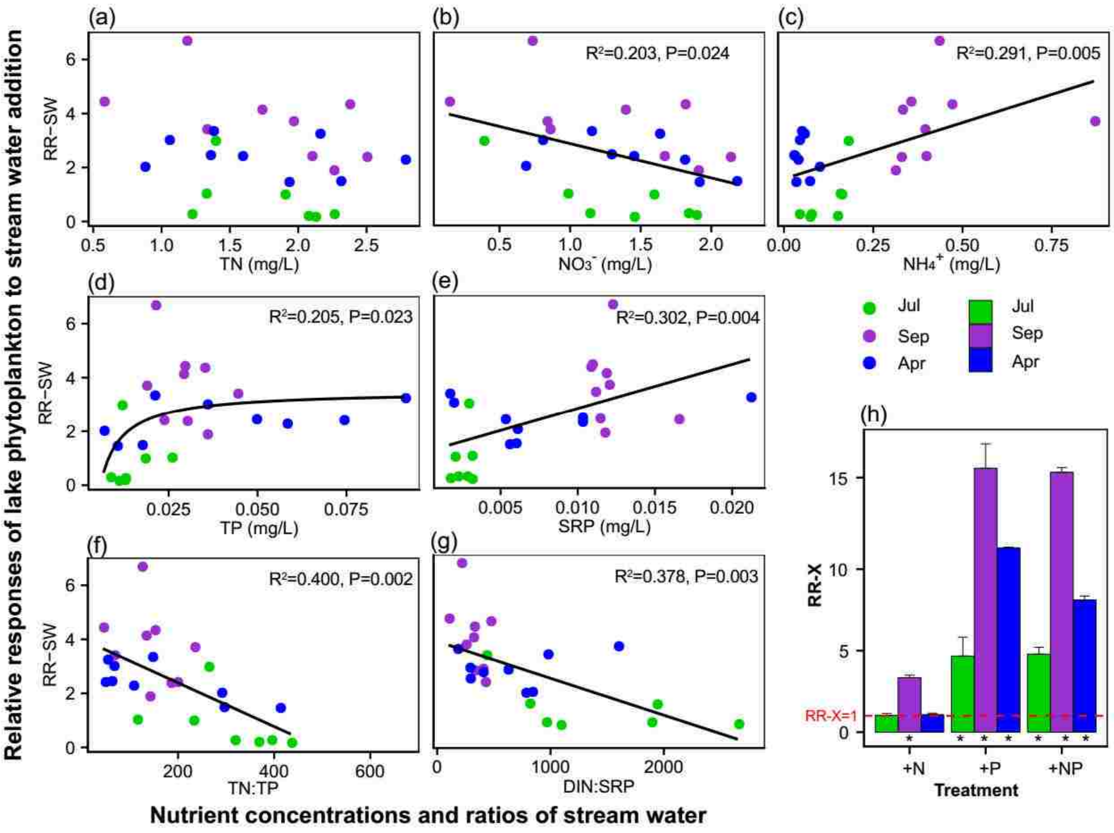

Figure 4.5 (a-g) Regression analyses for the relative responses of lake phytoplankton to the addition of streamwater from different sub-basins in July 2017, September 2017, and April 2018. The x-axis represents nutrient concentrations of the streamwater used. Y-axis (RR-

SW) represents the relative responses of lake phytoplankton to streamwater addition. Each point represents one bioassay with three replicates. The solid line represents relationships with $p<0.05$. (h) Nutrient limitation bioassays for the phytoplankton in Qinghai Lake. Y-axis (RR-X) represents the relative responses of lake phytoplankton to nutrient enrichment. The whisker represents the standard deviation (n=3 for each treatment). "*" represents $P<0.05$ for t-test of RR-X=1 for all responses.

Discussion

Our analyses show that NDVI varies spatially in the Qinghai Lake basin and has decreased rapidly in the past decade, suggesting acute grassland degradation in some areas with rapid degradation across the whole watershed. This degradation appears to be associated with large-scale changes in nutrient cycling in the basin, as N concentrations and N:P ratios in streamwater decreased while P concentrations increased with decreases in NDVI (a proxy for grassland degradation). These results suggest that grassland degradation (low NDVI) has the potential to differentially decrease N availability and increase P availability in streams, resulting in decreased N:P ratios, especially in early and late growing seasons. These changes can be understood as grassland degradation impacts soil properties and vegetation in the watershed, which alters terrestrial biogeochemical processes, and, as a consequence, alters aquatic biogeochemistry (Figueiredo et al., 2010; Nielsen et al., 2012). More specifically, we suggest that, as grassland degradation increases, aboveground vegetation declines together with deterioration of soil physical, chemical, and biological properties, such as soil moisture, nitrogen content, and microbial activity (Abril and Bucher, 1999; Viragh et al., 2011; Li et al., 2012; Dlamini et al., 2014). Soil and vegetation as well as soil microbes are the key factors influencing nutrient accumulation and export (Canham et al., 2012), which then influence nutrient availability and movement potential in a watershed (Fraterrigo and Downing, 2008). It has been demonstrated that high grass productivity increases soil N stocks by N fixation and enhances N mineralization (Fornara and Tilman, 2008; Oelmann et al., 2011; Cong et al., 2014). N is transported from top soil to deep soil and streamwaters predominantly via inorganic forms, such as NO_3^--N due to their high mobility (Perakis and Hedin, 2002). This scenario is supported in our study by our observation that N in streamwater was mainly in the form of NO_3^--N and had high concentrations in the streams with high NDVI (intact grassland) in the catchment. In degraded grassland ecosystems, low plant productivity likely leads to low N content in soil and thus less N is available to be lost to streams. Moreover, in degraded grassland ecosystems, loss of vegetation and deterioration of soil texture aggravate soil erosion, enhancing the loss of soil nutrients and especially particle-bound P to adjacent aquatic ecosystems (Dong et al., 2012; Su et al., 2015). For example, P generally enters aquatic ecosystems through surface runoff and soil erosion by binding to soil particles and organic matter (Jacobson et al., 2011) and thus particulate P is often the most important P loss from catchments to aquatic ecosystems (Pärn et al., 2012). In our study, SRP only accounted for 29% of TP on average, suggesting particulate P appears to account for a large proportion of TP in stream (samples for analysis of dissolved organic P pools are not available). Thus, in our study, grassland degradation was associated with increased P but decreased N and N:P in streamwater in general.

In our study, stream periphyton was mainly limited by P in July and co-limited by N&P in September and April. However, the responses of stream periphyton to P addition and combined N and P addition appeared to be dampened in streams in low-NDVI sub-basins, likely because grassland degradation differentially increased P availability and decreased N availability in streamwaters. In stream ecosystems, periphyton are tightly linked to watershed conditions because landcover significantly controls the export of C, N, and P from catchments to aquatic ecosystems, potentially shifting *in situ* periphyton community structure and influencing primary production (Abell et al., 2011; Erol and Randhir, 2013; Umbanhowar et al., 2015). Previous studies have suggested that the community composition, biomass, and stoichiometry of stream periphyton change significantly in response to landcover-induced changes in available nutrients (Fanta et al., 2010; Hill et al., 2011; Drake et al., 2012). C:N and C:P ratios of stream periphyton generally decrease with increasing nutrient availability, shifting the nutrient limitation regime (Frost et al., 2005; Kohler et al., 2011). Thus, our results suggest that grassland degradation differentially affecting nutrients in streamwater, potentially alleviating P limitation of stream periphyton.

Nutrient enrichment bioassays indicated that phytoplankton growth in Qinghai Lake was strongly P limited. Previous research has shown that Qinghai Lake has relatively high concentrations of nitrogen and carbon (both DIC and DOC are high) but very low phosphorus concentrations (Yang et al., 2005; Zhang et al., 2010; Ao et al., 2014; Meng et al., 2014), consistent with P limitation of the lake phytoplankton as we observed. It is generally recognized that P is usually the main nutrient limiting lake primary production (Schindler, 1974, 1977; Sterner, 2008) because P has no gaseous atmospheric cycle and there are no biological mechanisms to redress P deficiencies (Schindler, 1974, 1977; Sterner, 2008). Lake ecosystems respond rapidly to nutrient alterations caused by land use change (Vanni et al., 2011), particularly in oligotrophic lakes where a small change in absolute concentration can mean a large change in relative availability (Brahney et al., 2015). Relatively subtle changes in the supplies of N and P might shift phytoplankton between N- and P-limited growth (Elser et al., 2009) or primary producers might be synergistically limited by supplies of N and P (Elser et al., 2007; Harpole et al., 2011). Our stream data suggest that grassland degradation can differentially affect the nutrients delivered to streams by increasing P but decreasing N and N:P, leading us to expect that P-limited Qinghai Lake phytoplankton would be more strongly stimulated by streamwater from sub-catchments with low NDVI (higher grassland degradation). Consistent with this, the response of phytoplankton in Qinghai Lake to streamwater amendment was positively related with streamwater P concentrations and negatively with NO_3^--N concentration and N:P ratios, supporting a conclusion that grassland degradation alleviates P limitation of the lake and stimulates P-limited lake phytoplankton growth. These impacts are important because, in lakes, phytoplankton form the base of food webs supporting higher trophic levels (Elser et al., 2010). The growth, reproduction, elemental composition, and even community structure of primary producers strongly depends on the supplies of N and P (Sterner and Elser, 2002; Klausmeier et al., 2004). Moreover, according to the concepts of ecological stoichiometry (Sterner and Elser, 2002), P limitation of primary production can create stoichiometric imbalances between consumers and primary producers and transfer P limitation to herbivores (Elser et al., 2000, 2009), influencing the herbivore success, the stability of aquatic food webs, and a variety of other ecological processes (Elser et al., 2002; Sterner and Elser, 2002; Mueller et al., 2007; Rellstab et al., 2007). Thus, grassland degradation has the potential to affect lake ecosystem structure and function by shifting nutrient limitation patterns of primary producers.

Qinghai Lake is saline and has high pH. Thus, our results may not pertain to other lakes of lower salinity and neutral pH. Indeed, pH is a vital variable in aquatic systems affecting nutrient uptake and controlling chemical species of most nutrient ions for primary producers (Tilman et al., 1982) while salinity also affects phytoplankton communities in lakes (Larson and Belovsky, 2013; Wurtsbaugh and Barnes, 2015). However, saline lakes represent 45% of the volume of all inland lakes in the world (Van der Leeden, 1990). For example, there are many saline lakes in arid regions, such as the Aral Sea and Lake Balkhash in Central Asia and Great Salt Lake and Salton Sea in the USA. On the Qinghai-Tibet Plateau, saline lakes make up 42% of the total area of all lakes (Zheng, 1997). Thus, our study is potentially important regionally and globally.

Our study addressed the objectives we proposed originally by highlighting several potential influences of grassland degradation on nutrient concentrations and nutrient limitation in aquatic ecosystems in the grassland watershed of Qinghai Lake. However, more in-depth work is required to overcome some of our study's limitations. For example, this study was conducted in a 1-year timeframe and neglects potential inter-annual variation. Longer-term assessments can avoid the potential influence of random climatic events and reveal effects of grassland degradation across a wider temporal scale. Moreover, in addition to the influence of catchment properties on nutrient concentrations and ratios in stream (Frost et al., 2009), watershed discharge is another important factor influencing the balance of elements in streams, leading to seasonal variations (Frost et al., 2009). More intensive intra-annual sampling and experimentation would be useful in establishing seasonal patterns of grassland influence on stream and lake nutrient limitation.

Conclusions

Aquatic ecosystems are facing increasing pressures and threats from disturbances of landcover in upland watersheds due to global climate change and human activities. These landcover disturbances are prevalent in grassland ecosystems where climate change and human activities such as livestock grazing contribute to widespread degradation. Such landcover changes not only alter terrestrial biogeochemical processes but can also affect nutrient concentrations and stoichiometry and nutrient limitation in the aquatic ecosystems to which those terrestrial habitats are linked. Our study indicates that stream nutrient concentrations and stoichiometric ratios are affected by grassland conditions in the Qinghai Lake watershed, a degrading grassland ecosystem on the Qinghai-Tibet Plateau. Specifically, N concentrations decline, and P concentrations increase, potentially alleviating P limitation of primary producers both in the basin's streams but also in Qinghai Lake itself, contributing to lake eutrophication. These results provide insights into how landcover disturbances affect nutrient balance and limitation in aquatic ecosystems in a rapidly changing world.

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References

Abell JM, Oezkundakci D, Hamilton DP, Miller SD. 2011. Relationships between land use and nitrogen and phosphorus in New Zealand lakes. Marine and Freshwater Research, 62: 162-175.

Abril A, Bucher EH. 1999. The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. Applied Soil Ecology, 12: 159-167.

An ZS, Ai L, Song YG, Colman SM. 2006. Lake Qinghai scientific drilling project. Scientific Drilling, 2: 20-22.

Ao H, Wu C, Xiong X, Jing L, Huang X, Zhang K, Liu J. 2014. Water and sediment quality in Qinghai Lake, China: a revisit after half a century. Environmental Monitoring and Assessment, 186: 2121-2133.

Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology, 14: 251-263.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57: 289-300.

Bergstrom A, Faithfull C, Karlsson D, Karlsson J. 2013. Nitrogen deposition and warming - effects on phytoplankton nutrient limitation in subarctic lakes. Global Change Biology, 19: 2557-2568.

Bergström AK, Karlsson D, Karlsson J, Vrede T. 2015. N‐limited consumer growth and low nutrient regeneration N:P ratios in lakes with low N deposition. Ecosphere, 6: 1-13.

Brahney J, Mahowald N, Ward DS, Ballantyne AP, Neff JC. 2015. Is atmospheric phosphorus pollution altering global alpine lake stoichiometry? Global Biogeochemical Cycles, 29: 1369-1383.

Bring A, Destouni G. 2009. Hydrological and hydrochemical observation status in the pan-Arctic drainage basin. Polar Research, 28: 327-338.

Canham CD, Pace ML, Weathers KC, McNeil EW, Bedford BL, Murphy L, Quinn S. 2012. Nitrogen deposition and lake nitrogen concentrations: a regional analysis of terrestrial controls and aquatic linkages. Ecosphere, 3: 1-16.

Cao SK, Cao GC, Chen KL, Xie JA, Zhu JF, Wang JM. 2013. Research in the variation characteristics of temperature and precipitation for lately 50 years in the Qinghai Lake basin. Journal of Qinghai Normal University (Natural Science), 2: 37-41.

Conant RT, Paustian K, Elliott ET. 2001. Grassland management and conversion into grassland: effects on soil carbon. Ecological Applications, 11: 343-355.

Cong W, van Ruijven J, Mommer L, De Deyn GB, Berendse F, Hoffland E. 2014. Plant species richness promotes soil carbon and nitrogen stocks in grasslands without legumes. Journal of Ecology, 102: 1163-1170.

Cronan CS. 2012. Biogeochemistry of the Penobscot River watershed, Maine, USA: nutrient export patterns for carbon, nitrogen, and phosphorus. Environmental Monitoring and Assessment, 184: 4279-4288.

Daily GC. 1995. Restoring value to the world's degraded lands. Science, 269: 350-354.

Deegan LA, Neill C, Haupert CL, Ballester MVR, Krusche AV, Victoria RL, Thomas SM, de Moor E. 2011. Amazon deforestation alters small stream structure, nitrogen biogeochemistry and connectivity to larger rivers. Biogeochemistry, 105: 53-74.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma, 235-236: 372-381.

Dodson SI, Lillie RA, Will-Wolf S. 2005. Land use, water chemistry, aquatic vegetation, and zooplankton community structure of shallow lakes. Ecological Applications, 15: 1191-1198.

Dong SK, Wen L, Yi YY, Wang XX, Zhu L, Li XY. 2012. Soil-quality effects of land degradation and restoration on the Qinghai－Tibetan plateau. Soil Science Society of America Journal, 76: 2256-2264.

Drake WM, Scott JT, Evans-White M, Haggard B, Sharpley A, Rogers CW, Grantz EM. 2012. The effect of periphyton stoichiometry and light on biological phosphorus immobilization and release in streams. Limnology, 13: 97-106.

Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecology Letters, 10: 1135-1142.

Elser JJ, Fagan WF, Denno RF, Dobberfuhl DR, Folarin A, Huberty A, Interlandi S, Kilham SS, McCauley E, Schulz KL, Siemann EH, Sterner RW. 2000. Nutritional constraints in terrestrial and freshwater food webs. Nature, 408: 578-580.

Elser JJ, Frost P, Kyle M, Urabe J, Andersen T. 2002. Effects of light and nutrients on plankton stoichiometry and biomass in a P-limited lake. Hydrobiologia, 481: 101-112.

Elser JJ, Hayakawa K, Urabe J. 2001. Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. Ecology, 82: 898-903.

Elser JJ, Kyle M, Steger L, Nydick KR, Baron JS. 2009. Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. Ecology, 90: 3062-3073.

Elser JJ, Peace AL, Kyle M, Wojewodzic M, McCrackin ML, Andersen T, Hessen DO. 2010. Atmospheric nitrogen deposition is associated with elevated phosphorus limitation of lake zooplankton. Ecology Letters, 13: 1256-1261.

Erol A, Randhir TO. 2013. Watershed ecosystem modeling of land-use impacts on water quality. Ecological Modelling, 270: 54-63.

Fanta SE, Hill WR, Smith TB, Roberts BJ. 2010. Applying the light: nutrient hypothesis to stream periphyton. Freshwater Biology, 55: 931-940.

Figueiredo RO, Markewitz D, Davidson EA, Schuler AE, Dos S. Watrin O, de Souza Silva P. 2010. Land-use effects on the chemical attributes of low-order streams in the eastern Amazon. Journal of Geophysical Research, 115: 1-14.

Fornara DA, Tilman D. 2008. Plant functional composition influences rates of soil carbon and nitrogen accumulation. Journal of Ecology, 96: 314-322.

Fraterrigo JM, Downing JA. 2008. The influence of land use on lake nutrients varies with watershed transport capacity. Ecosystems, 11: 1021-1034.

Frost PC, Cross WF, Benstead JP. 2005. Ecological stoichiometry in freshwater benthic ecosystems: an introduction. Freshwater Biology, 50: 1781-1785.

Frost PC, Kinsman LE, Johnston CA, Larson JH. 2009. Watershed discharge modulates relationships between landscape components and nutrient ratios in stream seston. Ecology, 90: 1631-1640.

Gibeau GG, Miller MC. 1989. A micro-bioassay for epilithon using nutrient-diffusing substrata. Journal of Freshwater Ecology, 5: 171-176.

Godwin CM, Carrick HJ. 2008. Spatio-temporal variation of periphyton biomass and accumulation in a temperate spring-fed stream. Aquatic Ecology, 42: 583--595.

Hall RO, Likens GE, Malcom HM. 2001. Trophic basis of invertebrate production in 2 streams at the Hubbard Brook Experimental Forest. Journal of the North American Benthological Society, 20: 432-447.

Hao X. 2008. A green fervor sweeps the Qinghai-Tibetan Plateau. Science, 321: 633-635.

Harpole WS, Ngai JT, Cleland EE, Seabloom EW, Borer ET, Bracken MES, Elser JJ, Gruner DS, Hillebrand H, Shurin JB, Smith JE. 2011. Nutrient co-limitation of primary producer communities. Ecology Letters, 14: 852-862.

Harris RB. 2010. Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. Journal of Arid Environments, 74: 1-12.

Hauer FR, Lamberti GA. 2011. Methods in stream ecology. Second Edition. Academic Press. San Diego, USA.

Hill WR, Rinchard J, Czesny S. 2011. Light, nutrients and the fatty acid composition of stream periphyton. Freshwater Biology, 56: 1825-1836.

Hoffmann MD, Dodson SI. 2005. Land use, primary productivity, and lake area as descriptors of zooplankton diversity. Ecology, 86: 255-261.

Holmes RM, McClelland JW, Peterson BJ, Tank SE, Bulygina E, Eglinton TI, Gordeev VV, Gurtovaya TY, Raymond PA, Repeta DJ, Staples R, Striegl RG, Zhulidov AV, Zimov SA. 2012. Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic ocean and surrounding seas. Estuaries and Coasts, 35: 369-382.

Jacobson LM, David MB, Drinkwater LE. 2011. A spatial analysis of phosphorus in the Mississippi River basin. Journal of Environment Quality, 40: 931-941.

Jin LY, Ganopolski A, Chen FH, Claussen M, Wang HJ. 2005. Impacts of snow and glaciers over Tibetan Plateau on Holocene climate change: Sensitivity experiments with a coupled model of intermediate complexity. Geophysical Research Letters, 32: L1770917.

Klausmeier CA, Litchman E, Daufresne T, Levin SA. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature, 429: 171-174.

Kohler TJ, Murdock JN, Gido KB, Dodds WK. 2011. Nutrient loading and grazing by the minnow *Phoxinus erythrogaster* shift periphyton abundance and stoichiometry in mesocosms. Freshwater Biology, 56: 1133-1146.

Larned ST. 2010. A prospectus for periphyton: recent and future ecological research. Journal of the North American Benthological Society, 29: 182-206.

Larson CA, Belovsky GE. 2013. Salinity and nutrients influence species richness and evenness of phytoplankton communities in microcosm experiments from Great Salt Lake, Utah, USA. Journal of Plankton Research, 35: 1154-1166.

Li S, Verburg PH, Lv S, Wu J, Li X. 2012. Spatial analysis of the driving factors of grassland degradation under conditions of climate change and intensive use in Inner Mongolia, China. Regional Environmental Change, 12: 461-474.

Li XY, Ma YJ, Xu HY, Wang JH, Zhang DS. 2009. Impact of land use and land cover change on environmental degradation in Lake Qinghai watershed, northeast Qinghai-Tibet Plateau. Land Degradation and Development, 20: 69-83.

Li XY, Xu HY, Sun YL, Zhang DS, Yang ZP. 2007. Lake-level change and water balance analysis at Lake Qinghai, west China during recent decades. Water Resources Management, 21: 1505-1516.

Liu BW, Jiang ZG. 2004. Dietary overlap between Przewalski's gazelle and domestic sheep in the Qinghai Lake region and implications for rangeland management. Journal of Wildlife Management, 68: 241-246.

Luo CF, Xu CJ, You HY, Jin SH. 2013. Analysis on grassland degradation in Qinghai Lake Basin during 2000-2010. Acta Ecologica Sinica, 33: 4450-4459 (In Chinese).

Mayer MS, Likens GE. 1987. The importance of algae in a shaded headwater stream as food for an abundant caddisfly (Trichoptera). Journal of the North American Benthological Society, 6: 262-269.

Meng XL, He YB, Song ZY, Ao HY, Zhang H, Jiang XM. 2014. Structure and spatial distribution patterns of macrozoobenthos in Qinghai Lake Area. Acta Hydrobiologica Sinica, 38: 819-827.

Mueller R, Breitenstein M, Bia MM, Rellstab C, Kirchhofer A. 2007. Bottom-up control of whitefish populations in ultra-oligotrophic Lake Brienz. Aquatic Sciences, 69: 271-288.

Nielsen A, Trolle D, Sondergaard M, Lauridsen TL, Bjerring R, Olesen JE, Jeppesen E. 2012. Watershed land use effects on lake water quality in Denmark. Ecological Applications, 22: 1187-1200.

O'Brien PJ, Wehr JD. 2010. Periphyton biomass and ecological stoichiometry in streams within an urban to rural land-use gradient. Hydrobiologia, 657: 89-105.

Oelmann Y, Buchmann N, Gleixner G, Habekost M, Roscher C, Rosenkranz S, Schulze E, Steinbeiss S, Temperton VM, Weigelt A, Weisser WW, Wilcke W. 2011. Plant diversity effects on aboveground and belowground N pools in temperate grassland ecosystems: Development in the first 5 years after establishment. Global Biogeochemical Cycles, 25: GB2014.

Pärn J, Pinay G, Mander Ü. 2012. Indicators of nutrients transport from agricultural catchments under temperate climate: A review. Ecological Indicators, 22: 4-15.

Perakis SS, Hedin LO. 2002. Nitrogen loss from unpolluted South American forests mainly via dissolved organic compounds. Nature, 415: 416-419.

Piao S, Cui M, Chen A, Wang X, Ciais P, Liu J, Tang Y. 2011. Altitude and temperature dependence of change in the spring vegetation green-up date from 1982 to 2006 in the Qinghai-Xizang Plateau. Agricultural and Forest Meteorology, 151: 1599-1608.

Qiu J. 2008. China: The third pole. Nature, 454: 393-396.

R Core Team. 2017. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Rellstab C, Maurer V, Zeh M, Buergi HR, Spaak P. 2007. Temporary collapse of the *Daphnia* population in turbid and ultra-oligotrophic Lake Brienz. Aquatic Sciences, 69: 257-270.

Sadro S, Nelson CE, Melack JM. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. Ecosystems, 15: 363-386.

Schiller DV, Marti E, Riera JL, Sabater F. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. Freshwater Biology, 52: 891-906.

Schindler DW. 1974. Eutrophication and recovery in experimental lakes: implications for lake management. Science, 184: 897-899.

Schindler DW. 1977. Evolution of Phosphorus Limitation in Lakes. Science, 195: 260-262.

Schlesinger WH, Bernhardt ES. 2013. Biogeochemistry: an analysis of global change. Academic press, San Diego, California, USA.

Scott JT, Lang DA, King RS, Doyle RD. 2009. Nitrogen fixation and phosphatase activity in periphyton growing on nutrient diffusing substrata: evidence for differential nutrient limitation in stream periphyton. Journal of the North American Benthological Society, 28: 57-68.

Scurlock J, Hall DO. 1998. The global carbon sink: a grassland perspective. Global Change Biology, 4: 229-233.

Sterner RW. 2008. On the phosphorus limitation paradigm for lakes. International Review of Hydrobiology, 93: 433-445.

Sterner RW, Elser JJ. 2002. Ecological stoichiometry: the biology of elements from molecules to the biosphere. Princeton University Press, Princeton.

Su X, Wu Y, Dong S, Wen L, Li Y, Wang X. 2015. Effects of grassland degradation and re-vegetation on carbon and nitrogen storage in the soils of the Headwater Area Nature Reserve on the Qinghai-Tibetan Plateau, China. Journal of Mountain Science, 12: 582-591.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the World. No. 34. Food and Agriculture Organization of the United Nations. Rome, Italy.

Tan K, Ciais P, Piao S, Wu X, Tang Y, Vuichard N, Liang S, Fang J. 2010. Application of the ORCHIDEE global vegetation model to evaluate biomass and soil carbon stocks of Qinghai-Tibetan grasslands. Global Biogeochemical Cycles, 24: GB1013.

Tank JL, Dodds WK. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology, 48: 1031-1049.

Thiam AK. 2003. The causes and spatial pattern of land degradation risk in southern Mauritania using multitemporal AVHRR-NDVI imagery and field data. Land Degradation and Development, 14: 133-142.

Tilman D, Kilham SS, Kilham P. 1982. Phytoplankton community ecology: the role of limiting nutrients. Annual Review of Ecology and Systematics, 13: 349-372.

Tischer A, Potthast K, Hamer U. 2014. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. Oecologia, 175: 375-393.

Umbanhowar C, Camill P, Edlund M, Geiss C, Henneghan P, Passow K. 2015. Lake-landscape connections at the forest-tundra transition of northern Manitoba. Inland Waters, 5: 57-74.

Van der Leeden F. 1990. The water encyclopedia. CRC Press. Boca Raton, USA.

Vanni MJ, Renwick WH, Bowling AM, Horgan MJ, Christian AD. 2011. Nutrient stoichiometry of linked catchment-lake systems along a gradient of land use. Freshwater Biology, 56: 791-811.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences, 37: 130-137.

Viragh K, Toth T, Somodi I. 2011. Effect of slight vegetation degradation on soil properties in *Brachypodium pinnatum* grasslands. Plant and Soil, 345: 303-313.

Wang X, Dong S, Yang B, Li Y, Su X. 2014. The effects of grassland degradation on plant diversity, primary productivity, and soil fertility in the alpine region of Asia's headwaters. Environmental Monitoring and Assessment, 186: 6903-6917.

Wasserstein RL, Lazar NA. 2016. The ASA's statement on p-values: context, process, and purpose. The American Statistician, 70: 129-133.

Wen L, Dong S, Li Y, Wang X, Li X, Shi J, Dong Q. 2013. The impact of land degradation on the C pools in alpine grasslands of the Qinghai-Tibet Plateau. Plant and Soil, 368: 329-340.

Wiesmair M, Feilhauer H, Magiera A, Otte A, Waldhardt R. 2016. Estimating vegetation cover from high-resolution satellite data to assess grassland degradation in the Georgian Caucasus. Mountain Research and Development, 36: 56-65.

Williamson CE, Dodds W, Kratz TK, Palmer MA. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment, 6: 247-254.

Wurtsbaugh WA, Barnes BD. 2015. The effects of salinity on plankton and benthic communities in the Great Salt Lake, Utah, USA: a microcosm experiment. Canadian Journal of Fisheries and Aquatic Sciences, 72: 807-817.

Yang JX, Qi HF, Shi JQ, Chen DQ, Huang FX, Tang HY. 2005. Hydrochemistry property and water quality analysis of Qinghaihu Lake. Freshwater Fisheries, 35: 28-32.

Yao T, Thompson LG, Mosbrugger V, Zhang F, Ma Y, Luo T, Xu B, Yang X, Joswiak DR, Wang W, Joswiak ME, Devkota LP, Tayal S, Jilani R, Fayziev R. 2012. Third Pole Environment (TPE). Environmental Development, 3: 52-64.

Yengoh GT, Dent D, Olsson L, Tengberg A, Tucker C. 2014. The use of the Normalized Difference Vegetation Index (NDVI) to assess land degradation at multiple scales a review of the current status, future trends, and practical considerations. Lund University Center for Sustainability Studies (LUCSUS), and the Scientific and Technical Advisory Panel of the Global Environment Facility (STAP/GEF). Lund, Sweden.

Yi WJ, Li XY, Cui BL, Ma YJ. 2010. Climate change and impact on water level of the Qinghai Lake watershed. Journal of Arid Meteorology, 28: 375-383.

Zhang K, Lan JH, Shen ZX, Xu H. 2010. The chemical composition and quality evaluation of surface water in Qinghai Lake areas. Journal of Earth Environment, 1: 162-168.

Zhang S, Hua D, Meng X, Zhang Y. 2011a. Climate change and its driving effect on the runoff in the "Three-River Headwaters" region. Journal of Geographical Sciences, 21: 963-978.

Zhang T. 2011b. Distance-decay patterns of nutrient loading at watershed scale: Regression modeling with a special spatial aggregation strategy. Journal of Hydrology, 402: 239-249.

Zhao YZ, Zou XY, Cheng H, Jia HK, Wu YQ, Wang GY, Zhang CL, Gao SY. 2006. Assessing the ecological security of the Tibetan plateau: methodology and a case study for Lhaze County. Journal of Environmental Management, 80: 120-131.

Zheng M. 1997. An introduction to saline Lakes on the Qinghai-Tibet plateau. Kluwer Academic Publisher, Dordrecht, Netherlands.

Supplementary Information

Figure S4.1 The design of nutrient diffusion substrata to determine nutrient limitation in stream periphyton and to determine periphyton responses to nutrient additions. (a) Schematic diagram. (b) Field photograph. Centrifuge tubes (50 mL) were filled with agar and covered by a GF/F filter to serve as a substratum for periphyton colonization. Agar was amended with nitrate (+N), phosphate (+P), nitrate and phosphate (+NP) or no nutrients (Control).

Figure S4.2 (a) Nutrient limitation bioassay to determine which nutrient (N vs P) is limiting. Nutrients were added to the bottles to increase N-concentrations by 100 µg/L and/or P-concentrations by 10 µg/L. (b) Streamwater bioassay to determine lake phytoplankton response to streamwater additions. “62-µm LW” represents 62-µm screened lake water. “0.2-µm LW” represents lake water filtered by 0.2 µm GF/F filter. “0.2-µm SW_n” represents water from stream n spanning a gradient of grassland degradation (n represents S11, S31, S41, S61, S71, S81, S91, SD, and SE) filtered by 0.2 µm GF/F filter. Each treatment was in triplicate.

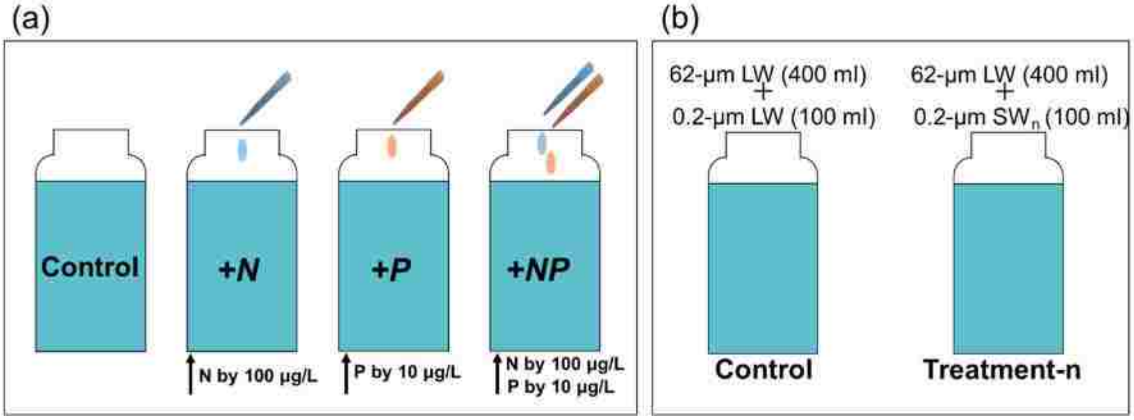

Figure S4.3 Relationships between NDVI and *in situ* measured (a) aboveground biomass, (b) fractional vegetation cover, and (c) grass species richness. Grey shadow represents 95 % confidence limits.

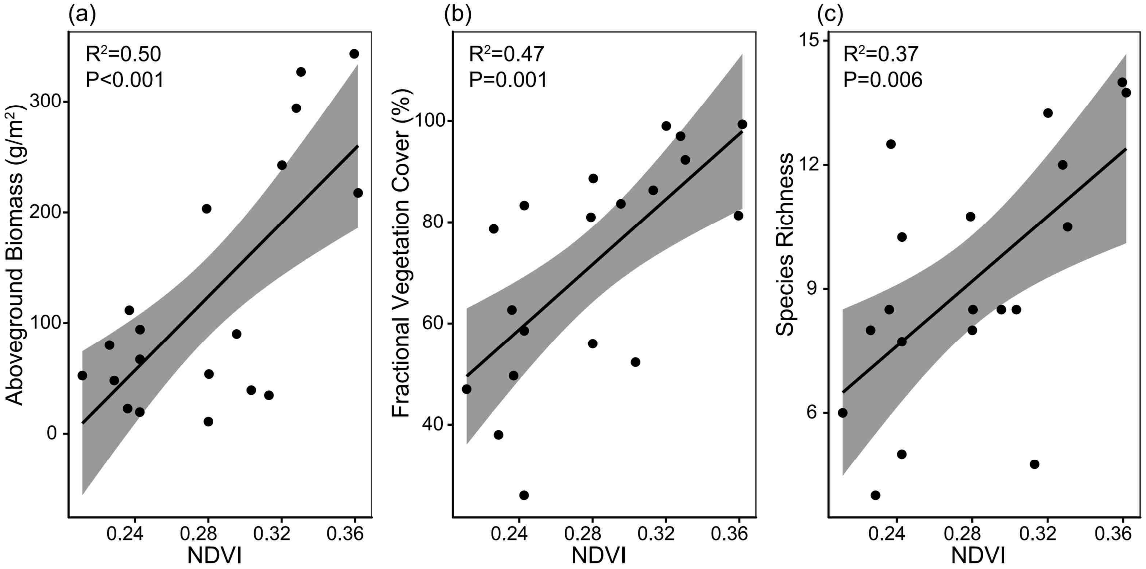

Figure S4.4 Scatter plots of elevation versus grassland vegetation parameters, including aboveground biomass, fractional vegetation cover, grass species richness, and NDVI. R^2 and P-value of linear regression are shown.

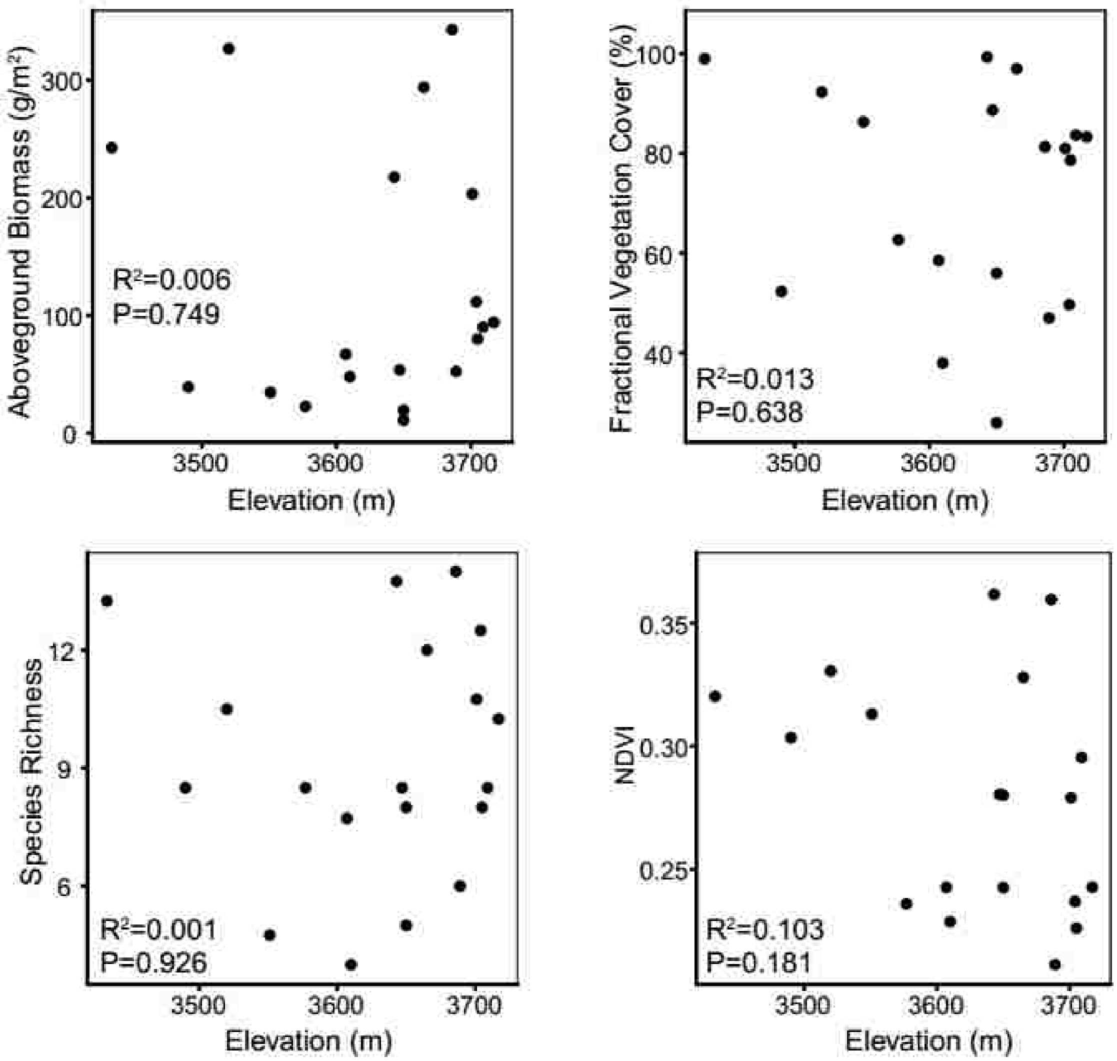

Figure S4.5 Relative responses (RR-X) of stream periphyton to nutrient enrichment (+N, +P, +NP) in (a) July 2017, (b) September 2017, and (c) April 2018 to determine nutrient limitation of periphyton in 9 streams spanning a gradient of grassland degradation as determined by normalized difference vegetation index (NDVI). The red dashed line represents RR-X=1. The sub-basins on the X-axis are ordered based on NDVI.17 from high to low. The italic letters above the bar represent the type of nutrient limitation. " • " and "*" represent P<0.1 and P<0.05 for t-test of RR-X=1 for all responses. "na" represents no nutrient limitation; "N" represents N limitation; "P" represents "P limitation"; "NP" represents NP colimitation. The whisker represents standard deviation (n=5 for each treatment)

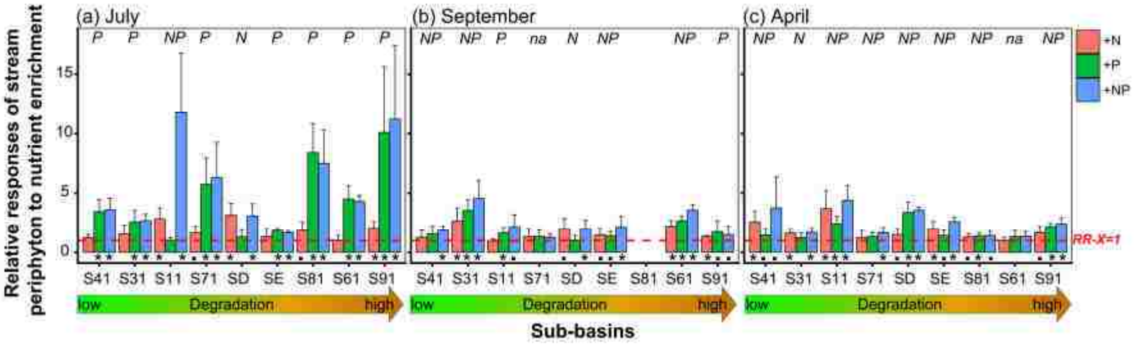

Table S4.1 Basic information for the study 22 sub-basins (mean ± SD, SD represents standard deviation). NDVI.17 value indicates grassland status of the study sub-basins in 2017, the year of the field sampling and experiments.

Sub-Basin	Area (km^2)	Elevation of the sample site (m)	Elevation of the sub-basin (m)	Slope	NDVI.17
S11	126	3488	3787 ± 175	10.8 ± 6.5	0.302 ± 0.053
S12	80	3581	3875 ± 153	12 ± 6.8	0.314 ± 0.051
S13	33	3672	4072 ± 850	2.6 ± 1.4	0.256 ± 0.032
S31	192	3501	3891 ± 158	12.4 ± 6.7	0.304 ± 0.072
S32	79	3608	3982 ± 146	12.9 ± 6.8	0.344 ± 0.029
S33	57	3696	4026 ± 134	13.2 ± 6.7	0.279 ± 0.068
S41	245	3460	3847 ± 250	16.1 ± 10.3	0.305 ± 0.088
S42	157	3505	3991 ± 217	4.9 ± 3.8	0.297 ± 0.036
S43	93	3587	3973 ± 197	18.4 ± 10.4	0.260 ± 0.075
S61	1317	3540	4166 ± 299	11.4 ± 8.9	0.206 ± 0.069
S62	1115	3600	4510 ± 120	4.6 ± 3.1	0.191 ± 0.062
S63	868	3657	4743 ± 248	12.4 ± 7.6	0.167 ± 0.049
S71	53	3566	3929 ± 169	5.4 ± 5.2	0.293 ± 0.059
S81	1029	3373	4046 ± 245	12.8 ± 9.0	0.218 ± 0.063
S82	921	3504	4094 ± 206	13.1 ± 9.1	0.211 ± 0.062
S83	697	3647	4139 ± 185	12.7 ± 9.1	0.189 ± 0.045
S91	80	3590	4432 ± 145	16.3 ± 4.3	0.198 ± 0.068
S101	98	3652	4051 ± 204	11.7 ± 8.1	0.199 ± 0.051
SB	123	3659	4113 ± 219	15.5 ± 8.7	0.216 ± 0.052
SC	80	3623	4046 ± 241	15.8 ± 9.8	0.253 ± 0.050
SD	85	3568	4006 ± 180	13.1 ± 7.0	0.252 ± 0.056
SE	273	3547	4015 ± 187	12.1 ± 7.2	0.240 ± 0.065

Table S4.2 Basic information for Qinghai Lake measured in this study (mean±SD). SD represents standard deviation

Environmental Variable	July	September	April
pH	9.01 ± 0.045	9.01 ± 0.060	9.04 ± 0.036
DO (mg/L)	6.85 ± 0.447	6.89 ± 0.513	7.37 ± 0.380
Temperature (℃)	13.40 ± 0.265	8.43 ± 0.208	1.63 ± 0.416
Conductivity (mS/cm)	15.26 ± 0.344	14.83 ± 0.513	14.8 ± 0.300
TN (mg/L)	0.952 ± 0.087	0.848 ± 0.016	0.535 ± 0.052
NO_3^--N (mg/L)	0.267 ± 0.014	0.082 ± 0.004	0.168 ± 0.016
NH_4^+-N (mg/L)	0.119 ± 0.008	0.096 ± 0.008	0.142 ± 0.010
TP (mg/L)	0.014 ± 0.001	0.016 ± 0.002	0.020 ± 0.002
SRP (mg/L)	0.003 ± 0.001	0.003 ± 0.000	0.004 ± 0.000
DOC (mg/L)	18.96 ± 10.95	12.75 ± 2.966	52.06 ± 0.043
Chla (μg/L)	0.657 ± 0.081	1.06 ± 0.098	0.336 ± 0.012

Table S4.3 Model comparisons for the regression analyses of NDVI (y) versus nutrient concentrations in streamwater (x). P-value corrections (Benjamini-Hochberg method) were conducted. P-values <0.10 are shown in bold

Nutrient	Model	April			July			September		
		R^2	P	AIC	R^2	P	AIC	R^2	P	AIC
TN	y=ax + b	0.207	**0.038**	-39.9	0.143	**0.082**	-50.7	0.252	**0.034**	-45.8
TN	y=ax^{-1} + b	0.170	**0.063**	44.5	0.109	0.134	38.9	0.213	**0.031**	44.0
NO$_3^-$-N	y=ax + b	0.199	**0.043**	-47.5	0.155	**0.070**	-46.9	0.118	0.118	-42.2
NO$_3^-$-N	y=ax^{-1} + b	0.149	**0.084**	37.3	0.118	0.118	42.7	0.094	0.164	47.1
NH$_4^+$-N	y=ax + b	0.029	0.462	-167	0.341	**0.009**	-163	0.273	**0.013**	-113
NH$_4^+$-N	y=ax^{-1} + b	0.012	0.639	-83.9	0.263	**0.015**	-71.9	0.263	**0.015**	-23.7
TP	y=ax + b	0.014	0.608	-170	0.098	0.156	-232	0.122	0.112	-229
TP	y=ax^{-1} + b	0.012	0.643	-86.5	0.088	0.181	-143	0.199	**0.038**	-142
SRP	y=ax + b	0.051	0.323	-244	0.008	0.700	-335	0.251	**0.018**	-306
SRP	y=ax^{-1} + b	0.030	0.453	-160	0.003	0.803	-246	0.266	**0.014**	-218
TN:TP	y=ax + b	0.009	0.676	175	0.011	0.642	196	0.384	**0.002**	144
TN:TP	y=ax^{-1} + b	0.009	0.683	259	0.008	0.692	285	0.382	**0.002**	233
DIN:SRP	y=ax + b	0.024	0.501	241	0.134	**0.093**	266	0.447	**0.001**	179
DIN:SRP	y=ax^{-1} + b	0.011	0.657	325	0.104	0.143	356	0.400	**0.002**	269

Table S4.4 Results of ANOVA assessing whether the relative responses of stream periphyton for each nutrient treatment (RR-X) differs between different seasons. *Post hoc* pairwise comparisons and P-value corrections (Benjamini-Hochberg method) were conducted. P-values <0.10 are shown in bold.

Parameter	Factor	F-value	P-value
RR-N	July vs. September	1.229	0.488
	July vs. April	0.021	0.886
	September vs. April	0.878	0.528
RR-P	July vs. September	20.270	**< 0.001**
	July vs. April	23.340	**< 0.001**
	September vs. April	0.128	0.811
RR-NP	July vs. September	23.220	**< 0.001**
	July vs. April	23.750	**< 0.001**
	September vs. April	0.602	0.566

Table S4.5 Results of ANOVA comparing the effects of different nutrient enrichment (+N, +P, +NP) on stream periphyton. *Post hoc* pairwise comparisons and P-value corrections (Benjamini-Hochberg method) were conducted. P-values <0.10 are shown in bold.

Time Period	Factor	F-value	P-value
July	RR-N vs. RR-P	22.010	**< 0.001**
	RR-N vs. RR-NP	34.340	**< 0.001**
	RR-P vs. RR-NP	2.145	0.189
September	RR-N vs. RR-P	1.196	0.313
	RR-N vs. RR-NP	7.072	**0.017**
	RR-P vs. RR-NP	2.809	0.147
April	RR-N vs. RR-P	0.031	0.860
	RR-N vs. RR-NP	7.909	**0.014**
	RR-P vs. RR-NP	9.329	**0.009**

Table S4.6 Model comparisons for the regression analyses of relative responses of stream periphyton to nutrient enrichment (RR-N, RR-P, and RR-NP) and the relative responses of lake phytoplankton to streamwater (RR-SW) versus streamwater nutrients. P-value corrections (Benjamini-Hochberg method) were conducted. P-values <0.10 are shown in bold.

Nutrient	Model	RR-N			RR-P			RR-NP			RR-SW		
		R^2	P	AIC	R^2	P	AIC	R^2	P	AIC	R^2	P	AIC
TN	$y=ax + b$	0.035	0.363	78.5	0.004	0.755	138	0.003	0.777	149	0.091	0.144	116
TN	$y=ax^{-1} + b$	0.011	0.618	62.1	0.006	0.713	121	0.000	0.959	132	0.109	0.107	95.6
NO_3^--N	$y=ax + b$	0.004	0.755	78.8	0.000	0.993	138	0.004	0.760	148	0.203	**0.024**	112
NO_3^--N	$y=ax^{-1} + b$	0.000	0.988	62.4	0.067	0.200	120	0.023	0.462	131	0.131	**0.075**	95.0
NH_4^+-N	$y=ax + b$	0.045	0.301	28.5	0.013	0.585	88.4	0.045	0.298	98.1	0.291	**0.005**	59.2
NH_4^+-N	$y=ax^{-1} + b$	0.106	0.105	59.5	0.036	0.351	120	0.041	0.323	131	0.081	0.169	96.4
TP	$y=ax + b$	0.083	0.153	-89.4	0.059	0.230	-29.7	0.002	0.830	-17.5	0.081	0.169	-48.0
TP	$y=ax^{-1} + b$	0.088	0.141	56.0	0.127	**0.074**	118	0.010	0.620	132	0.205	**0.023**	92.8
SRP	$y=ax + b$	0.008	0.669	-159	0.087	0.143	-102	0.145	**0.055**	-93.3	0.302	**0.004**	-124
SRP	$y=ax^{-1} + b$	0.000	0.975	62.4	0.165	**0.039**	117	0.319	**0.009**	122	0.240	**0.020**	91.7
TN:TP	$y=ax + b$	0.076	0.172	372	0.180	**0.031**	428	0.029	0.402	443	0.400	**0.002**	375
TN:TP	$y=ax^{-1} + b$	0.136	**0.064**	58.6	0.062	0.221	120	0.000	0.945	132	0.186	**0.031**	93.4
DIN:SRP	$y=ax + b$	0.002	0.827	457	0.202	**0.021**	510	0.256	**0.012**	519	0.378	**0.003**	459
DIN:SRP	$y=ax^{-1} + b$	0.028	0.413	61.6	0.050	0.272	120	0.099	0.118	129	0.398	**0.001**	85.9

Table S4.7 Results of ANOVA comparing the effects of different nutrient enrichment (+N, +P, +NP) on lake phytoplankton. *Post hoc* pairwise comparisons and P-value corrections (Benjamini-Hochberg method) were conducted. P-values <0.10 are shown in bold.

Time Period	Factor	F-value	P-value
July	RR-N vs. RR-P	29.39	**< 0.001**
	RR-N vs. RR-NP	222.7	**< 0.001**
	RR-P vs. RR-NP	0.026	0.243
September	RR-N vs. RR-P	219.2	0.352
	RR-N vs. RR-NP	4557	**0.031**
	RR-P vs. RR-NP	0.077	0.221
April	RR-N vs. RR-P	31506	0.860
	RR-N vs. RR-NP	2174	**0.031**
	RR-P vs. RR-NP	499.3	**0.027**

Chapter 5 Grassland degradation shifts bacterial communities in soil on the Qinghai-Tibet Plateau

Abstract

Grasslands are among the largest terrestrial biomes in the world and are experiencing severe degradation, impacting both aboveground vegetation and soil properties. However, the influences of grassland degradation on bacterial communities in soil are not well-understood. In this study, we used high throughput 16S rRNA gene sequencing to characterize soil bacterial communities in a degrading grassland on the Qinghai-Tibet Plateau. The normalized difference vegetation index (NDVI) was calculated to represent grassland status and indicate grassland degradation (decreasing NDVI). Soil physicochemical properties were also analyzed. The results showed that bacterial alpha diversity had negative relationships with soil moisture, soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP). Bacterial community structure was significantly associated with NDVI, the change rate of NDVI, moisture, pH, SOC, TN, as well as soil C:N and C:P ratios as different bacterial phyla had different relationships with these environmental variables. For example, Proteobacteria were positively correlated with soil moisture, SOC, TN, and soil C:N and N:P ratios while Chloroflexi were negatively correlated with those variables. Overall, these results identify grassland degradation as a driving force to shift soil bacterial communities.

Keywords NDVI, 16S rRNA, soil property, moisture, Qinghai Lake, grassland

Introduction

In terrestrial ecosystems, the soil microbial community is a major component of biodiversity and plays crucial roles in various ecosystem functions and processes (Trevors, 1998; Kirk et al., 2004; Lozupone and Knight, 2007; Philippot et al., 2013). For example, soil microorganisms drive biogeochemical cycles of carbon and nutrients, contributing to plant growth and maintaining soil structure (Smith and Goodman, 1999; Yao et al., 2000; O'Donnell et al., 2001; Young and Crawford, 2004). Given the wide distribution and pivotal roles of microbes, the influences of external disturbances on soil microbial communities have long been interests of ecological studies (Yao et al., 2000; Zhou et al., 2016; Pérez Castro et al., 2019; Weiser et al., 2019).

Soil bacterial communities are regulated by various abiotic and biotic factors (Zhou et al., 2002; Fierer and Jackson, 2006; Fierer et al., 2007a), such as soil pH, moisture, soil organic carbon, and soil nutrient availabilities (Rousk et al., 2010; Fierer et al., 2012a; Delgado-Baquerizo et al., 2017; Ochoa-Hueso et al., 2018). For example, bacterial diversity and richness are highest in soil with neutral pH and are usually positively correlated with soil moisture and resource availability (Fierer and Jackson, 2006; Barnard et al., 2015; Zhang et al., 2016; Delgado-Baquerizo et al., 2018). For different taxa groups, evolutionary histories and trophic strategies may lead to differential responses to environmental variation (Fierer et al., 2007b; Delgado-Baquerizo et al., 2017; Aanderud et al., 2018). For example, old taxa (such as Chloroflexi) evolved on a young Earth and include oligotrophic taxa (such as *Bacillus*) that may prefer soils with low organic carbon and nutrient contents, while younger phyla and copiotrophic taxa (such as some members of Proteobacteria) may be superior competitors in soils with high organic matter content (Battistuzzi and Hedges, 2009; Fierer et al., 2012a; Delgado-Baquerizo et al., 2017). Moreover, plant species and biomass have strong effects on soil bacterial communities (Stephan et al., 2000; Berg and Smalla, 2009). A well-studied example is that rhizobia form symbioses with legumes. In turn, the interactions and feedbacks between plant and soil bacteria determine ecosystem functioning and processing in both aboveground and belowground components of terrestrial ecosystems (Clarholm, 1985; Wardle et al., 2004; Glick, 2010; Wagg et al., 2014). Thus, changes in landcover that significantly affect the structure, function, and elemental balances of terrestrial ecosystems also potentially shift microbial diversities, activities, and community composition (Anderson et al., 2011; Li et al., 2014; Ng et al., 2014; Tischer et al., 2014).

Grasslands are among the largest terrestrial biomes in the world (Suttie et al., 2005) but are facing widespread degradation (Gang et al., 2014). Defined as a process of retrogressive ecosystem succession, grassland degradation is largely driven by climate change and anthropogenic activities such as overgrazing (Daily, 1995; Gang et al., 2014). The declines of aboveground vegetation species richness, coverage, and biomass are conspicuous consequences of grassland degradation (Bai et al., 2004; Deng et al., 2014). Meanwhile, grassland degradation impacts soil physical (e.g. soil moisture and texture), chemical (e.g. soil pH, organic carbon, and nutrients), and biological (e.g. microbial community and activity) properties (Abril and Bucher, 1999; Viragh et al., 2011; Li et al., 2012; Dlamini et al., 2014). For example, soil moisture, organic matter, and nitrogen significantly decrease with grassland degradation (Wu and Tiessen, 2002; Steffens et al., 2008). Such impacts are likely associated with major changes in soil bacterial communities. However, despite the well-known ecological influences of grassland degradation on vegetation, the patterns and driving forces of soil bacterial communities in degrading grassland ecosystems are not well-characterized.

Grassland is the dominant landcover on the Qinghai-Tibet Plateau (QTP), covering more than 60% of the plateau (Li and Zhou, 1998). Known as the “Third Pole” of the Earth, the QTP grassland ecosystem is extremely sensitive and vulnerable to human activities and climate change (Piao et al., 2006; Wang et al., 2016), and is experiencing serious degradation in recent decades (Harris, 2010). However, external pressures on QTP grassland ecosystems continue to increase, influencing above-ground and below-ground components (Dong et al., 2015; Zhang et al., 2016; Ma et al., 2018). Understanding how grassland degradation impacts soil bacterial communities is essential for predicting responses of soil ecosystems under global change and anthropogenic activities.

In this study, we aim to address the following question: how do soil bacterial communities respond to grassland degradation? According to mechanisms described above, we hypothesized that shifts in bacterial community structure are driven by grassland degradation via alterations in soil physicochemical properties that are associated with ongoing degradation that also lead to decreases in bacterial alpha diversity and differential responses of different groups of bacteria. To test this hypothesis, we examined the soil bacterial communities in a grassland watershed on the Qinghai-Tibet Plateau across a gradient of grassland degradation. We assessed the bacterial communities using high-throughput 16S rRNA gene sequencing. If the hypothesis is correct, we predict that (1) bacterial alpha diversity will decrease and community structure will shift with increased grassland degradation and concomitant changes in soil properties, (2) different taxonomic groups will respond differently to environmental variations according to their evolutionary history and trophic strategy.

Methods

Study area

This study was conducted in the Qinghai Lake watershed (Figure 5.1). Qinghai Lake (36°32′ - 37°15′ N, 99°36′ - 100°47′ E), the largest lake in China, is a remote endorheic saline lake located at 3194 m above sea level on the Qinghai-Tibet Plateau. The area of Qinghai Lake watershed is 29,660 km^2 and grassland is the main landcover type, accounting for 75% area of the watershed. The watershed receives mean annual precipitation of 363 mm and experiences an average annual evaporation 4 times greater than precipitation (Yi et al., 2010). The annual mean temperature is -0.7°C (Yi et al., 2010) with a linear warming rate of 0.28°C per decade (Chen et al., 2011). The Qinghai Lake region contains a variety of terrestrial and aquatic ecosystems that are sensitive to global climate change and other anthropogenic impacts (An et al., 2006; Hao, 2008; Li et al., 2009). Indeed, its grassland is seriously deteriorating due to overgrazing and climate change (Li et al., 2007, 2009; Chen et al., 2011; Luo et al., 2013), with around half of the grassland classified as degraded in 2010 (Luo et al., 2013).

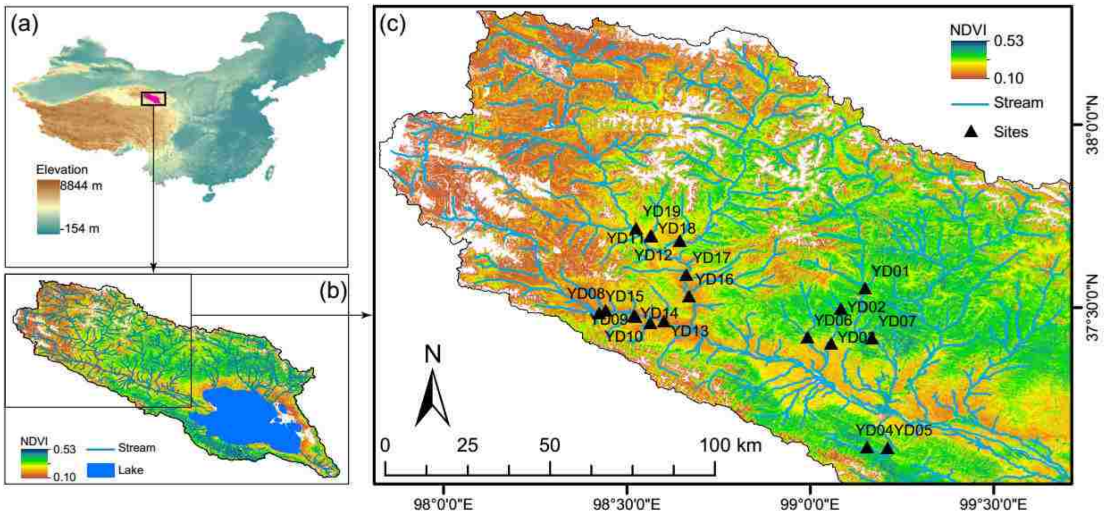

Figure 5.1 (a) The location of study area, Qinghai Lake watershed. (b) The distribution of normalized difference vegetation index (NDVI) across Qinghai Lake watershed. (c) The location of sample sites (n=19). This map is from Ren et al. (in preparation, Chapter 2)

Field sampling and sample analyses

Soil samples were collected from 19 soil sites (Figure 5.1) during July 2017, the peak growing season of the grassland there. At each sample site, three plots (1m×1m) with 15-m intervals were established. In each plot, five representative points (four vertices and one center) of the topsoil (0-10 cm in depth) were sampled using a drill (10 cm in diameter). After each sampling, the drill was cleaned with sterile water and then air dried. Samples for each plot were pooled into one composite sample and passed through a 2-mm sieve. Subsamples were stored at 4°C for the measurement of soil biogeochemical properties and in liquid nitrogen for soil DNA extraction.

Soil pH was measured in 1:2.5 soil to distilled water ratio using a pH meter. Soil moisture was determined gravimetrically with 10 g fresh soil into aluminum dishes that was oven-dried at 105±1°C for 48 h. Soil organic carbon (SOC) was measured via oxidation with potassium dichromate (HJ615-2011). Soil total nitrogen content (TN) was measured via the modified Kjeldahl Method (HJ717-2014). Soil total phosphorus (TP) was measured using the spectrophotometric ammonium molybdate-ascorbic acid method. C, N, and P in microbial biomass were determined by the fumigation extraction method (Brookes et al., 1985; Vance et al., 1987).

Normalized difference vegetation index and grassland degradation

The normalized difference vegetation index (NDVI) was calculated based on the reflectance of near-infrared light (NIR) and red light (RED): NDVI=(NIR-RED)/(NIR+RED) (Rouse et al., 1973). Landsat-8 images acquired in July 2017 and July 2009 were downloaded (https://earthexplorer.usgs.gov/) and processed in ArcGIS 14.0 for NDVI calculation. For each sample site, the average NDVI in a 300-m radius buffer with the sample site as the center was calculated. NDVI values of 2017 (indicated as NDVI in the following analyses) were visualized on the map (Figure 5.1) to represent contemporary grassland status of our sampling year. NDVI values of 2009 were calculated to represent historical grassland status (indicated as NDVI.h in the following analyses). The average annual change of NDVI from 2009 to 2017 was calculated as the rate of change of NDVI (indicated as NDVI.r in the following analyses). In our study area, there is large spatial variation in NDVI (Figure 5.1) as well as other vegetation variables, including aboveground biomass, fractional vegetation coverage, and vegetation species richness (Ren et al., 2019). However, these vegetation indices did not show clear patterns with changing altitude (Ren et al., 2019). Moreover, as shown in Chapter 1, the relationship between NDVI difference and geographic distance of paired sample sites was statistically significant (P=0.002) but relatively weak (R^2=0.039), suggesting that spatial non-independence should not contribute strongly to our space-for-time approach. NDVI generally exhibits intimate relationships with above-ground vegetation variables (Carlson and Ripley, 1997; Eastwood et al., 1997) and is commonly used to indicate vegetation status and grassland degradation (Thiam, 2003; Akiyama and Kawamura, 2007; Li et al., 2013; Hilker et al., 2014). As an alternative approach in ecological studies of long-term phenomena, "space-for-time" substitution has been widely and successfully used in systems experiencing sustained disturbance (Pickett, 1989).Thus, the spatial variation of grassland status permits us to use "space-for-time" substitution (Pickett, 1989; Blois et al., 2013) to study the impacts of grassland degradation based on the spatial pattern of contemporary grassland status indicated by NDVI (Ren et al., 2019).

DNA extraction, PCR, and sequencing

DNA was extracted from soil samples (0.5 g frozen soil per sample) using the TIANGEN-DP336 soil DNA Kit (TIANGEN-Biotech, Beijing, China) following manufacturer protocols. The V3-V4 regions of the 16S rRNA gene were amplified using forward primers containing the sequence 5'-CCTACGGRRBGCASCAGKVRVGAAT-3' and reverse primers containing the sequence 5'-GGACTACNVGGGTWTCTAATCC-3'. PCR was performed with a thermal cycler (ABI GeneAmp® 9700, USA) using the following program: initial denaturation at 94 °C for 3 min, 24 cycles of denaturation at 94 °C for 30 s followed by annealing at 57 °C for 90 s and extension at 72 °C for 10 s, and final extension step at 72 °C for 10 min. Amplified DNA was verified by electrophoresis of PCR mixtures in 1.0% agarose in 1X TAE buffer and purified using the Gel Extraction Kit (Qiagen, Hilden, Germany). DNA libraries were validated by Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), and quantified by Qubit 3.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument (Illumina, San Diego, CA, USA) according to manufacturer's instructions.

Sequence analysis

Raw sequence data were processed using the software package QIIME 1.9.1 (Caporaso et al., 2010). The forward and reverse reads were merged and then the sequences were assigned to samples based on the barcode and truncated by cutting off the barcode and primer sequence. Quality filtering on merged sequences were performed and sequences which did not meet the criteria were discarded. This involved sequences shorter than 200 bp, containing ambiguous bases, and exhibiting a mean quality score below 20. Then the chimeric sequences were detected and removed by comparing with the reference database (RDP Gold database) using UCHIME algorithm (Edgar et al., 2011). The effective sequences were grouped into operational taxonomic units (OTUs) against the SILVA132 database (Quast et al., 2013) at 99% sequence identity level. The singletons, chloroplast sequences, and unclassified OTUs were removed using the *filter_otus_from_otu_table.py* script. The *single_rarefaction.py* script was used to randomly select subset of 29,814 sequence per sample to normalize surveying effort at the same level (Figure S5.1). Alpha diversity indices (Chao 1, observed OTUs, Shannon, and PD whole tree) were calculated by using the *alpha_diversity.py* script. The raw sequence data was deposited in the database of National Center for Biotechnology Information (PRJNA526110).

Statistical analysis

In order to test the grassland degradation hypothesis, Pearson correlation analyses were conducted using R package stats v3.6.1 (R Core Team, 2017) to assess the relationships between environmental variables versus bacterial alpha diversity and relative abundances of the dominant phyla. P-values of the correlations were adjusted using FDR adjustment (Benjamini and Hochberg, 1995). Non-metric multidimensional scaling (NMDS) with contour lines was conducted using R package vegan 2.5-5 (Oksanen et al., 2007) to assess bacterial distributions along the NDVI gradient. NMDS is used to collapse information from high dimension into low dimension, making complex multivariate data easier to be visualized and interpreted. Distance-based redundancy analysis (dbRDA), a constrained ordination method using non-Euclidean distance measures, was conducted using R package vegan 2.5-5 (Oksanen et al., 2007) to assess the relationships between environmental variables and soil bacterial community structure. All the analyses were carried out in R 3.4.1 (R Core Team, 2017).

Results

Dynamics of alpha diversity

In total, 12550 OTUs were detected in soil bacterial communities at a 99% nucleotide sequence identity threshold after quality filtering. On average, the number of observed OTUs was 5871±619, the richness estimated by Chao1 was 8196±790, Shannon diversity was 11.06±0.34, and the phylogenetic diversity was 316±24. Correlation analysis showed that these alpha diversity indices had significantly negative relationships with soil moisture, SOC, and TP ($P<0.05$, Table 5.1). Shannon diversity and phylogenetic diversity also had significantly negative relationships with TN ($P<0.05$, Table 5.1). Alpha diversity was not associated with NDVI, historical NDVI, the change rate of NDVI, altitude, and pH (Table 5.1). Moreover, bacterial alpha diversity was negatively ($P<0.1$) correlated with soil C:P and microbial biomass C:N, but was positively ($P<0.05$) correlated with microbial biomass N:P (Table S5.1). Thus, our hypothesis that grassland degradation decreases bacterial alpha diversity was not supported.

Table 5.1 Relationships between alpha diversity indices and environmental variables. P-values are adjusted using FDR adjustment. * represents $P<0.05$. ** represents $P<0.01$.

Alpha Diversity	NDVI	NDVI.h	NDVI.r	Altitude	Moisture	pH	SOC	TN	TP
Observed OTUs	-0.021	-0.047	-0.089	-0.080	**-0.585****	-0.035	**-0.471***	-0.445	**-0.468***
Chao1	-0.050	-0.104	-0.194	0.025	**-0.618****	0.011	**-0.497***	-0.472	**-0.486***
Shannon	-0.086	-0.158	-0.272	0.035	**-0.691****	0.039	**-0.581***	**-0.553***	**-0.609***
PD Whole Tree	-0.099	-0.138	-0.191	0.061	**-0.626****	0.122	**-0.564***	**-0.554***	**-0.515***

Bacterial community shifts

In soil bacterial communities, the dominant phyla (relative abundance >1%) were (in order of prevalence): Acidobacteria, Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Bacteroidetes, and Rokubacteria (Figure 5.2). Correlation analyses showed that the relative abundance of the bacterial phyla responded differently to environmental variables (Table 5.2). For example, Alphaproteobacteria were positively but Chloroflexi negatively correlated with NDVI, soil moisture, SOC, and TN (Table 5.2). Similarly, Alphaproteobacteria were positively but Gammaproteobacteria negatively correlated with historical NDVI and the rate of change in NDVI (Table 5.2). Gemmatimonadetes and Bacteroidetes positively correlated with soil pH (Table 5.2). Actinobacteria negatively while Rokubacteria positively correlated with soil moisture (Table 5.2). Moreover, Bacteroidetes and Chloroflexi were negatively correlated with soil C:P and N:P while Alphaproteobacteria and Rokubacteria had a positive correlation (Table S5.2). These results indicate that grassland degradation and resulting changes of soil properties imposed different influences on different groups of soil bacteria. Thus, our hypothesis that different groups of soil bacteria respond differently to grassland degradation was supported.

NMDS showed that soil bacterial communities generally distributed along NDVI gradients across the study area (Figure 5.3a). To gain more insight into factors behind these distributions, dbRDA was performed to evaluate relationships between environmental variables and bacterial community distributions (Figure 5.3b). NDVI, NDVI.h, NDVI.r, soil moisture, pH, SOC, and TN had significant relationships ($P < 0.05$) with the distribution of soil bacterial communities. This indicates that our hypothesis that grassland degradation shifts soil bacterial community structure was supported.

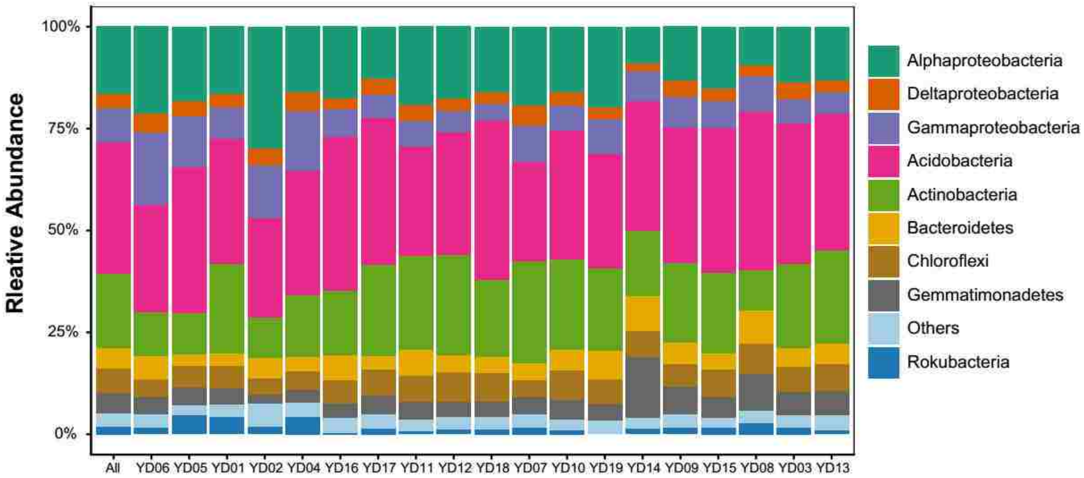

Figure 5.2 Community composition of soil bacteria at the phylum level. Only the phyla with a relative abundance >1% are shown. “All” represents the whole dataset including all samples.

Table 5.2 Correlation relationships between environmental factors and the relative abundance of the dominant phyla. The numbers represent Pearson’s correlation coefficients. P-values are adjusted using FDR adjustment. * represents P<0.05. ** represents P<0.01.

	NDVI	NDVI.h	NDVI.r	Altitude	Moisture	pH	SOC	TN	TP
Alphaproteobacteria	**0.584***	**0.655***	**0.628***	-0.059	**0.602***	-0.436	**0.615***	**0.562***	0.364
Deltaproteobacteria	0.402	0.399	0.359	-0.454	0.468	-0.325	0.405	0.450	0.252
Gammaproteobacteria	**0.643***	0.506	0.391	-0.297	**0.688****	-0.149	0.440	0.437	0.363
Acidobacteria	-0.274	-0.421	-0.453	0.031	-0.399	0.261	-0.369	-0.315	-0.198
Actinobacteria	-0.413	-0.166	-0.028	0.126	**-0.587***	-0.166	-0.326	-0.321	-0.477
Bacteroidetes	-0.411	-0.507	-0.507	0.293	-0.247	**0.726****	-0.444	-0.492	-0.134
Chloroflexi	**-0.609***	-0.500	-0.398	0.445	**-0.669****	0.383	**-0.566***	**-0.577***	-0.365
Gemmatimonadetes	-0.491	**-0.624***	**-0.630***	0.070	-0.305	**0.593***	-0.420	-0.405	-0.009
Rokubacteria	0.472	0.468	0.422	-0.277	**0.554***	-0.463	0.523	0.525	0.398

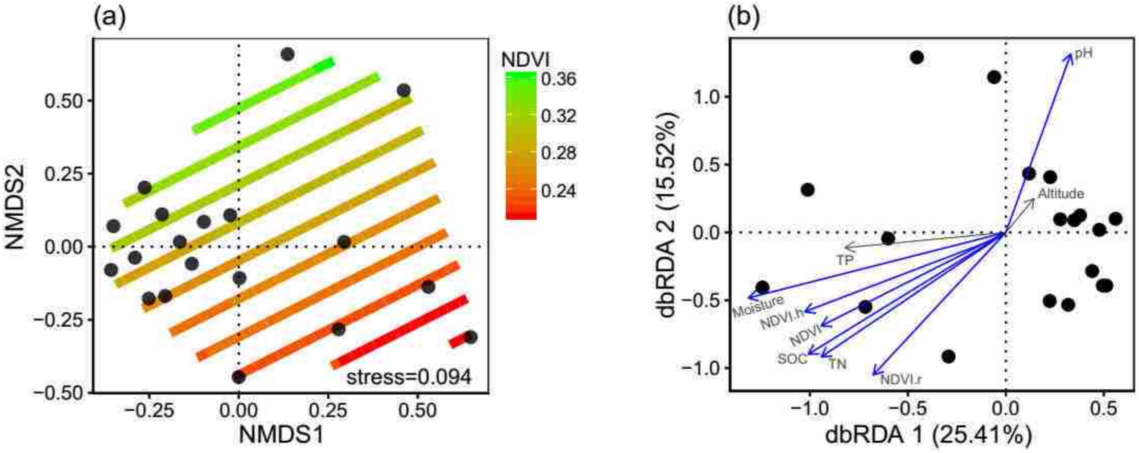

Figure 5.3 (a) Non-metric multidimensional scaling (NMDS) ordination showing the distribution of bacterial communities along the gradient of normalized difference vegetation index (NDVI) based on Bray-Curtis distances. The contour lines of NDVI are shown. (b) Distance-based redundancy analyses (dbRDA) of the relationships between environmental variables and soil bacterial communities. Bacterial communities are shown in points. Environmental variables are shown in arrows and the significant ones ($P<0.05$ by envfit function) are colored as blue.

Discussion

Influences of grassland degradation on bacterial communities

Consistent with our grassland degradation hypothesis, bacterial community structure was significantly associated with NDVI and NDVI.r, as well as with associated soil variables such as moisture, pH, SOC, TN, as well as soil C:N and N:P ratios (Figure 5.3). Moreover, different bacterial phyla had different relationships with environmental variables (Table 5.2 and Table S5.2). The results indicate that grassland degradation can substantially shift bacterial communities in soil. A variety of studies have shown that soil bacterial diversity and community composition can be regulated by various biotic and abiotic factors, such as altitude (Ren et al., 2018), temperature (Zhou et al., 2016; Yu et al., 2018), drought (Barnard et al., 2015; Ochoa-Hueso et al., 2018), pH (Fierer and Jackson, 2006), SOC (Curd et al., 2018; Tian et al., 2018), nutrients (Fierer et al., 2012a; Yuan et al., 2016), and aboveground vegetation (Högberg et al., 2007; Berg and Smalla, 2009). Bacterial communities usually have multiple drivers and different taxonomic groups respond differently to environmental variations (Fierer and Jackson, 2006; Wang et al., 2015). Aboveground vegetation change is the most obvious consequence of grassland degradation, including decreased vegetation coverage, reduction of above and below ground biomass, and lower species richness. Such changes should affect microbes as soil bacterial communities have intimate interactions with plants (Schimel and Bennett, 2004; Berg and Smalla, 2009; Thoms et al., 2010; Eisenhauer et al., 2011). Changes in plant community properties could affect soil bacteria through many pathways, such shifts in root-derived resources, competition for resources, mycorrhizal activities, decomposable organic matter, and soil properties (Schimel and Bennett, 2004; Berg and Smalla, 2009; Thoms et al., 2010; Eisenhauer et al., 2011). NDVI is an integrative index to represent vegetation status and indicate land degradation (Thiam, 2003; Akiyama and Kawamura, 2007; Li et al., 2013; Hilker et al., 2014). The close relationships between NDVI and the rate of change of NDVI with bacterial community structure highlight the potential for vegetation-mediated influences (such as changes in the quality and quantity of leaf litter supplies) on bacterial communities. However, grassland degradation also increases soil pH (Ren et al., in preparation, Chapter 2; Viragh et al., 2011), which has been widely demonstrated as one of the most important factors driving soil bacterial communities (Fierer and Jackson, 2006; Huang et al., 2016).

Moisture is also among one of the major drivers for soil microbial assemblages (Fierer and Jackson, 2006; Barnard et al., 2015; Wang et al., 2015; Ochoa-Hueso et al., 2018). It has been demonstrated that Actinobacteria and Chloroflexi phyla increase their relative abundance in more arid areas, while Proteobacteria and Rokubacteria decline with increasing drought (Pointing et al., 2009; Fierer et al., 2012b). This is because Actinobacteria and Chloroflexi resist desiccation and can outcompete other bacterial taxa under drought (Battistuzzi and Hedges, 2009; Maestre et al., 2015). Reduction of soil moisture is one of the consequence of grassland degradation (Ren et al., in preparation, Chapter 2; Barrow, 1991). In our study in the Qinghai Lake watershed, soil moisture was significantly associated with the structuring soil bacterial communities (Figure 5.3). Alphaproteobacteria, Betaproteobacteria, and Rokubacteria were positively correlated while Actinobacteria and Chloroflexi were negatively correlated with soil moisture (Table 5.2). These results suggest that one major mechanism by which grassland degradation affects soil bacterial communities is by reducing soil moisture.

Different evolutionary histories and trophic strategies of bacterial taxa can also influence their distributions as a function of the availability of key soil resources, such as organic carbon and nutrient contents as well as their stoichiometry (Fierer et al., 2007b; Delgado-Baquerizo et al., 2017; Aanderud et al., 2018). For example, some members of Proteobacteria and Bacteroidetes are considered copiotrophic (Fierer et al., 2007b, 2012a; Leff et al., 2015) and function as initial metabolizers of soil organic matter (Padmanabhan et al., 2003). Thus, they increase their relative abundance in soils with high C and N contents (Wang et al., 2015; Curd et al., 2018). Moreover, Proteobacteria is one of the comparatively young bacterial phyla and has likely evolved in well-developed soils and thus prefers high organic matter contents and high C:nutrient ratios (Battistuzzi and Hedges, 2009; Delgado-Baquerizo et al., 2017). On the contrary, as one of the old bacterial phyla evolved in mineral soils on a young Earth, Chloroflexi appear to prefer soils with low organic matter content and low C:nutrient ratio, allowing them to outcompete other phyla in such environments (Battistuzzi and Hedges, 2009; Delgado-Baquerizo et al., 2017). Many studies have shown that SOC, N, as well as C:nutrient stoichiometry are strong drivers for microbial communities (Fierer et al., 2012a; Delgado-Baquerizo et al., 2017; Curd et al., 2018; Tian et al., 2018). Grassland degradation reduces organic carbon and nutrient pools in soil and leads to decreases of C:nutrient ratios (Ren et al., in preparation, Chapter 2; Wu and Tiessen, 2002; Viragh et al., 2011; Dlamini et al., 2014). In our study, SOC, TN, as well as soil C:N and N:P ratios were significantly associated with variation of bacterial community structure (Figure 5.3). Moreover, Alphaproteobacteria was positively correlated while Chloroflexi was negatively correlated with SOC, TN, and soil C:N and N:P (Table 5.2 and Table S5.2). These results suggest that grassland degradation also influences soil bacterial communities by changing soil resource availabilities and stoichiometric ratios.

Influence of grassland degradation on bacterial alpha diversity

Soil moisture and resource availability are usually positively correlated with bacterial diversity (Fierer and Jackson, 2006; Barnard et al., 2015). It is interesting that, in our study, bacterial alpha diversity did not follow this pattern. Our results showed that soil moisture, SOC, TN, and TP had negative relationships with bacterial alpha diversity (Table 5.1). There are several possible explanations. First, in addition to shifting the composition and structure of bacterial communities *per se*, moisture and nutrients might also influence fungal communities. Reductions of SOC and C:nutrient ratios have been reported to reduce the abundances of fungi (Eiland et al., 2001; Rousk and Bååth, 2009) and appear to have stronger influences on fungi than on bacteria (Bardgett and Mcalister, 1999; de Vries et al., 2012). In response to drought, fungi decrease in abundance and biomass (Barnard et al., 2015; Ochoa-Hueso et al., 2018). Thus, a hypothetical reduction of fungal abundance would reduce their competition with bacteria, promoting bacterial alpha diversity. This explanation needs to be further verified by studying relative changes of bacterial and fungal communities in response to grassland degradation. One the other hand, soil bacteria living in dry areas have strong potential to be activated by even a small rainfall event (Collins et al., 2008; Barnard et al., 2015; Wang et al., 2015). Indeed, high species richness is usually fostered under intermediate disturbance (Connell, 1978; Hobbs and Huenneke, 1992). Thus, acting as a disturbance, precipitation is likely to enhance bacterial alpha diversity in dry areas.

Implication of grassland degradation for ecosystem functions

Soil bacterial communities play an essential role in mediating biogeochemical processes of C and N (Smith and Goodman, 1999; Young and Crawford, 2004; Fierer et al., 2012b), influencing soil fertility and promoting plant growth (Trevors, 1998; Kirk et al., 2004; Lozupone and Knight, 2007; Philippot et al., 2013). As degradation proceeds, grassland ecosystem functions are impaired (Daily, 1995), with decline of vegetation, increased erodibility of topsoil, as well as loss of soil moisture, organic matter, and nutrients (Viragh et al., 2011; Li et al., 2012; Dlamini et al., 2014). Through multiple statistical analysis methods, our study confirmed that grassland degradation is associated with shifts in soil bacterial communities, likely via deterioration of vegetation status (indicated as NDVI) and soil properties (e.g. pH, moisture, and nutrients). It has been demonstrated that bacterial functions are strongly correlated with the taxonomic composition of the community (Gilbert et al., 2010; Bryant et al., 2012). Thus, grassland degradation could impair bacterial activities, such as mineralization and respiration (Abril and Bucher, 1999; Wu et al., 2014), influencing biogeochemical processes and vegetation status. For example, Actinobacteria are abundant in soils (32% in our study). They are heterotrophic or chemoautotrophic using a wide variety of organic resources (Zimmermann, 1990). Most Actinobacteria are soil saprophytes, playing crucial roles in nutrient cycling, and some genera are plant symbionts or pathogens (Barka et al., 2015). However, their growth is largely limited, and can even be halted, in soils with low moisture and nutrients (Barka et al., 2015), conditions that are favored by grassland degradation. Proteobacteria are considered a dominant bacterial clade in many environments and can control nitrogen availability by nitrogen fixation. Proteobacteria also play many other critical functional roles, such as organic compound degradation and oxidation of sulfur and iron. However, Proteobacteria are vulnerable to reductions of soil moisture and nutrients caused by grassland degradation, affecting ecological functions processed by Proteobacteria.

As discussed above, climate change and various direct human activities have significantly affected grassland ecosystems across the world (Wiesmair et al., 2016). In particular, grassland ecosystems on the QTP appear to be extremely sensitive to external disturbances and thus have been experiencing serious degradation (Harris, 2010) that has impacted the structure of soil microbial communities. Further study of the impacts of these shifts on bacterial function would provide more insights into the mechanisms underlying the interactions among bacterial communities, soil nutrients, and above-ground vegetation.

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References

Aanderud ZT, Saurey S, Ball BA, Wall DH, Barrett JE, Muscarella ME, Griffin NA, Virginia RA, Barberán A, Adams BJ. 2018. Stoichiometric shifts in soil C:N:P promote bacterial taxa dominance, maintain biodiversity, and deconstruct community assemblages. Frontiers in Microbiology, 9: e1401.

Abril A, Bucher EH. 1999. The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. Applied Soil Ecology, 12: 159-167.

Akiyama T, Kawamura K. 2007. Grassland degradation in China: methods of monitoring, management and restoration. Grassland Science, 53: 1-17.

An ZS, Ai L, Song YG, Colman SM. 2006. Lake Qinghai scientific drilling project. Scientific Drilling, 2: 20-22.

Anderson CR, Condron LM, Clough TJ, Fiers M, Stewart A, Hill RA, Sherlock RR. 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. Pedobiologia, 54: 309-320.

Bai YF, Han XG, Wu JG, Chen ZZ, Li LH. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature, 431: 181-184.

Bardgett RD, McAlister E. 1999. The measurement of soil fungal: bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils, 29: 282-290.

Barka EA, Vatsa P, Sanchez L, Gaveau-Vaillant N, Jacquard C, Klenk H, Clément C, Ouhdouch Y, van Wezel GP. 2015. Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and Molecular Biology Reviews, 80: 1-43.

Barnard RL, Osborne CA, Firestone MK. 2015. Changing precipitation pattern alters soil microbial community response to wet-up under a Mediterranean-type climate. ISME Journal, 9: 946-957.

Barrow CJ. 1991. Land degradation: development and breakdown of terrestrial environments. Cambridge University Press, New York.

Battistuzzi FU, Hedges SB. 2009. A major clade of prokaryotes with ancient adaptations to life on land. Molecular Biology and Evolution, 26: 335-343.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57: 289-300.

Berg G, Smalla K. 2009. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. FEMS Microbiology Ecology, 68: 1-13.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences, 110: 9374-9379.

Brookes PC, Landman A, Pruden G, Jenkinson DS. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry, 17: 837-842.

Bryant JA, Stewart FJ, Eppley JM, Delong EF. 2012. Microbial community phylogenetic and trait diversity declines with depth in a marine oxygen minimum zone. Ecology, 93: 1659-1673.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7: 335-336.

Carlson TN, Ripley DA. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. Remote Sensing of Environment, 62: 241-252.

Chen L, Chen KL, Liu BK, Hou GL, Cao SK, Han YL, Yang L, Wu YP. 2011. Characteristics of climate variation in Qinghai Lake basin during the recent 50 years. Journal of Arid Meteorology, 29: 483-487.

Clarholm M. 1985. Interactions of bacteria, protozoa and plants leading to mineralization of soil nitrogen. Soil Biology and Biochemistry, 17: 181-187.

Collins SL, Sinsabaugh RL, Crenshaw C, Green L, Porras Alfaro A, Stursova M, Zeglin LH. 2008. Pulse dynamics and microbial processes in aridland ecosystems. Journal of Ecology, 96: 413-420.

Connell JH. 1978. Diversity in tropical rain forests and coral reefs. Science, 199: 1302-1310.

Curd EE, Martiny JB, Li H, Smith TB. 2018. Bacterial diversity is positively correlated with soil heterogeneity. Ecosphere, 9: e02079.

Daily GC. 1995. Restoring value to the world's degraded lands. Science, 269: 350-354.

de Vries FT, Manning P, Tallowin JR, Mortimer SR, Pilgrim ES, Harrison KA, Hobbs PJ, Quirk H, Shipley B, Cornelissen JH. 2012. Abiotic drivers and plant traits explain landscape‐scale patterns in soil microbial communities. Ecology Letters, 15: 1230-1239.

Delgado-Baquerizo M, Reich PB, Khachane AN, Campbell CD, Thomas N, Freitag TE, Abu Al-Soud W, Sørensen S, Bardgett RD, Singh BK. 2017. It is elemental: soil nutrient stoichiometry drives bacterial diversity. Environmental Microbiology, 19: 1176-1188.

Delgado-Baquerizo M, Reith FDPG, Hamonts K, Powell JR, Young A, Singh BK, Bissett A. 2018. Ecological drivers of soil microbial diversity and soil biological networks in the Southern Hemisphere. Ecology, 99: 583-596.

Deng L, Zhang Z, Shangguan Z. 2014. Long-term fencing effects on plant diversity and soil properties in China. Soil and Tillage Research, 137: 7-15.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma, 235-236: 372-381.

Dong SK, Wang XX, Liu SL, Li YY, Su XK, Wen L, Zhu L. 2015. Reproductive responses of alpine plants to grassland degradation and artificial restoration in the Qinghai-Tibetan Plateau. Grass and Forage Science, 70: 229-238.

Eastwood JA, Yates MG, Thomson AG, Fuller RM. 1997. The reliability of vegetation indices for monitoring saltmarsh vegetation cover. International Journal of Remote Sensing, 18: 3901-3907.

Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. 2011. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27: 2194-2200.

Eiland F, Klamer M, Lind AM, Leth M, Bååth E. 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. Microbial Ecology, 41: 272-280.

Eisenhauer N, Milcu A, Sabais AC, Bessler H, Brenner J, Engels C, Klarner B, Maraun M, Partsch S, Roscher C. 2011. Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. PLoS One, 6: e16055.

Fierer N, Bradford MA, Jackson RB. 2007b. Toward an ecological classification of soil bacteria. Ecology, 88: 1354-1364.

Fierer N, Jackson RB. 2006. The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences, 103: 626-631.

Fierer N, Lauber CL, Ramirez KS, Zaneveld J, Bradford MA, Knight R. 2012b. Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. ISME Journal, 6: 1007-1017.

Fierer N, Leff JW, Adams BJ, Nielsen UN, Bates ST, Lauber CL, Owens S, Gilbert JA, Wall DH, Caporaso JG. 2012a. Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences, 109: 21390-21395.

Fierer N, Morse JL, Berthrong ST, Bernhardt ES, Jackson RB. 2007a. Environmental controls on the landscape-scale biogeography of stream bacterial communities. Ecology, 88: 2162-2173.

Gang C, Zhou W, Chen Y, Wang Z, Sun Z, Li J, Qi J, Odeh I. 2014. Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environmental Earth Sciences, 72: 4273-4282.

Gilbert JA, Field D, Swift P, Thomas S, Cummings D, Temperton B, Weynberg K, Huse S, Hughes M, Joint I, Somerfield PJ, Muehling M. 2010. The taxonomic and functional diversity of microbes at a temperate coastal site: a 'multi-omic' study of seasonal and diel temporal variation. PLoS One, 5: e15545.

Glick BR. 2010. Using soil bacteria to facilitate phytoremediation. Biotechnology Advances, 28: 367-374.

Hao X. 2008. A green fervor sweeps the Qinghai-Tibetan Plateau. Science, 321: 633-635.

Harris RB. 2010. Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. Journal of Arid Environments, 74: 1-12.

Hilker T, Natsagdorj E, Waring RH, Lyapustin A, Wang Y. 2014. Satellite observed widespread decline in Mongolian grasslands largely due to overgrazing. Global Change Biology, 20: 418-428.

Hobbs RJ, Huenneke LF. 1992. Disturbance, diversity, and invasion: implications for conservation. Conservation Biology, 6: 324-337.

Högberg MN, Högberg P, Myrold DD. 2007. Is microbial community composition in boreal forest soils determined by pH, C-to-N ratio, the trees, or all three? Oecologia, 150: 590-601.

Huang R, Zhao D, Zeng J, Shen F, Cao X, Jiang C, Huang F, Feng J, Yu Z, Wu QL. 2016. pH affects bacterial community composition in soils across the Huashan Watershed, China. Canadian Journal of Microbiology, 62: 726-734.

Kirk JL, Beaudette LA, Hart M, Moutoglis P, Khironomos JN, Lee H, Trevors JT. 2004. Methods of studying soil microbial diversity. Journal of Microbiological Methods, 58: 169-188.

Leff JW, Jones SE, Prober SM, Barberan A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schuetz M, Steenbock C, Stevens CJ, Fierer N. 2015. Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences, 112: 10967-10972.

Li P, Yang Y, Han W, Fang J. 2014. Global patterns of soil microbial nitrogen and phosphorus stoichiometry in forest ecosystems. Global Ecology and Biogeography, 23: 979-987.

Li S, Verburg PH, Lv S, Wu J, Li X. 2012. Spatial analysis of the driving factors of grassland degradation under conditions of climate change and intensive use in Inner Mongolia, China. Regional Environmental Change, 12: 461-474.

Li WH, Zhou XM. 1998. Ecosystems of Qinghai-Xizang (Tibetan) Plateau and approach for their sustainable management. Guangdong Science and Technology Press, Guangzhou.

Li XL, Gao J, Brierley G, Qiao YM, Zhang J, Yang YW. 2013. Rangeland degradation on the Qinghai‐Tibet plateau: Implications for rehabilitation. Land Degradation and Development, 24: 72-80.

Li XY, Ma YJ, Xu HY, Wang JH, Zhang DS. 2009. Impact of land use and land cover change on environmental degradation in Lake Qinghai watershed, northeast Qinghai-Tibet Plateau. Land Degradation and Development, 20: 69-83.

Li XY, Xu HY, Sun YL, Zhang DS, Yang ZP. 2007. Lake-level change and water balance analysis at Lake Qinghai, west China during recent decades. Water Resources Management, 21: 1505-1516.

Lozupone CA, Knight R. 2007. Global patterns in bacterial diversity. Proceedings of the National Academy of Sciences, 104: 11436-11440.

Luo CF, Xu CJ, You HY, Jin SH. 2013. Analysis on grassland degradation in Qinghai Lake Basin during 2000-2010. Acta Ecologica Sinica, 33: 4450-4459 (In Chinese).

Ma L, Yao Z, Zheng X, Zhang H, Wang K, Zhu B, Wang R, Zhang W, Liu C. 2018. Increasing grassland degradation stimulates the non-growing season CO_2 emissions from an alpine meadow on the Qinghai－Tibetan Plateau. Environmental Science and Pollution Research, 25: 26576-26591.

Maestre FT, Delgado-Baquerizo M, Jeffries TC, Eldridge DJ, Ochoa V, Gozalo B, Quero JL, Garcia-Gomez M, Gallardo A, Ulrich W. 2015. Increasing aridity reduces soil microbial diversity and abundance in global drylands. Proceedings of the National Academy of Sciences, 112: 15684-15689.

Ng EL, Patti AF, Rose MT, Schefe CR, Wilkinson K, Cavagnaro TR. 2014. Functional stoichiometry of soil microbial communities after amendment with stabilised organic matter. Soil Biology and Biochemistry, 76: 170-178.

Ochoa-Hueso R, Collins SL, Delgado-Baquerizo M, Hamonts K, Pockman WT, Sinsabaugh RL, Smith MD, Knapp AK, Power SA. 2018. Drought consistently alters the composition of soil fungal and bacterial communities in grasslands from two continents. Global Change Biology, 24: 2818-2827.

Oksanen J, Kindt R, Legendre PO, Hara B, Stevens MHH, Oksanen MJ, Suggests MASS. 2007. The vegan package. Community Ecology Package, 10: 631-637.

Padmanabhan P, Padmanabhan S, DeRito C, Gray A, Gannon D, Snape J, Tsai C, Park W, Jeon C, Madsen E. 2003. Respiration of ^{13}C-labeled substrates added to soil in the field and subsequent 16S rRNA gene analysis of ^{13}C-labeled soil DNA. Applied and Environmental Microbiology, 69: 1614-1622.

Pérez Castro S, Cleland EE, Wagner R, Sawad RA, Lipson DA. 2019. Soil microbial responses to drought and exotic plants shift carbon metabolism. ISME Journal, 13: 1776-1787.

Philippot L, Spor A, Henault C, Bru D, Bizouard F, Jones CM, Sarr A, Maron P. 2013. Loss in microbial diversity affects nitrogen cycling in soil. ISME Journal, 7: 1609-1619.

Piao S, Fang J, Zhou L, Ciais P, Zhu B. 2006. Variations in satellite-derived phenology in China's temperate vegetation. Global Change Biology, 12: 672-685.

Pickett ST. 1989. Space-for-time substitution as an alternative to long-term studies. In Long-term studies in ecology. Springer, New York, NY.

Pointing SB, Chan Y, Lacap DC, Lau MC, Jurgens JA, Farrell RL. 2009. Highly specialized microbial diversity in hyper-arid polar desert. Proceedings of the National Academy of Sciences, 106: 19964-19969.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41: 590-596.

R Core Team. 2017. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Ren C, Zhang W, Zhong Z, Han X, Yang G, Feng Y, Ren G. 2018. Differential responses of soil microbial biomass, diversity, and compositions to altitudinal gradients depend on plant and soil characteristics. Science of the Total Environment, 610: 750-758.

Ren Z, Niu DC, Ma PP, Wang Y, Fu H, Elser JJ. 2019. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. Ecology, 100: e02755.

Ren Z, Wang Y, Ma PP, Wang ZM, Niu DC, Fu H, Elser JJ. in preparation, Chapter 2. Effects of grassland degradation on ecological stoichiometry of soil ecosystems on the Qinghai-Tibet Plateau.

Rouse JW, Haas RH, Schell JA, Deering DW. 1973. Monitoring vegetation systems in the Great Plains with ERTS. Third Earth Resources Technology Satelite-1 Symposium. 309-317.

Rousk J, Bååth E. 2009. Fungal and bacterial growth in soil with plant materials of different C/N ratios. FEMS Microbiology Ecology, 62: 258-267.

Rousk J, Bååth E, Brookes PC, Lauber CL, Lozupone C, Caporaso JG, Knight R, Fierer N, MEMEG, Lund U, Lunds U. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. ISME Journal, 4: 1340-1351.

Schimel JP, Bennett J. 2004. Nitrogen mineralization: Challenges of a changing paradigm. Ecology, 85: 591-602.

Smith KP, Goodman RM. 1999. Host variation for interactions with beneficial plant-associated microbes. Annual Review of Phytopathology, 37: 473-491.

Steffens M, Kölbl A, Totsche KU, Kögel-Knabner I. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). Geoderma, 143: 63-72.

Stephan A, Meyer AH, Schmid B. 2000. Plant diversity affects culturable soil bacteria in experimental grassland communities. Journal of Ecology, 88: 988-998.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the World. No. 34. Food and Agriculture Organization of the United Nations. Rome, Italy.

Thiam AK. 2003. The causes and spatial pattern of land degradation risk in southern Mauritania using multitemporal AVHRR-NDVI imagery and field data. Land Degradation and Development, 14: 133-142.

Thoms C, Gattinger A, Jacob M, Thomas FM, Gleixner G. 2010. Direct and indirect effects of tree diversity drive soil microbial diversity in temperate deciduous forest. Soil Biology and Biochemistry, 42: 1558-1565.

Tian J, He N, Hale L, Niu S, Yu G, Liu Y, Blagodatskaya E, Kuzyakov Y, Gao Q, Zhou J. 2018. Soil organic matter availability and climate drive latitudinal patterns in bacterial diversity from tropical to cold temperate forests. Functional Ecology, 32: 61-70.

Tischer A, Potthast K, Hamer U. 2014. Land-use and soil depth affect resource and microbial stoichiometry in a tropical mountain rainforest region of southern Ecuador. Oecologia, 175: 375-393.

Trevors JT. 1998. Bacterial biodiversity in soil with an emphasis on chemically-contaminated soils. Water Air and Soil Pollution, 101: 45-67.

Vance ED, Brookes PC, Jenkinson DS. 1987. An extraction method for measuring soil microbial biomass C. Soil Biology and Biochemistry, 19: 703-707.

Viragh K, Toth T, Somodi I. 2011. Effect of slight vegetation degradation on soil properties in *Brachypodium pinnatum* grasslands. Plant and Soil, 345: 303-313.

Wagg C, Bender SF, Widmer F, van der Heijden MG. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. Proceedings of the National Academy of Sciences, 111: 5266-5270.

Wang X, Van Nostrand JD, Deng Y, Lü X, Wang C, Zhou J, Han X. 2015. Scale-dependent effects of climate and geographic distance on bacterial diversity patterns across northern China's grasslands. FEMS Microbiology Ecology, 91: fiv133.

Wang Z, Zhang Y, Yang Y, Zhou W, Gang C, Zhang Y, Li J, An R, Wang K, Odeh I, Qi J. 2016. Quantitative assess the driving forces on the grassland degradation in the Qinghai-Tibet Plateau, in China. Ecological Informatics, 33: 32-44.

Wardle DA, Bardgett RD, Klironomos JN, Setala H, van der Putten WH, Wall DH. 2004. Ecological linkages between aboveground and belowground biota. Science, 304: 1629-1633.

Weiser MD, Ning D, Buzzard V, Michaletz ST, He Z, Enquist BJ, Waide RB, Zhou J, Kaspari M. 2019. Thermal disruption of soil bacterial assemblages decreases diversity and assemblage similarity. Ecosphere, 10: e02598.

Wiesmair M, Feilhauer H, Magiera A, Otte A, Waldhardt R. 2016. Estimating vegetation cover from high-resolution satellite data to assess grassland degradation in the Georgian Caucasus. Mountain Research and Development, 36: 56-65.

Wu G, Ren G, Dong Q, Shi J, Wang Y. 2014. Above- and belowground response along degradation gradient in an alpine grassland of the Qinghai- Tibetan Plateau. CLEAN-Soil Air Water, 42: 319-323.

Wu RG, Tiessen H. 2002. Effect of land use on soil degradation in alpine grassland soil, China. Soil Science Society of America Journal, 66: 1648-1655.

Yao H, He Z, Wilson MJ, Campbell CD. 2000. Microbial biomass and community structure in a sequence of soils with increasing fertility and changing land use. Microbial Ecology, 40: 223-237.

Yi WJ, Li XY, Cui BL, Ma YJ. 2010. Climate change and impact on water level of the Qinghai Lake watershed. Journal of Arid Meteorology, 28: 375-383.

Young IM, Crawford JW. 2004. Interactions and self-organization in the soil-microbe complex. Science, 304: 1634-1637.

Yu H, Deng Y, He Z, Van Nostrand JD, Wang S, Jin D, Wang A, Wu L, Wang D, Tai X, Zhou J. 2018. Elevated CO_2 and warming altered grassland microbial communities in soil top-layers. Frontiers in Microbiology, 9: e1790.

Yuan X, Knelman JE, Gasarch E, Wang D, Nemergut DR, Seastedt TR. 2016. Plant community and soil chemistry responses to long- term nitrogen inputs drive changes in alpine bacterial communities. Ecology, 97: 1543-1554.

Zhang Y, Dong S, Gao Q, Liu S, Zhou H, Ganjurjav H, Wang X. 2016. Climate change and human activities altered the diversity and composition of soil microbial community in alpine grasslands of the Qinghai-Tibetan Plateau. Science of the Total Environment, 562: 353-363.

Zhou J, Deng Y, Shen L, Wen C, Yan Q, Ning D, Qin Y, Xue K, Wu L, He Z, Voordeckers JW, Van Nostrand JD, Buzzard V, Michaletz ST, Enquist BJ, Weiser MD, Kaspari M, Waide R, Yang Y, Brown JH. 2016. Temperature mediates continental-scale diversity of microbes in forest soils. Nature Communications, 7: e12083.

Zhou JZ, Xia BC, Treves DS, Wu LY, Marsh TL, O'Neill RV, Palumbo AV, Tiedje JM. 2002. Spatial and resource factors influencing high microbial diversity in soil. Applied and Environmental Microbiology, 68: 326-334.

Zimmermann W. 1990. Degradation of lignin by bacteria. Journal of Biotechnology, 13: 119-130.

Supplementary Information

Figure S5.1 Rarefaction curve of operational taxonomic units (OTUs) in each sample site at 99% nucleotide sequence identity threshold.

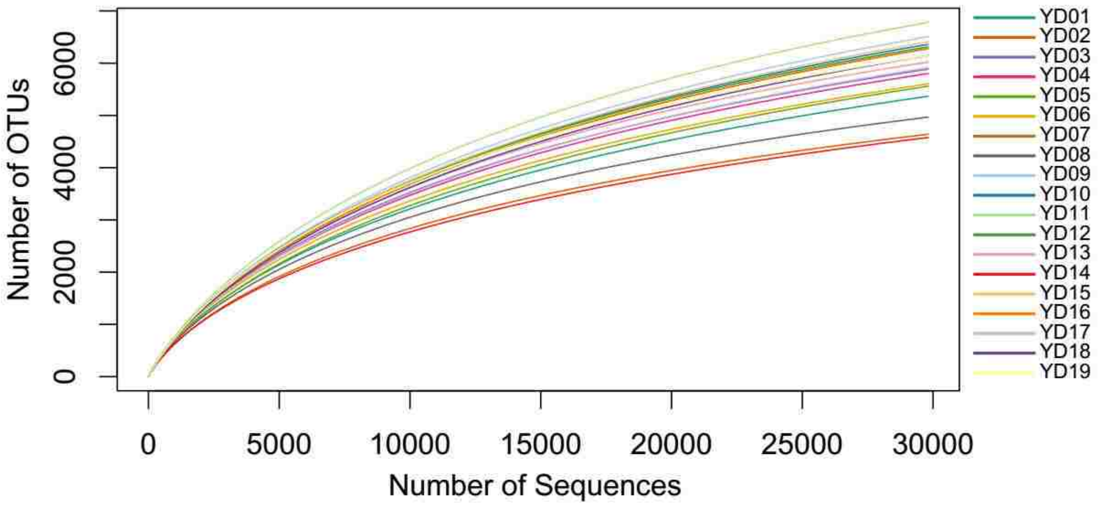

Table S5.1 Correlation relationships between alpha diversity of soil bacterial communities and C:N:P stoichiometry of soil and soil microbial biomass. P-values are adjusted using FDR adjustment.

Alpha diversity	**Soil**						**Soil Microbial Biomass**					
	C:N		C:P		N:P		C:N		C:P		N:P	
	r	p	r	p	r	p	r	p	r	p	r	p
Observed OTUs	-0.066	0.949	-0.395	0.095	-0.362	0.128	-0.402	0.094	0.116	0.843	**0.538**	**0.023**
Chao1	-0.018	0.949	-0.435	0.084	-0.403	0.116	-0.419	0.094	0.095	0.843	**0.543**	**0.023**
Shannon	-0.016	0.949	-0.479	0.076	-0.445	0.113	-0.471	0.094	0.049	0.843	**0.517**	**0.024**
PD Whole Tree	0.079	0.949	-0.528	0.076	-0.516	0.095	-0.395	0.094	0.124	0.843	**0.570**	**0.023**

Table S5.2 Correlation relationships between bacterial phyla and C:N:P stoichiometry of soil and soil microbial biomass. P-values are adjusted using FDR adjustment.

	Soil						**Soil Microbial Biomass**					
Phylum	C:N		C:P		N:P		C:N		C:P		N:P	
	r	p	r	p	r	p	r	p	r	p	r	p
Alphaproteobacter	0.331	0.796	**0.531**	**0.040**	0.467	0.081	0.367	0.452	0.215	0.629	-0.021	0.948
Deltaproteobacter	-0.036	0.989	0.396	0.123	0.458	0.081	0.194	0.712	0.275	0.629	0.091	0.888
Gammaproteobacter	0.169	0.980	0.390	0.123	0.381	0.134	0.197	0.712	-0.006	0.979	-0.223	0.888
Acidobacteria	-0.374	0.796	-0.329	0.187	-0.261	0.311	-0.444	0.452	-0.541	0.169	-0.266	0.888
Actinobacteria	-0.080	0.989	-0.209	0.391	-0.198	0.415	-0.132	0.830	0.281	0.629	0.461	0.472
Bacteroidetes	0.284	0.796	**-0.546**	**0.040**	**-0.608**	**0.039**	0.096	0.830	0.114	0.919	0.100	0.888
Chloroflexi	0.036	0.989	**-0.529**	**0.040**	**-0.546**	**0.039**	-0.355	0.452	-0.223	0.629	0.123	0.888
Gemmatimonadetes	-0.185	0.980	-0.451	0.088	-0.437	0.087	0.079	0.830	0.077	0.944	-0.016	0.948
Rokubacteria	0.022	0.989	**0.588**	**0.040**	**0.580**	**0.039**	-0.047	0.849	-0.260	0.629	-0.330	0.839

Chapter 6 Bacterial communities in stream biofilms in a degrading grassland watershed on the Qinghai-Tibet Plateau

Abstract

Grassland is among the largest terrestrial biomes and is experiencing serious degradation, especially on the Qinghai-Tibet Plateau (China). However, the influences of grassland degradation on bacterial communities in stream biofilms are largely unknown. Using 16S rRNA gene sequencing, we investigated the bacterial communities in stream biofilms in sub-basins with different grassland status in the Qinghai Lake watershed. Grassland status in the sub-basins was quantified using the normalized difference vegetation index (NDVI). Proteobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia were the dominant bacterial phyla. 7050 OTUs were detected in total, within which 19 were abundant taxa and 6922 were rare taxa. Bacterial alpha diversity was positively correlated with carbon (C), nitrogen (N), and phosphorus (P) in biofilms *per se*. The variation of bacterial communities in stream biofilms was closely associated with the rate of change in NDVI, pH, conductivity, as well as C, N, P contents and C:N ratio of the biofilms. Abundant subcommunities were more influenced by environmental variables relative to the whole community and to rare subcommunities. These results suggest that the history of grassland degradation (indicated as the rate of change in NDVI) influences bacterial communities in stream biofilms. By documenting the properties of bacterial communities in stream biofilms in a degrading grassland watershed, our study adds to our knowledge of the potential influences of grassland degradation on aquatic ecosystems.

Keywords 16S rRNA, stream biofilm, NDVI, grassland degradation, Qinghai Lake

Introduction

Landcover change, which has generally been considered a local environmental issue, is becoming a force of global importance with an outcome of degraded environmental conditions (Foley et al., 2005). Due to intimate connections between aquatic and terrestrial ecosystems (Williamson et al., 2008), aquatic environments are strongly influenced by changes in the surrounding landscape (Vannote et al., 1980; Figueiredo et al., 2010; Deegan et al., 2011). As one of the largest terrestrial biomes in the world (Suttie et al., 2005), grasslands are facing widespread degradation (Gang et al., 2014), largely driven by anthropogenic activities (e.g. overgrazing) (Daily, 1995; Gang et al., 2014). Defined as a retrogressive ecosystem succession, grassland degradation disrupts aboveground vegetation (Bai et al., 2004; Deng et al., 2014) as well as soil physical, chemical, and biological properties (Abril and Bucher, 1999; Viragh et al., 2011; Li et al., 2012; Dlamini et al., 2014). These influences are likely transferred to adjacent aquatic ecosystems (Ren et al., 2019). Since grassland streams contribute approximately one-fifth of continental run-off on the Earth (Dodds, 1997), it's important to better understand the influences of grassland degradation on grassland streams.

As the primary receiver of organic matter and nutrient inputs from terrestrial ecosystems and the main channel linking upland watersheds with downstream aquatic ecosystems such as lakes and estuaries (Vannote et al., 1980; Cronan, 2012), streams play a critical role in biogeochemical processes (Figueiredo et al., 2010; Deegan et al., 2011). Streams are strongly influenced by the landscapes through which they flow (Vannote et al., 1980; Allan, 2004) and thus are particularly sensitive to terrestrial disturbance (Bring and Destouni, 2009; Holmes et al., 2012). Landcover change can alter stream ecosystem properties through a variety of pathways (Burgos-Caraballo et al., 2014). Aquatic impacts of changing landcover can often be first observed via their effects on stream biofilms, which play an integral role in biogeochemical cycling in stream ecosystems through nutrient uptake, transfer of nutrients to higher trophic levels, and remineralization (Schiller et al., 2007). Stream biofilms support complex microbial communities, including bacteria, fungi, algae, and protists (Schiller et al., 2007; Battin et al., 2016) and are likely linked to watershed conditions because landcover controls the export of C, N, and P from terrestrial systems to streams (Abell et al., 2011; Erol and Randhir, 2013; Umbanhowar et al., 2015).

Due to the key roles of microbes in biogeochemical processes, assessment of microbial diversity is key for understanding the influences of external disturbances and environmental changes on aquatic ecosystems (Hosen et al., 2017). Previous studies have shown that microbial diversity in streams is affected by catchment landcover (Burgos-Caraballo et al., 2014). For example, by increasing streamwater nutrients, urbanization has been shown to increase microbial diversity in stream biofilms (Burgos-Caraballo et al., 2014) although other studies have shown decreased microbial diversity (Kohler et al., 2011) because of increased nutrient loading and other anthropogenic disturbances (Vitousek et al., 1997; Dodds and Welch, 2000). Furthermore, Ren et al. (2019) has shown that grassland degradation differentially impacts inputs of N and P to streams, making it difficult to predict how microbial diversity would respond to grassland degradation.

Beyond impacts on microbial community structure, landcover also likely impacts microbial functions. Microbial processes in stream ecosystems are influenced by multiple environmental variables (Zeglin, 2015), such as nutrient availability (Levi et al., 2017; Qu et al., 2017), organic matter quantity and quality (Molinero and Burke, 2009; Mann et al., 2014), and hydrological factors (Valett et al., 1997; Ren et al., 2017). In turn, these factors are determined by catchment landcover. Thus, it is reasonable to predict that microbial communities respond to landcover change due to the changes of these environmental variables. Previous studies have shown that microbial biomass and stoichiometry change significantly when landcover shifts from forest to agriculture or urban area (Walker and Pan, 2006; Smucker et al., 2013a; Teittinen et al., 2015; Qu et al., 2017). Our previous study has reported potential influence of grassland degradation on nutrient availabilities in streams water and nutrient limitation of stream biofilms (Ren et al., 2019). However, we still have very limited knowledge about the influences of grassland degradation on bacterial communities in these and similar streams that are affected by grassland deterioration.

Indeed, grassland degradation is a pervasive threat. Approximately 90% of the grassland in China has already been degraded due to a combination of anthropogenic and natural factors (Harris, 2010). On the Qinghai-Tibet Plateau (QTP), grassland is the dominant landcover and has undergone serious degradation in recent decades (Dong et al., 2015; Zhang et al., 2016; Ma et al., 2018). In degraded grassland systems, large losses of nutrients can occur due to erosion, loss of vegetation cover, and changes of soil texture (Dong et al., 2012; Su et al., 2015). Such impacts are likely associated with major changes in bacterial communities in adjacent streams. In this study, our aim was to document the structural properties of bacterial communities in stream biofilms and address the question: how do bacterial diversity and community respond to grassland degradation? According to mechanisms and previous studies described above, we hypothesized that grassland degradation and associated changes in environmental variables decrease bacterial alpha diversity and shift the structure of bacterial communities in stream biofilms. To get a better understanding of bacterial community properties in stream biofilms and to test these hypotheses, we investigated the bacterial communities in stream biofilms in the Qinghai Lake watershed on the northeast edge of the QTP within sub-catchments that are experiencing different degrees of degradation. If the hypothesis is correct, we predict that bacterial alpha diversity will decrease and community structure will shift with increased grassland degradation in the sub-catchments due to the disruptions of stream physicochemical properties.

Methods

Sample collection and analyses

The study area, the Qinghai Lake Watershed (36°15′ - 38°20′ N and 97°50′ - 101°20′ E), is located on the northeast edge of the QTP (Figure 6.1). Qinghai Lake Watershed is situated in a semiarid and cold climate zone with a high altitude from 3194 to 5174 m above the sea level. The mean annual temperature is -0.7℃ (Yi et al., 2010). The mean annual precipitation and evaporation are 363 mm and 1,300-2,000 mm, respectively (Yi et al., 2010). As an endorheic saline lake, Qinghai Lake is the largest lake in China feeding by more than 40 tributaries, within which the Buha River is the largest (Figure 6.1). The watershed covers an area of 29,660 km^2 of which 75% is covered by grassland. However, due to overgrazing, climate change, and other external disturbance, more than half of the grassland had been classified as degraded in 2010 (Luo et al., 2013).

Samples were collected from 22 stream sites during July 2017 (Figure 6.1). At each sample site, pH and conductivity (Cond) were measured *in situ* using a YSI handheld meter (model 80; YSI, Yellow Springs, Ohio). Altitude was measured using a GPS unit (Triton 500, Magellan, Santa Clara, California). At each stream sampling point, 6 to 9 submerged rocks were randomly chosen along the river cross section. The benthic biofilm in a 4.5-cm-diameter area was removed from the upper surface of each rock by rigorously brushing with a sterilized nylon brush. The slurry was rinsed using 500 mL sterile water. Approximately 10-mL of the mixed slurry was filtered onto a 0.2-µm polycarbonate membrane filter (Whatman, UK) that was immediately frozen in liquid nitrogen in the field. Twenty milliliters of mixed slurry were filtered onto pre-combusted glass fiber (GF/F) filters (six replicates) for later chemical analysis. 500-mL water samples were also collected for chemical analyses.

Total nitrogen (TN) in the water samples was quantified by ion chromatography after persulfate oxidation (EPA 300.0). Total phosphorus (TP) was analyzed using the ascorbate acid colorimetric method after oxidation (EPA 365.3). DOC was analyzed on filtered (using pre-combusted GF/F filters) streamwater using a Shimadzu TOC Analyzer (TOC-VCPH, Shimadzu Scientific Instruments, Columbia, Maryland). Biofilm carbon (BFC) and nitrogen (BFN) were analyzed using an elemental analyzer (LECO 628). Biofilm phosphorus (BFP) was analyzed as soluble reactive phosphorus (SRP) after digestion with potassium persulfate (EPA 365.3).

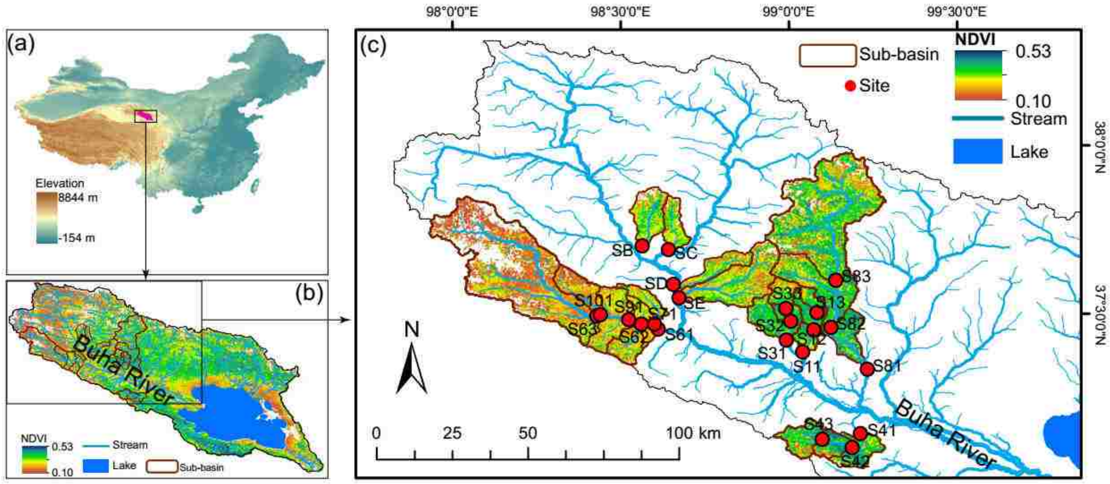

Figure 6.1 Study area and sample sites. (a) Qinghai Lake is the largest lake in China and is located on the northeast edge of Qinghai-Tibet Plateau. (b) Buha river is the largest tributary of Qinghai Lake, which is an endorheic saline lake. (c) Water and benthic biofilm samples were collected at 22 sites.

Normalized difference vegetation index

Grassland status of the Qinghai Lake watershed was assessed using the normalized difference vegetation index (NDVI) based on Landsat images downloaded from USGS (https://earthexplorer.usgs.gov/) (Figure 6.1). NDVI is the most commonly used vegetation index and is often used as a measure of landcover status to monitor land degradation (Thiam, 2003; Yengoh et al., 2014). NDVI is calculated based on the absorption of red light and the reflection of infrared radiation by vegetation (Rouse et al., 1973). NDVI is calculated as (NIR-RED)/(NIR+RED), where NIR is near infrared reflectance and RED is visible red reflectance. Bare areas of soil, rock, and sand are represented by very low NDVI values (0~0.1). Negative NDVI represents water bodies. It has been demonstrated that NDVI exhibits close relationships with above-ground vegetation biomass and coverage (Carlson and Ripley, 1997; Eastwood et al., 1997) and thus is commonly used to estimate regional vegetation coverage (Carlson and Ripley, 1997) and to indicate grassland degradation (Thiam, 2003; Akiyama and Kawamura, 2007; Li et al., 2013; Hilker et al., 2014).

In this study, Landsat satellite images acquired in July 2017 and July 2009 were used to calculate NDVI in ArcGIS 14.0. The average NDVI of the sub-basin above the sample sites were calculated. Contemporary grassland status was represented by NDVI value in 2017 (shown as NDVI in the following analyses). Historical grassland status was represented by the NDVI value in 2009 (shown as NDVI.h in the following analyses). The average annual change of NDVI from 2009 to 2017 was calculated to indicate the change rate of NDVI in the recent past (shown as NDVI.r in the following analyses). In the Qinghai Lake watershed, NDVI had a large spatial variation that was closely associated with *in situ* measured vegetation status (e.g. aboveground biomass, grass species richness, and fractional vegetation coverage rate) but was not associated with altitude (Ren et al., 2019). Moreover, the relationship between NDVI difference and geographic distance of paired sample sites was statistically significant ($P=0.002$) but explained little variance ($R^2=0.039$), indicating that potential spatial non-independence of the observations should not strongly complicate the space-for-time approach. Thus, using “space-for-time” substitution (Pickett, 1989; Blois et al., 2013), we could study the influences of grassland degradation on stream ecosystems according to spatial patterns of contemporary NDVI (Ren et al., 2019).

DNA extraction, PCR, and sequencing

DNA in stream biofilm samples was extracted using the TIANGEN-DP336 soil DNA Kit (TIANGEN-Biotech, Beijing, China) following the manufacturer's recommendations. The forward primer (CCTACGGRRBGCASCAGKVRVGAAT) and reverse primer (GGACTACNVGGGTWTCTAATCC) were used to amplify the V3 and V4 regions of the 16S rRNA genes. Samples were amplified using an initial denaturation of 3 min at 94 °C, followed by 24 cycles of 30 s denaturation at 94 °C, 90 s annealing at 57 °C, 10 s extension at 72 °C, and a final elongation for 10 min at 72 °C. PCR products were verified on a 2% agarose gel and quantified using a Qubit 3.0 Fluorometer (Life Technologies, Germany). DNA libraries were sent to GENEWIZ, Inc. (Suzhou, China) for sequencing on an Illumina MiSeq platform (Illumina, San Diego, CA, USA).

Sequence analysis

The resulting 16S rRNA raw sequences (available at NCBI, PRJNA526110) were processed and analyzed with QIIME 1.9.1 (Caporaso et al., 2010). The *join_paired_ends.py* script was used to join forward and reverse reads. The paired-end sequences were assigned to samples based on barcode, trimmed by removing barcode and primer sequences, and quality filtered using *split_libraries.py* script. Low quality sequences and the chimeric sequences were removed. Operational taxonomic units (OTUs) were clustered and assigned at 99% genetic similarity against the SILVA 132 database (Quast et al., 2013) using *pick_open_reference_otus.py* script. The *filter_otus_from_otu_table.py* script was used to remove the singletons, Archaea, and unknown OTUs (Kozich et al., 2013). To normalize surveying effort at the sample level, the *single_rarefaction.py* script was used with the depth of 25,884 sequence per sample (Figure S6.1). The *alpha_diversity.py* script was used to calculate alpha diversity indices, including Chao 1, observed OTUs, Shannon, and PD whole tree.

Statistical analysis

Abundant and rare taxa were defined based on their relative abundances (Pedrós-Alió, 2012; Logares et al., 2014). Following recent studies (Liu et al., 2015a; Xue et al., 2018), OTUs with a relative abundance ≥1% in all samples and OTUs with a relative abundance ≥1% in at least one sample and ≥0.01% in all samples were classified as abundant taxa (i.e. “AT” or “abundant subcommunity” hereafter). OTUs with a relative abundance <0.01% in all samples and OTUs with a relative abundance <0.01% in at least one sample and <1% in all samples were classified as rare taxa (RT) and constituted the “rare subcommunity”. OTUs with a relative abundance ≥0.01% and <1% were classified as moderate taxa (MT). OTUs with a relative abundance <0.01% and ≥1% in at least one sample were classified as Conditionally rare and abundant taxa (CRAT).

The relationships between environmental variables and bacterial alpha diversity were assessed using Pearson correlation with the R stats v3.6.1 package (R Core Team, 2017). P-values were adjusted for false discovery using Benjamini and Hochberg (BH) methods (Benjamini and Hochberg, 1995). Bacterial community distributions along the NDVI gradient were visualized using non-metric multidimensional scaling (NMDS) with vegan 2.5-5 package (Oksanen et al., 2007), which is a method to collapse information from high dimension into low dimension, making the data easier to visualize and interpret. The relationships between environmental variables and bacterial community structure were assessed using distance-based redundancy analysis (dbRDA) with vegan 2.5-5 package (Oksanen et al., 2007), which is a method to carry out constrained ordinations on data using non-Euclidean distance measures. Furthermore, variation partitioning analyses (VPA) were performed to dissect the relative contributions of three group factors to the variations of bacterial community structure. These three group factors were vegetation (NDVI, NDVI.h, and NDVI.r), nutrients (DOC, TN, TP, BFC, BFN, and BFP), and others (conductivity, altitude, and pH).

Results

Alpha diversity and community composition

In total, 7050 OTUs were detected from 569,448 high-quality sequences at the 99% similarity level. Within the whole data set, 19 (0.3% of the total number) OTUs were classified as abundant (Table S6.1) while 6922 (98.2% of the total number) OTUs were rare, representing 16.2% and 61.6% of total sequences, respectively (Figure 6.2). Across all sample sites, the observed OTUs, Chao 1, Shannon diversity, and phylogenetic diversity (PD whole tree) were 3299±700, 4400±884, 9.41±0.77, and 166±27, respectively. Correlation analysis showed that the number of observed OTUs had significant positive relationships with BFC and BFP, Chao 1 had a significant positive relationship with BFP, and phylogenetic diversity had significant positive relationships with BFC, BFN, and BFP (Table 6.1). However, alpha diversity was not associated with NDVI, historical NDVI (NDVI.h), rate of change in NDVI (NDVI.r), altitude, any of the tested physicochemical variables of streamwater, nor the C:N:P ratios in streamwater and biofilms. Thus, our hypothesis that grassland degradation negatively affects bacterial alpha diversity of stream biofilms was not supported.

In the stream biofilm bacterial communities, the dominant phyla (relative abundance >1%) were Proteobacteria (67.9%), followed by Bacteroidetes (20.1%) Cyanobacteria/Chloroplast (7.0%), and Verrucomicrobia (1.5%) (Figure 6.2). Abundant taxa were affiliated to Alphaproteobacteria (8.6%, n=11), Gammaproteobacteria (4.4%, n=5), Bacteroidetes (2.2%, n=2), and Cyanobacteria (1.0%, n=1) (Figure 6.2 and Table S6.1). The relative abundances of those bacterial phyla were not associated with environmental variables. As shown by the mean Bray-Curtis distance between all pairs of samples, abundant subcommunities had a significantly lower mean Bray-Curtis distance value while rare subcommunities had a significantly higher mean value than the overall community (Figure S6.2), suggesting lower variabilities for abundant subcommunities but larger variabilities for rare subcommunities.

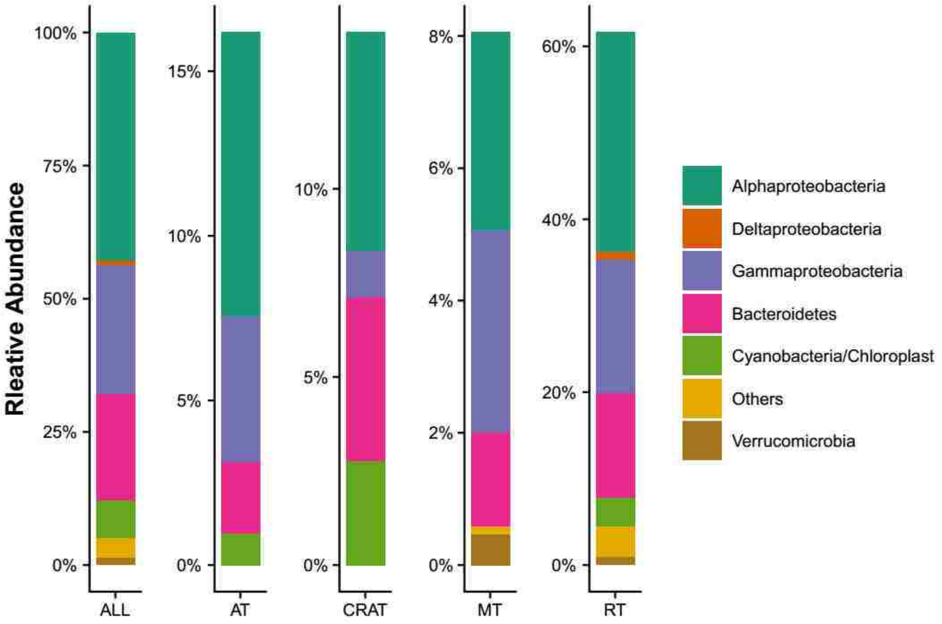

Figure 6.2 Composition structure of bacterial communities in stream biofilms. Only the dominant phyla with relative abundance >1% are shown. "All" represents the whole communities. "AT", "CRAT", "MT", and "RT" represent subcommunities of abundant taxa, conditionally rare and abundant taxa, moderate taxa, and rare taxa, respectively.

Table 6.1 Correlation relationships between alpha diversity versus carbon, nitrogen, and phosphorus concentrations in stream biofilms. P-values are adjusted using Benjamini and Hochberg (BH) methods. Significant associations (P<0.05) are shown in bold.

	Chao 1		Observed OTUs		Shannon		PD Whole Tree	
	r	p	r	p	r	p	r	p
BFC	0.437	**0.042**	0.394	0.069	0.255	0.252	0.473	**0.026**
BFN	0.387	0.075	0.347	0.114	0.227	0.310	0.440	**0.040**
BFP	0.455	**0.033**	0.431	**0.045**	0.337	0.125	0.454	**0.034**

Cyanobacteria and Chloroplast

In the Cyanobacteria/Chloroplast group, the total number of OTUs was 512, within which, 343 OTUs were affiliated to cyanobacteria and 169 OTUs were identified as chloroplast sequences (indicative of eukaryotic algae). Cyanobacteria had a much higher relative abundance than chloroplasts (mean relative abundance of 5.8% vs. 1.2%, Figure S6.3). The ratio of cyanobacteria to chloroplast sequences ranged from 0.37 to 74.8 (Figure S6.3).

There were no significant relationships of environmental variables with the relative abundances of cyanobacteria and chloroplast sequences nor with the ratio between them (except negative relationships between pH and the Cyanobacteria:Chloroplast ratio, Table S6.2). *Nostocales,* an order than includes N-fixers, was one of the dominant cyanobacterial groups in those streams (Figure S6.4). However, environmental variables were not significantly correlated with the relative abundance of any of the cyanobacterial orders (Table S6.3).

Driving factors of spatial patterns of community structure

Bacterial communities did not show clear patterns with NDVI across the sub-basins (Figure S6.5). However, distance-based redundancy analyses (dbRDA) showed that NDVI.r, pH, conductivity, as well as C, N, and P contents in stream biofilms had significant relationships ($P < 0.05$) with the distribution of bacterial communities (Figure 6.3). The first two axes represented 29.1%, 38.9%, and 29.1% of the variances in terms of all, abundant, and rare communities/subcommunities, respectively (Figure 6.3). Variation partitioning analyses (VPA) showed that the environmental variables explained 27.2%, 37.8%, and 24.0% of the compositional variances of these bacterial communities, respectively (Figure 6.3). Thus, our hypothesis that grassland degradation and associated environmental change affect bacterial communities in stream biofilms was supported.

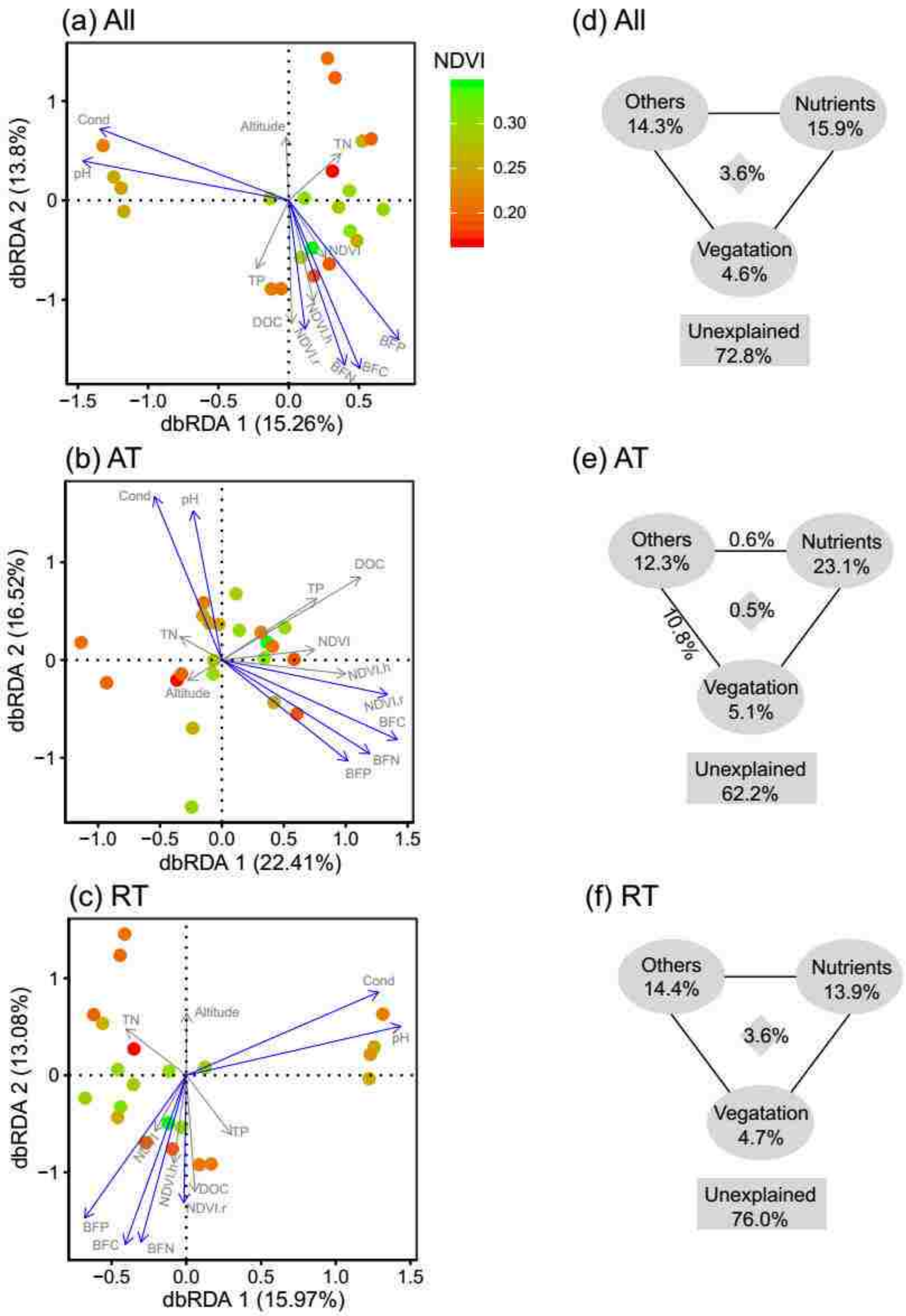

Figure 6.3 (a-c) Distance-based redundancy analyses (dbRDA) depicting the relationships between bacterial communities (points) and environmental variables (arrows). Points are colored by NDVI. Significant variables ($P<0.05$ by envfit function) are shown in blue. (d-f) Variance partition analysis (VPA) determined the relative contributions of three group factors and the interactions between two or three of the factors. These three group factors

are vegetation (NDVI, NDVI.h, and NDVI.r), nutrients (DOC, TN, TP, BFC, BFN, and BFP), and others (conductivity, altitude, and pH). The relative variance proportions that the corresponding components could explain are shown in percentages. "All", "AT", and "RT" represent the whole communities and the subcommunities of abundant and rare OTUs, respectively.

Discussion

In the biofilm bacterial communities of the QTP streams we sampled, the dominant phyla were Proteobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia, which are typical in freshwater ecosystems (Tamames et al., 2010; Wilhelm et al., 2013). The relative abundances of these phyla were not associated with any of the tested environmental variables in our study. However, the variation of overall bacterial community composition was significantly associated with rate of change in NDVI, pH, conductivity, as well as C, N, P contents and C:N ratio in the biofilm biomass. Many studies have shown that the properties of stream biofilms are influenced by activities in upstream catchments (Fanta et al., 2010; O'Brien and Wehr, 2010; Kohler et al., 2011). In grassland watersheds, large proportions of organic carbon and nutrient stocks are concentrated in the top soil layers (Gill et al., 1999). External disturbances likely cause dramatic soil nutrient depletion and soil texture changes (Steffens et al., 2008; Ruiz-Sinoga and Romero Diaz, 2010; Dong et al., 2012; Dlamini et al., 2014). In degraded grassland ecosystems, increased soil erosion, loss of vegetation cover, and soil texture changes aggravate scour from surface flow, causing large losses of soil organic matter and nutrients to adjacent aquatic ecosystems (Dong et al., 2012; Su et al., 2015). This is of potential importance because terrestrial inputs are often the most important organic matter and nutrient sources, especially in small streams (Howarth and Fisher, 1976; Meyer et al., 1981; Saunders et al., 2006; Tank et al., 2010). These inputs can be accumulated and stored in benthic biofilms, influencing bacterial diversity, activities, and community composition (Kamjunke et al., 2015; Aubeneau et al., 2016). Overall our results are consistent with a previous study showing that diversity and composition of microbial communities in stream biofilms are more controlled by site-specific conditions than factors at the watershed scale (Burgos-Caraballo et al., 2014). Moreover, grassland degradation increases pH and influences free ion activities in soil (Viragh et al., 2011). These impacts could be transferred to adjacent stream ecosystems and affect streamwater chemical conditions and, ultimately, their biological communities (Figueiredo et al., 2010; Zeglin, 2015). Thus, our results suggest that the history of grassland degradation (indicated as the rate of change in NDVI) influences the bacterial communities in stream biofilms in this catchment, although the influence is not observed in relative abundances at the phylum level. These shifts in stream bacterial communities support a view of inland waters as sentinels and integrators of terrestrial processes due to their close connections via transport and storage of water, nutrients, and energy.

In our 16S data, primers picked up some chloroplast reads, which accounted to 1.2% of the total reads on average. These reads were grouped into 169 OTUs. Eukaryotic algae are important components of stream biofilms and also change with shifts in landcover and other environmental factors (Busse et al., 2006; Niyogi et al., 2007; Smucker et al., 2013b). In our study, the relative abundance of chloroplasts varied across the study streams but only accounted for a very small proportion of the total number of OTUs. Furthermore, the relative abundance of chloroplasts as well as the ratio of Cyanobacteria:Chloroplast were not associated with measured environmental variables (except negative relationships between pH and the Cyanobacteria:Chloroplast ratio), suggesting that the distributions of eukaryotic algae and cyanobacteria did not respond to grassland status *per se* in the sub-catchment nor any of the measured environmental variables. Previous studies have found that nutrients and temperature can also affect the relative abundance of cyanobacteria and chloroplast reads in 16S surveys. For example, chloroplasts contributed a higher proportion of sequence reads than cyanobacteria in environments with high nitrogen, high N:P, and low temperature (Peipoch et al., 2015; Zwirglmaier et al., 2015; Scherer et al., 2017; Varkey et al., 2018). However, in our studied streams, the ratio of Cyanobacteria:Chloroplast was not significantly associated with nutrient concentrations and nutrient ratios. However, as discussed above, grassland degradation increases soil pH (Viragh et al., 2011), which might increase streamwater pH and further shift Cyanobacteria:Chloroplast ratio. It has been found that cyanobacteria are strong competitors in comparison with eukaryotic algae at high pH and associated low CO_2

because cyanobacteria have effective CO_2-concentrating mechanisms to augment their intracellular CO_2 level by taking up CO_2 and bicarbonate (Caraco and Miller, 1998; Price et al., 2007; Burnap et al., 2015). However, eukaryotic algae can also exhibit effective CO_2-concentrating mechanisms to adapt to low CO_2 level and increased pH (Moroney and Ynalvez, 2007; Meyer and Griffiths, 2013; Ji et al., 2017). In fact, cyanobacteria and eukaryotic algae are not only affected by a single environmental variable but by coupled physicochemical and hydrological factors (Hart et al., 1990; Dahm et al., 2003; Velasco et al., 2006; Bray et al., 2008).

In our studied bacterial communities, more than 98% of the OTUs were rare taxa that accounted for 61.6% of total sequences, while only 0.3% OTUs were abundant, accounting for 16.2% of total sequences. Biological communities normally contain a small number of abundant taxa and many rare species (Magurran and Henderson, 2003). This is also true of microbial communities, for which most taxa are extremely rare (Logares et al., 2014). Abundant and rare microbial subcommunities may have intrinsic characteristics that result in different contributions to community dynamics and ecosystem functions (Logares et al., 2014). Abundant taxa are the core taxa that contribute the most ecosystem functions, such as nutrient cycling, biomass production, and energy flow, and are maintained because of their high growth rate and close fit to particular habitats (Pedros-Alio, 2006). Drastic variations in abundant taxa could cause significant effects on ecosystem processes (Logares et al., 2014). In contrast, rare taxa may have low growth rates but encompass a broad diversity, explaining large amounts of microbial community dynamics and dissimilarities (Campbell et al., 2011; Pedrós-Alió, 2012). It has been proposed that rare taxa include ecologically redundant taxa that could maintain ecosystem functioning and contribute to community stability during environmental perturbations that impact dominant taxa (Caron and Countway, 2009; Pedrós-Alió, 2012). In our study, the rare subcommunities showed similar associations with environmental variables as the whole communities, while the abundant subcommunities were more affected by those environmental variables (Figure 6.3). Thus, we infer that grassland degradation and associated environmental changes influence stream ecosystem functions via effects on both abundant and rare subcommunities but with more intense effects on abundant subcommunities.

Stream biofilms are very complex systems composed of various organisms, including bacteria, algae, fungi, protozoa, and other taxa (Battin et al., 2016). These organisms can have strong interactions involving both cooperation and competition (Fitter and Hillebrand, 2009; Rendueles and Ghigo, 2015; Liu et al., 2015b) that are driven by a large number of environmental factors, including but not limited to nutrient availability (Tank and Dodds, 2003; Myrstener et al., 2018), temperature (Boyero et al., 2011), light (Hillebrand et al., 2004), hydrological factors (Valett et al., 1997; Ren et al., 2017), and land use (Burgos-Caraballo et al., 2014; Qu et al., 2017). Even terrestrial bacteria can contribute to the complexity of bacterial communities in stream biofilms (Crump et al., 2012; Ruiz-Gonzalez et al., 2015). Our data indicate that these interactions can be strongly affected by grassland degradation in the QTP by changing bacterial community composition.

We acknowledge that there are limitations in our interpretation, which are based on limited spatiotemporal scales and a subset of potential environmental variables. More studies, including experimental manipulations, are needed. For example, more streams and longer-term investigations will be helpful in alleviating stochastic influences. Inclusion of more environmental variables, such as hydrological, geological, and climatic factors, may also help explain community variations. Controlled experiments will help reveal the underlying mechanisms of community response to grassland degradation. However, we believe our study has added useful knowledge about how bacterial communities in stream biofilms respond to undergoing grassland degradation in a watershed.

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References

Abell JM, Oezkundakci D, Hamilton DP, Miller SD. 2011. Relationships between land use and nitrogen and phosphorus in New Zealand lakes. Marine and Freshwater Research, 62: 162-175.

Abril A, Bucher EH. 1999. The effects of overgrazing on soil microbial community and fertility in the Chaco dry savannas of Argentina. Applied Soil Ecology, 12: 159-167.

Akiyama T, Kawamura K. 2007. Grassland degradation in China: methods of monitoring, management and restoration. Grassland Science, 53: 1-17.

Allan JD. 2004. Landscapes and riverscapes: The influence of land use on stream ecosystems. Annual Review of Ecology, Evolution, and Systematics, 35: 257-284.

Aubeneau AF, Hanrahan B, Bolster D, Tank J. 2016. Biofilm growth in gravel bed streams controls solute residence time distributions. Journal of Geophysical Research: Biogeosciences, 121: 1840-1850.

Bai YF, Han XG, Wu JG, Chen ZZ, Li LH. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature, 431: 181-184.

Battin TJ, Besemer K, Bengtsson MM, Romani AM, Packmann AI. 2016. The ecology and biogeochemistry of stream biofilms. Nature Reviews Microbiology, 14: 251-263.

Benjamini Y, Hochberg Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57: 289-300.

Blois JL, Williams JW, Fitzpatrick MC, Jackson ST, Ferrier S. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences, 110: 9374-9379.

Boyero L, Pearson RG, Gessner MO, Barmuta LA, Ferreira V, Graça MA, Dudgeon D, Boulton AJ, Callisto M, Chauvet E. 2011. A global experiment suggests climate warming will not accelerate litter decomposition in streams but might reduce carbon sequestration. Ecology Letters, 14: 289-294.

Bray JP, Broady PA, Niyogi DK, Harding JS. 2008. Periphyton communities in New Zealand streams impacted by acid mine drainage. Marine and Freshwater Research, 59: 1084-1091.

Bring A, Destouni G. 2009. Hydrological and hydrochemical observation status in the pan-Arctic drainage basin. Polar Research, 28: 327-338.

Burgos-Caraballo S, Cantrell SA, Ramirez A. 2014. Diversity of benthic biofilms along a land use gradient in tropical headwater streams, Puerto Rico. Microbial Ecology, 68: 47-59.

Burnap RL, Hagemann M, Kaplan A. 2015. Regulation of CO_2 concentrating mechanism in cyanobacteria. Life, 5: 348-371.

Busse LB, Simpson JC, Cooper SD. 2006. Relationships among nutrients, algae, and land use in urbanized southern California streams. Canadian Journal of Fisheries and Aquatic Sciences, 63: 2621-2638.

Campbell BJ, Yu L, Heidelberg JF, Kirchman DL. 2011. Activity of abundant and rare bacteria in a coastal ocean. Proceedings of the National Academy of Sciences, 108: 12776-12781.

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Pena AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Tumbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R. 2010. QIIME allows analysis of high-throughput community sequencing data. Nature Methods, 7: 335-336.

Caraco NF, Miller R. 1998. Effects of CO_2 on competition between a cyanobacterium and eukaryotic phytoplankton. Canadian Journal of Fisheries and Aquatic Sciences, 55: 54-62.

Carlson TN, Ripley DA. 1997. On the relation between NDVI, fractional vegetation cover, and leaf area index. Remote Sensing of Environment, 62: 241-252.

Caron DA, Countway PD. 2009. Hypotheses on the role of the protistan rare biosphere in a changing world. Aquatic Microbial Ecology, 57: 227-238.

Cronan CS. 2012. Biogeochemistry of the Penobscot River watershed, Maine, USA: nutrient export patterns for carbon, nitrogen, and phosphorus. Environmental Monitoring and Assessment, 184: 4279-4288.

Crump BC, Amaral-Zettler LA, Kling GW. 2012. Microbial diversity in arctic freshwaters is structured by inoculation of microbes from soils. ISME Journal, 6: 1629-1639.

Dahm CN, Baker MA, Moore DI, Thibault JR. 2003. Coupled biogeochemical and hydrological responses of streams and rivers to drought. Freshwater Biology, 48: 1219-1231.

Daily GC. 1995. Restoring value to the world's degraded lands. Science, 269: 350-354.

Deegan LA, Neill C, Haupert CL, Ballester MVR, Krusche AV, Victoria RL, Thomas SM, de Moor E. 2011. Amazon deforestation alters small stream structure, nitrogen biogeochemistry and connectivity to larger rivers. Biogeochemistry, 105: 53-74.

Deng L, Zhang Z, Shangguan Z. 2014. Long-term fencing effects on plant diversity and soil properties in China. Soil and Tillage Research, 137: 7-15.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma, 235-236: 372-381.

Dodds WK. 1997. Distribution of runoff and rivers related to vegetative characteristics, latitude, and slope: a global perspective. Journal of the North American Benthological Society, 16: 162-168.

Dodds WK, Welch EB. 2000. Establishing nutrient criteria in streams. Journal of the North American Benthological Society, 19: 186-196.

Dong SK, Wang XX, Liu SL, Li YY, Su XK, Wen L, Zhu L. 2015. Reproductive responses of alpine plants to grassland degradation and artificial restoration in the Qinghai-Tibetan Plateau. Grass and Forage Science, 70: 229-238.

Dong SK, Wen L, Yi YY, Wang XX, Zhu L, Li XY. 2012. Soil-quality effects of land degradation and restoration on the Qinghai–Tibetan plateau. Soil Science Society of America Journal, 76: 2256-2264.

Eastwood JA, Yates MG, Thomson AG, Fuller RM. 1997. The reliability of vegetation indices for monitoring saltmarsh vegetation cover. International Journal of Remote Sensing, 18: 3901-3907.

Erol A, Randhir TO. 2013. Watershed ecosystem modeling of land-use impacts on water quality. Ecological Modelling, 270: 54-63.

Fanta SE, Hill WR, Smith TB, Roberts BJ. 2010. Applying the light: nutrient hypothesis to stream periphyton. Freshwater Biology, 55: 931-940.

Figueiredo RO, Markewitz D, Davidson EA, Schuler AE, Dos S. Watrin O, de Souza Silva P. 2010. Land-use effects on the chemical attributes of low-order streams in the eastern Amazon. Journal of Geophysical Research, 115: 1-14.

Fitter A, Hillebrand H. 2009. Microbial food web structure affects bottom-up effects and elemental stoichiometry in periphyton assemblages. Limnology and Oceanography, 54: 2183-2200.

Foley JA, Defries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH. 2005. Global consequences of land use. Science, 309: 570-574.

Gang C, Zhou W, Chen Y, Wang Z, Sun Z, Li J, Qi J, Odeh I. 2014. Quantitative assessment of the contributions of climate change and human activities on global grassland degradation. Environmental Earth Sciences, 72: 4273-4282.

Gill RA, Burke DG, Milchunas DG, Lauenroth WK. 1999. Relationship between root biomass and soil organic matter pools in the short grass steppe of eastern Colorado. Ecosystems, 2: 226-236.

Harris RB. 2010. Rangeland degradation on the Qinghai-Tibetan plateau: A review of the evidence of its magnitude and causes. Journal of Arid Environments, 74: 1-12.

Hart BT, Bailey P, Edwards R, Hortle K, James K, McMahon A, Meredith C, Swadling K. 1990. Effects of salinity on river, stream and wetland ecosystems in Victoria, Australia. Water Research, 24: 1103-1117.

Hilker T, Natsagdorj E, Waring RH, Lyapustin A, Wang Y. 2014. Satellite observed widespread decline in Mongolian grasslands largely due to overgrazing. Global Change Biology, 20: 418-428.

Hillebrand H, de Montpellier G, Liess A. 2004. Effects of macrograzers and light on periphyton stoichiometry. Oikos, 106: 93-104.

Holmes RM, McClelland JW, Peterson BJ, Tank SE, Bulygina E, Eglinton TI, Gordeev VV, Gurtovaya TY, Raymond PA, Repeta DJ, Staples R, Striegl RG, Zhulidov AV, Zimov SA. 2012. Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic ocean and surrounding seas. Estuaries and Coasts, 35: 369-382.

Hosen JD, Febria CM, Crump BC, Palmer MA. 2017. Watershed urbanization linked to differences in stream bacterial community composition. Frontiers in Microbiology, 8: e1452.

Howarth RW, Fisher SG. 1976. Carbon, nitrogen, and phosphorus dynamics during leaf decay in nutrient-enriched stream microecosystems. Freshwater Biology, 6: 221-228.

Ji X, Verspagen JMH, Stomp M, Huisman J. 2017. Competition between cyanobacteria and green algae at low versus elevated CO_2: who will win, and why? Journal of Experimental Botany, 68: 3815-3828.

Kamjunke N, Mages M, Buttner O, Marcus H, Weitere M. 2015. Relationship between the elemental composition of stream biofilms and water chemistry-a catchment approach. Environmental Monitoring and Assessment, 187: e432.

Kohler TJ, Murdock JN, Gido KB, Dodds WK. 2011. Nutrient loading and grazing by the minnow *Phoxinus erythrogaster* shift periphyton abundance and stoichiometry in mesocosms. Freshwater Biology, 56: 1133-1146.

Kozich JJ, Westcott SL, Baxter NT, Highlander SK, Schloss PD. 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and Environmental Microbiology, 79: 5112-5120.

Levi PS, Starnawski P, Poulsen B, Baattrup-Pedersen A, Schramm A, Riis T. 2017. Microbial community diversity and composition varies with habitat characteristics and biofilm function in macrophyte-rich streams. Oikos, 126: 398-409.

Li S, Verburg PH, Lv S, Wu J, Li X. 2012. Spatial analysis of the driving factors of grassland degradation under conditions of climate change and intensive use in Inner Mongolia, China. Regional Environmental Change, 12: 461-474.

Li XL, Gao J, Brierley G, Qiao YM, Zhang J, Yang YW. 2013. Rangeland degradation on the Qinghai - Tibet plateau: Implications for rehabilitation. Land Degradation and Development, 24: 72-80.

Liu J, Prindle A, Humphries J, Gabalda-Sagarra M, Asally M, Lee DD, Ly S, Garcia-Ojalvo J, Sueel GM. 2015b. Metabolic co-dependence gives rise to collective oscillations within biofilms. Nature, 523: 550-555.

Liu L, Yang J, Yu Z, Wilkinson DM. 2015a. The biogeography of abundant and rare bacterioplankton in the lakes and reservoirs of China. ISME Journal, 9: 2068-2077.

Logares R, Audic S, Bass D, Bittner L, Boutte C, Christen R, Claverie JM, Decelle J, Dolan JR, Dunthorn M, Edvardsen B, Gobet A, Kooistra WH, Mahe F, Not F, Ogata H, Pawlowski J, Pernice MC, Romac S, Shalchian-Tabrizi K, Simon N, Stoeck T, Santini S, Siano R, Wincker P, Zingone A, Richards TA, de Vargas C, Massana R. 2014. Patterns of rare and abundant marine microbial eukaryotes. Current Biology, 24: 813-821.

Luo CF, Xu CJ, You HY, Jin SH. 2013. Analysis on grassland degradation in Qinghai Lake Basin during 2000-2010. Acta Ecologica Sinica, 33: 4450-4459 (In Chinese).

Ma L, Yao Z, Zheng X, Zhang H, Wang K, Zhu B, Wang R, Zhang W, Liu C. 2018. Increasing grassland degradation stimulates the non-growing season CO_2 emissions from an alpine meadow on the Qinghai﹣Tibetan Plateau. Environmental Science and Pollution Research, 25: 26576-26591.

Magurran AE, Henderson PA. 2003. Explaining the excess of rare species in natural species abundance distributions. Nature, 422: 714-716.

Mann PJ, Spencer RGM, Dinga BJ, Poulsen JR, Hernes PJ, Fiske G, Salter ME, Wang ZA, Hoering KA, Six J, Holmes RM. 2014. The biogeochemistry of carbon across a gradient of streams and rivers within the Congo Basin. Journal of Geophysical Research: Biogeosciences, 119: 687-702.

Meyer JL, Likens GE, Sloane J. 1981. Phosphorus, nitrogen and organic carbon flux in a headwater stream. Archiv fur Hydrobiologie, 99: 28-44.

Meyer M, Griffiths H. 2013. Origins and diversity of eukaryotic CO_2-concentrating mechanisms: lessons for the future. Journal of Experimental Botany, 64: 769-786.

Molinero J, Burke RA. 2009. Effects of land use on dissolved organic matter biogeochemistry in piedmont headwater streams of the Southeastern United States. Hydrobiologia, 635: 289-308.

Moroney JV, Ynalvez RA. 2007. Proposed carbon dioxide concentrating mechanism in *Chlamydomonas reinhardtii*. Eukaryotic Cell, 6: 1251-1259.

Myrstener M, Rocher-Ros G, Burrows RM, Bergstrom A, Giesler R, Sponseller RA. 2018. Persistent nitrogen limitation of stream biofilm communities along climate gradients in the Arctic. Global Change Biology, 24: 3680-3691.

Niyogi DK, Koren M, Arbuckle CJ, Townsend CR. 2007. Stream communities along a catchment land-use gradient: subsidy-stress responses to pastoral development. Environmental Management, 39: 213-225.

Oksanen J, Kindt R, Legendre PO, Hara B, Stevens MHH, Oksanen MJ, Suggests MASS. 2007. The vegan package. Community Ecology Package, 10: 631-637.

Pedros-Alio C. 2006. Marine microbial diversity: can it be determined? Trends in Microbiology, 14: 257-263.

Pedrós-Alió C. 2012. The rare bacterial biosphere. Annual Review of Marine Science, 4: 449-466.

Peipoch M, Jones R, Valett HM. 2015. Spatial patterns in biofilm diversity across hierarchical levels of river-floodplain landscapes. PLoS One, 10: e0144303.

Pickett ST. 1989. Space-for-time substitution as an alternative to long-term studies. In Long-term studies in ecology. Springer, New York, NY.

Price GD, Badger MR, Woodger FJ, Long BM. 2007. Advances in understanding the cyanobacterial CO_2-concentrating-mechanism (CCM): functional components, Ci transporters, diversity, genetic regulation and prospects for engineering into plants. Journal of Experimental Botany, 59: 1441-1461.

Qu XD, Ren Z, Zhang HP, Zhang M, Zhang YH, Liu XB, Peng WQ. 2017. Influences of anthropogenic land use on microbial community structure and functional potentials of stream benthic biofilms. Scientific Reports, 7: e15117.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glockner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research, 41: 590-596.

R Core Team. 2017. R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org.

Ren Z, Gao HK, Elser JJ. 2017. Longitudinal variation of microbial communities in benthic biofilms and association with hydrological and physicochemical conditions in glacier-fed streams. Freshwater Science, 36: 479-490.

Ren Z, Niu DC, Ma PP, Wang Y, Fu H, Elser JJ. 2019. Cascading influences of grassland degradation on nutrient limitation in a high mountain lake and its inflow streams. Ecology, 100: e02755.

Rendueles O, Ghigo J. 2015. Mechanisms of competition in biofilm communities. Microbiology Spectrum, 3: 1-18.

Rouse JW, Haas RH, Schell JA, Deering DW. 1973. Monitoring vegetation systems in the Great Plains with ERTS. Third Earth Resources Technology Satelite-1 Symposium. 309-317.

Ruiz-Gonzalez C, Pablo Nino-Garcia J, Del Giorgio PA. 2015. Terrestrial origin of bacterial communities in complex boreal freshwater networks. Ecology Letters, 18: 1198-1206.

Ruiz-Sinoga JD, Romero Diaz A. 2010. Soil degradation factors along a Mediterranean pluviometric gradient in Southern Spain. Geomorphology, 118: 359-368.

Saunders TJ, McClain ME, Llerena CA. 2006. The biogeochemistry of dissolved nitrogen, phosphorus, and organic carbon along terrestrial-aquatic flowpaths of a montane headwater catchment in the Peruvian Amazon. Hydrological Processes, 20: 2549-2562.

Scherer PI, Millard AD, Miller A, Schoen R, Raeder U, Geist J, Zwirglmaier K. 2017. Temporal dynamics of the microbial community composition with a focus on toxic cyanobacteria and toxin presence during harmful algal blooms in two South German lakes. Frontiers in Microbiology, 8: e2387.

Schiller DV, Marti E, Riera JL, Sabater F. 2007. Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses. Freshwater Biology, 52: 891-906.

Smucker NJ, Becker M, Detenbeck NE, Morrison AC. 2013b. Using algal metrics and biomass to evaluate multiple ways of defining concentration-based nutrient criteria in streams and their ecological relevance. Ecological Indicators, 32: 51-61.

Smucker NJ, Detenbeck NE, Morrison AC. 2013a. Diatom responses to watershed development and potential moderating effects of near-stream forest and wetland cover. Freshwater Science, 32: 230-249.

Steffens M, Kölbl A, Totsche KU, Kögel-Knabner I. 2008. Grazing effects on soil chemical and physical properties in a semiarid steppe of Inner Mongolia (P.R. China). Geoderma, 143: 63-72.

Su X, Wu Y, Dong S, Wen L, Li Y, Wang X. 2015. Effects of grassland degradation and re-vegetation on carbon and nitrogen storage in the soils of the Headwater Area Nature Reserve on the Qinghai-Tibetan Plateau, China. Journal of Mountain Science, 12: 582-591.

Suttie JM, Reynolds SG, Batello C. 2005. Grasslands of the World. No. 34. Food and Agriculture Organization of the United Nations. Rome, Italy.

Tamames J, Jose Abellan J, Pignatelli M, Camacho A, Moya A. 2010. Environmental distribution of prokaryotic taxa. BMC Microbiology, 10: e85.

Tank JL, Dodds WK. 2003. Nutrient limitation of epilithic and epixylic biofilms in ten North American streams. Freshwater Biology, 48: 1031-1049.

Tank JL, Rosi-Marshall EJ, Griffiths NA, Entrekin SA, Stephen ML. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. Journal of the North American Benthological Society, 29: 118-146.

Teittinen A, Taka M, Ruth O, Soininen J. 2015. Variation in stream diatom communities in relation to water quality and catchment variables in a boreal, urbanized region. Science of the Total Environment, 530: 279-289.

Thiam AK. 2003. The causes and spatial pattern of land degradation risk in southern Mauritania using multitemporal AVHRR-NDVI imagery and field data. Land Degradation and Development, 14: 133-142.

Umbanhowar C, Camill P, Edlund M, Geiss C, Henneghan P, Passow K. 2015. Lake-landscape connections at the forest-tundra transition of northern Manitoba. Inland Waters, 5: 57-74.

Valett H, Dahm C, Campana M, Morrice J, Baker MA, Fellows C. 1997. Hydrologic influences on groundwater-surface water ecotones: heterogeneity in nutrient composition and retention. Journal of the North American Benthological Society, 16: 239-247.

Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. Canadian Journal of Fisheries and Aquatic Sciences, 37: 130-137.

Varkey D, Mazard S, Jeffries TC, Hughes DJ, Seymour J, Paulsen IT, Ostrowski M. 2018. Stormwater influences phytoplankton assemblages within the diverse, but impacted Sydney Harbour estuary. PLoS One, 13: e0209857.

Velasco J, Millán A, Hernández J, Gutiérrez C, Abellán P, Sánchez D, Ruiz M. 2006. Response of biotic communities to salinity changes in a Mediterranean hypersaline stream. Saline Systems, 2: e12.

Viragh K, Toth T, Somodi I. 2011. Effect of slight vegetation degradation on soil properties in *Brachypodium pinnatum* grasslands. Plant and Soil, 345: 303-313.

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Schlesinger WH, Tilman D. 1997. Human alteration of the global nitrogen cycle: Sources and consequences. Ecological Applications, 7: 737-750.

Walker CE, Pan YD. 2006. Using diatom assemblages to assess urban stream conditions. Hydrobiologia, 561: 179-189.

Wilhelm L, Singer GA, Fasching C, Battin TJ, Besemer K. 2013. Microbial biodiversity in glacier-fed streams. ISME Journal, 7: 1651-1660.

Williamson CE, Dodds W, Kratz TK, Palmer MA. 2008. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Frontiers in Ecology and the Environment, 6: 247-254.

Xue Y, Chen H, Yang JR, Liu M, Huang B, Yang J. 2018. Distinct patterns and processes of abundant and rare eukaryotic plankton communities following a reservoir cyanobacterial bloom. ISME Journal, 12: e2263.

Yengoh GT, Dent D, Olsson L, Tengberg A, Tucker C. 2014. The use of the Normalized Difference Vegetation Index (NDVI) to assess land degradation at multiple scales a review of the current status, future trends, and practical considerations. Lund University Center for Sustainability Studies (LUCSUS), and the Scientific and Technical Advisory Panel of the Global Environment Facility (STAP/GEF). Lund, Sweden.

Yi WJ, Li XY, Cui BL, Ma YJ. 2010. Climate change and impact on water level of the Qinghai Lake watershed. Journal of Arid Meteorology, 28: 375-383.

Zeglin LH. 2015. Stream microbial diversity responds to environmental changes review and synthesis of existing research. Frontiers in Microbiology, 6: e454.

Zhang Y, Dong S, Gao Q, Liu S, Zhou H, Ganjurjav H, Wang X. 2016. Climate change and human activities altered the diversity and composition of soil microbial community in alpine grasslands of the Qinghai-Tibetan Plateau. Science of the Total Environment, 562: 353-363.

Zwirglmaier K, Keiz K, Engel M, Geist J, Raeder U. 2015. Seasonal and spatial patterns of microbial diversity along a trophic gradient in the interconnected lakes of the Osterseen Lake District, Bavaria. Frontiers in Microbiology, 6: e1168.

Supplementary Information

Figure S6.1 Rarefaction curves of bacterial communities in stream biofilms. Curves are colored by sample sites.

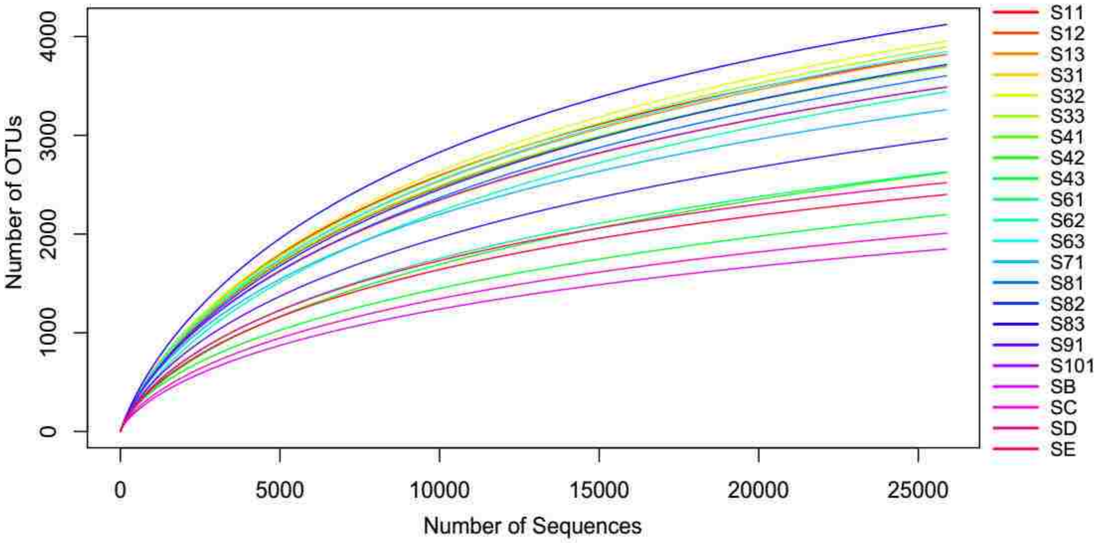

Figure S6.2 Box plot of pairwise Bray-Curtis distances of bacterial communities in stream biofilms. OTUs categories are defined by relative abundance. The top, middle, and bottom lines of the boxes represent the 25th, 50th, and 75th percentiles of the value. Different letters above each box indicate significant differences (ANOVA, $P<0.05$). “All” represents the whole communities. “AT”, “CRAT”, “MT”, and “RT” represent subcommunities of abundant taxa, conditionally rare and abundant taxa, moderate taxa, and rare taxa, respectively.

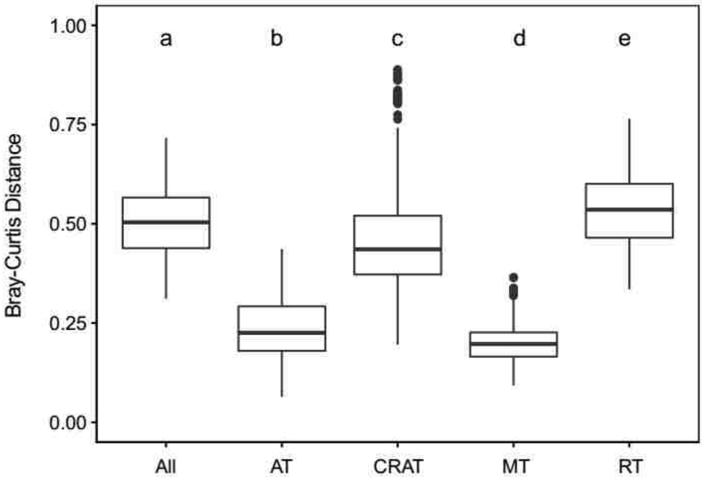

Figure S6.3 Relative abundances of cyanobacteria vs. chloroplast reads. The sample sites from left to right on the x-axis are ordered with decreasing NDVI.

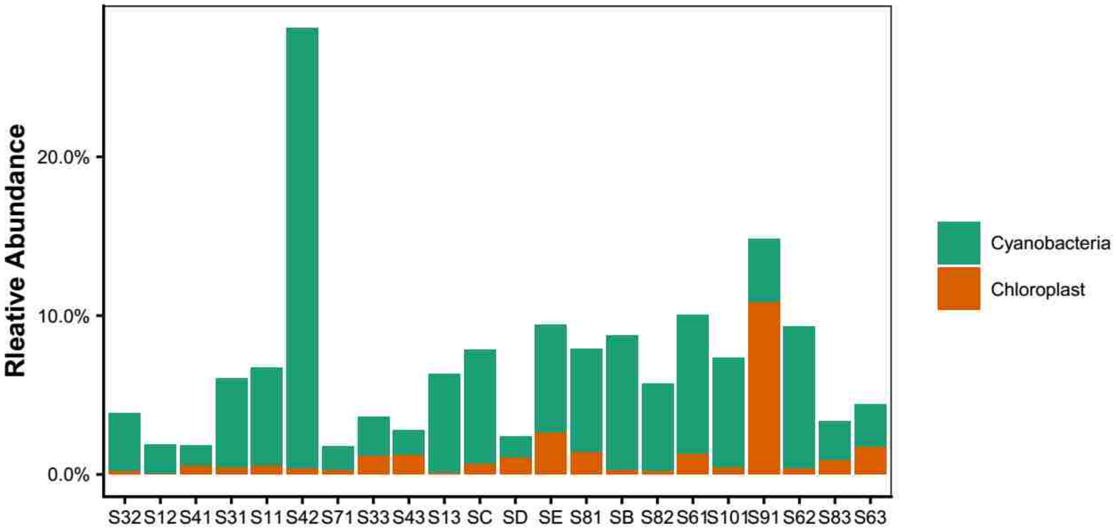

Figure S6.4 Relative abundances of major cyanobacterial orders. The sample sites from left to right on the x-axis are ordered with decreasing NDVI.

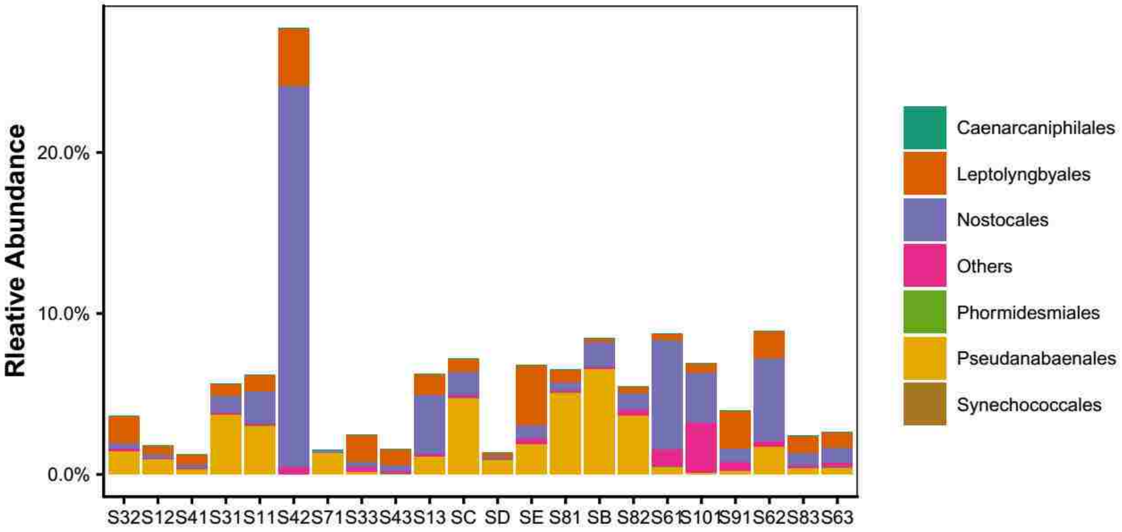

Figure S6.5 Non-metric multidimensional scaling (NMDS) ordination of bacterial communities in stream biofilms based on Bray-Curtis distances. The contour lines represent the value of NDVI. “All”, “AT”, and “RT” represent the whole communities and the subcommunities of abundant and rare OTUs, respectively.

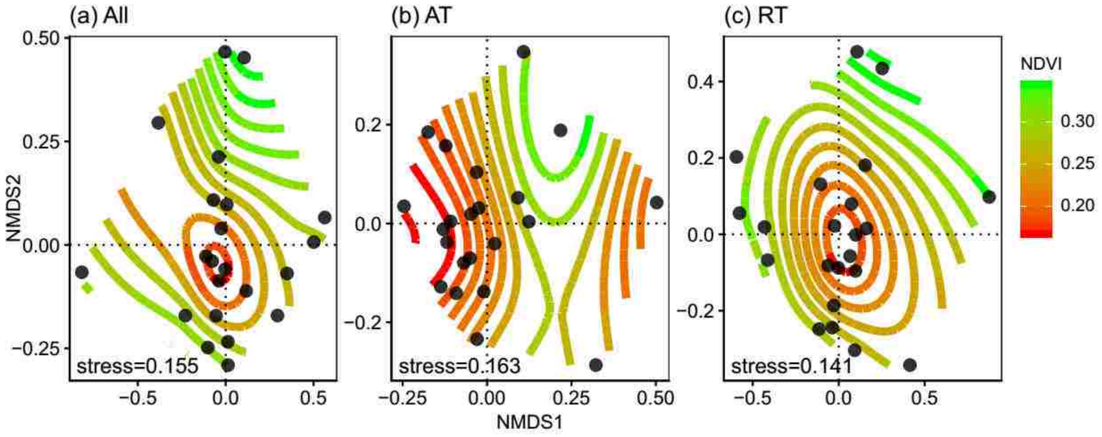

Table S6.1 Taxonomic information of the abundant taxa

OTU ID	Relative Abundance	Taxonomy
otu357022	0.36%	D0_Bacteria; D1_Bacteroidetes; D2_Bacteroidia; D3_Chitinophagales; D4_Chitinophagaceae; D5_Ferruginibacter; D6_uncultured bacterium
otu192389	1.83%	D0_Bacteria; D1_Bacteroidetes; D2_Bacteroidia; D3_Cytophagales; D4_Spirosomaceae; D5_Arcicella; D6_uncultured bacterium
otu8348	0.95%	D0_Bacteria; D1_Cyanobacteria; D2_Oxyphotobacteria; D3_Nostocales; D4_Phormidiaceae; D5_Tychonema CCAP 1459-11B
otu91364	0.63%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Caulobacterales; D4_Caulobacteraceae; D5_Brevundimonas
otu235616	0.29%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Rhodobacterales; D4_Rhodobacteraceae; D5_Pseudorhodobacter
otu72322	1.22%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Rhodobacterales; D4_Rhodobacteraceae; D5_Pseudorhodobacter; D6_uncultured bacterium
otu54665	0.97%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Rhodobacterales; D4_Rhodobacteraceae; D5_Pseudorhodobacter; D6_uncultured bacterium
otu76083	0.85%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_Polymorphobacter
otu85345	0.57%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_Sphingomonas
otu270397	0.35%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_Sphingomonas; D6_uncultured bacterium
otu354354	0.62%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_Sphingorhabdus
otu288327	1.82%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_Sphingorhabdus; D6_uncultured bacterium
otu93766	0.76%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_Sphingorhabdus; D6_uncultured bacterium
otu349146	0.55%	D0_Bacteria; D1_Proteobacteria; D2_Alphaproteobacteria; D3_Sphingomonadales; D4_Sphingomonadaceae; D5_uncultured; D6_uncultured bacterium
otu103015	1.01%	D0_Bacteria; D1_Proteobacteria; D2_Gammaproteobacteria; D3_Betaproteobacteriales; D4_Burkholderiaceae; D5_Hydrogenophaga
otu351024	0.71%	D0_Bacteria; D1_Proteobacteria; D2_Gammaproteobacteria; D3_Betaproteobacteriales; D4_Burkholderiaceae; D5_Hydrogenophaga
otu222488	0.59%	D0_Bacteria; D1_Proteobacteria; D2_Gammaproteobacteria; D3_Betaproteobacteriales; D4_Burkholderiaceae; D5_Polaromonas
otu136842	1.80%	D0_Bacteria; D1_Proteobacteria; D2_Gammaproteobacteria; D3_Betaproteobacteriales; D4_Burkholderiaceae; D5_Polaromonas; D6_uncultured bacterium
otu115425	0.30%	D0_Bacteria; D1_Proteobacteria; D2_Gammaproteobacteria; D3_Betaproteobacteriales; D4_Burkholderiaceae; D5_Rhodoferax

Table S6.2 Correlation analyses of the relative abundance of cyanobacteria and chloroplast as well as the ratio between them versus environmental variables. P-values were corrected using FDR methods. Significant results are indicated by bold.

	Cyanobacteria	Chloroplast	Cyanobacteria:Chloroplast Ratio
NDVI	0.040	-0.303	0.237
NDVI.h	0.070	-0.279	0.418
NDVI.r	0.073	-0.168	0.448
Altitude	-0.212	0.021	0.029
pH	-0.432	0.156	**-0.658**
Cond	-0.155	0.204	-0.174
DOC	-0.119	-0.229	-0.007
TN	0.022	0.125	-0.027
TP	-0.149	-0.056	-0.191
BFC	0.353	-0.345	0.359
BFN	0.383	-0.280	0.311
BFP	0.402	-0.309	0.351
DOC:TN	-0.165	-0.128	-0.144
DOC:TP	0.176	-0.165	0.252
TN:TP	0.344	0.139	0.277
BFC:BFN	-0.228	0.108	-0.119
BFC:BFP	-0.168	0.002	-0.101
BFN:BFP	-0.015	-0.055	-0.051

Table S6.3 Correlation analyses of the relative abundances of cyanobacteria orders with environmental variables. P-values were corrected using FDR methods. There were no significant correlations.

	Caenarcaniphilales	Leptolyngbyales	Nostocales	Others	Phormidesmiales	Pseudanabaenales	Synechococcales
NDVI	-0.118	0.054	0.066	-0.357	0.110	-0.040	-0.315
NDVI.h	-0.057	0.080	0.053	-0.448	-0.037	0.220	-0.370
NDVI.r	0.065	0.055	0.070	-0.478	0.020	0.262	-0.393
Altitude	0.139	-0.008	-0.139	0.197	-0.100	-0.263	0.171
pH	0.140	-0.255	-0.520	-0.149	0.385	0.315	-0.074
Cond	-0.022	-0.036	-0.228	-0.004	-0.270	0.164	0.028
DOC	0.241	-0.260	-0.214	-0.029	-0.249	0.361	0.05
TN	-0.163	0.191	0.007	0.145	-0.174	-0.164	0.247
TP	-0.221	-0.007	-0.185	-0.013	0.122	0.039	-0.055
BFC	0.056	0.155	0.328	-0.004	-0.402	0.079	0.251
BFN	0.047	0.252	0.337	0.003	-0.464	0.095	0.164
BFP	-0.090	0.218	0.398	0.328	-0.398	-0.084	0.465
DOC:TN	0.081	-0.161	-0.162	-0.134	0.165	0.092	-0.037
DOC:TP	0.495	-0.143	0.072	-0.042	-0.246	0.410	-0.029
TN:TP	0.081	0.245	0.335	0.081	-0.071	-0.073	0.132
BFC:BFN	-0.120	-0.261	-0.188	-0.127	0.667	-0.020	-0.01
BFC:BFP	0.310	-0.223	-0.219	-0.362	0.007	0.268	-0.185
BFN:BFP	0.453	0.026	-0.117	-0.331	-0.358	0.321	-0.258

Table S6.4 Mantel tests between bacterial communities versus C:N:P stoichiometry of streamwater and biofilms. Spearman correlations were calculated. P-values were adjusted using FDR method. * represents P<0.05. ** represents P<0.01. "All" represents the whole communities. "AT" represents abundant subcommunities. "RT" represents rare subcommunities.

	Water			Biofilms		
	DOC:TN	DOC:TP	TN:TP	BFC:BFN	BFC:BFP	BFN:BFP
All	0.067	-0.062	0.136	**0.498****	-0.093	-0.067
AT	-0.010	-0.136	0.098	**0.302****	-0.101	-0.111
RT	0.039	-0.059	0.114	**0.485****	-0.089	-0.060

Chapter 7 Summary

My dissertation provides insights into the influences of grassland degradation on terrestrial and aquatic ecosystems on the Qinghai-Tibet Plateau from the perspectives of C:N:P stoichiometry and nutrient limitation, as well insight into impacts on the taxonomic structure of bacterial communities in these habitats (Figure 7.1). Although this study was not built on long-term observations of grassland degradation processes, contemporary spatial patterns of NDVI were used to indicate grassland degradation according to the "space-for-time" substitution. Profound influences of grassland degradation on soil and aquatic ecosystems are indicated by the results. This research can contribute to the understanding, protection, and management of the fragile grassland ecosystems on the QTP.

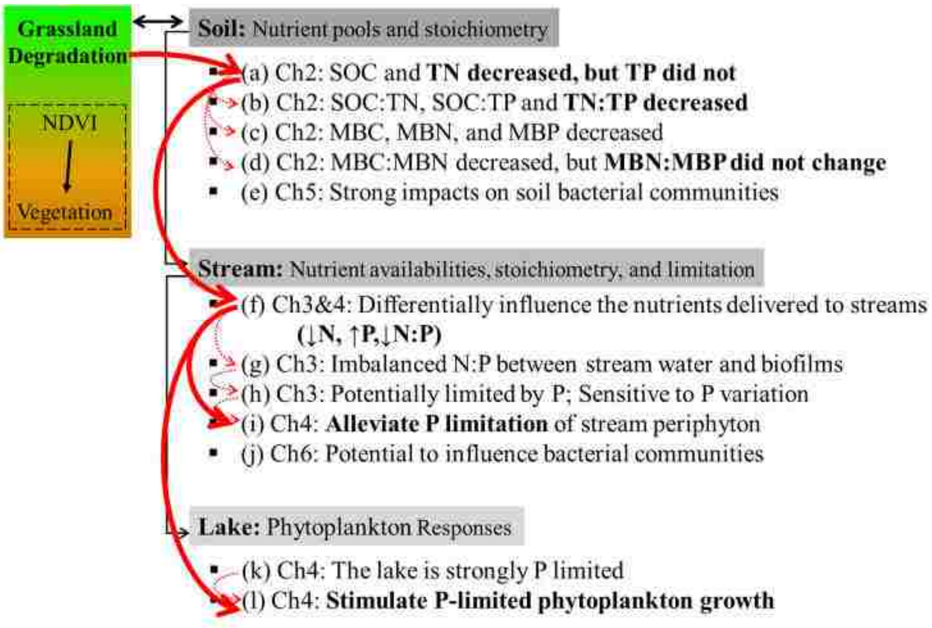

Figure 7.1 Summary of the main results from this study, showing potential influences of grassland degradation on the linked terrestrial-aquatic ecosystems in the Qinghai Lake basin. The arrows show the causal relationships connecting variations of nutrient concentrations, stoichiometry, and limitation in soil, stream, and lake ecosystems under the influence of grassland degradation in this watershed. The red solid arrows in bold show a pathway for cascading influences of grassland degradation transferred from soil to streams and finally to the lake.

In Chapter 2, we quantified normalized difference vegetation index (NDVI) to gauge grassland degradation. C, N, and P concentrations and their molar ratios in soil and in soil microbial biomass were also measured. The average soil C:N:P stoichiometry was 144:10:1. The results showed that grassland degradation decreased the concentrations of C and N, as well as the ratios of C:N, C:P, and N:P in soil (Figure 7.1a and b). Moreover, C, N, and P concentrations in soil microbial biomass decreased with increased grassland degradation (Figure 7.1c). C:N:P ratios of soil microbial biomass were highly constrained with an average value of 49:8:1. Microbial biomass C:N decreased with decreasing NDVI and soil C:N ratio, while microbial biomass N:P decreased with increasing soil N:P (Figure 7.1d). The results suggest that soil microorganisms exhibited strong homeostatic behavior while variations of microbial biomass C:N and N:P ratios suggest changes in microbial activities and community structure. Overall, the soil became relatively more P rich and thus N limitation is anticipated to be more apparent with grassland degradation.

In Chapter 3, C, N, and P concentrations and ratios of streamwater and benthic biofilms were assessed across this same set of catchments. The results showed that grassland degradation tended to decrease DOC, TN, C:N, and C:P of streamwater (Figure 7.1f), especially in September. DOC:TN:TP ratios of streamwater had high variabilities with an average of 387:169:1. C, N, P biomasses per unit area of stream biofilms also had high variabilities but their ratios were relatively constrained, with an average of 471:32:1. Moreover, biofilm C, N, and P concentrations and ratios were not closely associated with streamwater nutrients or grassland degradation. The complex chemical and biological composition of stream biofilms may make their stoichiometry difficult to interpret. However, the non-isometric relationships between biofilm C and P, as well as the large imbalance between C:N:P ratios of streamwater and biofilms do suggest that stream biofilms in this catchment are sensitive to P variation and potentially limited by DOC and P (Figure 7.1g and h). Thus, by changing DOC and nutrient supplies, grassland degradation may alleviate P limitation but aggravate carbon limitation in stream biofilms.

In Chapter 4, nutrient concentrations were sampled, and stream nutrient limitation was tested by conducting nutrient diffusing substrata (NDS) bioassays in streams flowing through sub-basins with different grassland status. To test nutrient limitation and the responses of lake phytoplankton to stream inflows, bioassays were conducted by adding different nutrients (N, P, and joint NP) as well as water from different streams to lake water with phytoplankton, respectively. The results showed that N concentrations as well as N:P ratios increased while P concentrations decreased with increasing normalized difference vegetation index (NDVI, an index of vegetation status), suggesting that grassland degradation (low NDVI) differentially decreased N availability and increased P availability in streams (Figure 7.1f). Consistent with this, relative responses (RR) of stream periphyton to P and combined NP enrichments in the NDS bioassays decreased with stream P concentrations and increased with streamwater N:P ratios. Lake phytoplankton responded strongly to P and combined NP addition, indicating strong P-limitation of lake phytoplankton (Figure 7.1k). Responses of lake phytoplankton to streamwater decreased with nitrate concentration and N:P ratio in streamwater and increased with the concentrations of ammonium, total phosphorus, and soluble reactive phosphorus, indicating that low N:P streamwater from degraded sub-catchments is associated with increased impact on P-limited lake phytoplankton. Overall, this study suggests that grassland degradation differentially influences the nutrients delivered to streams with substantial decreases in N:P, alleviating P limitation of stream periphyton (Figure 7.1i) and ultimately, stimulating P-limited phytoplankton growth in the lake (Figure 7.1l).

In Chapter 5, we used high throughput 16S rRNA gene sequencing to characterize soil bacterial communities as a function of grassland status and soil conditions. The results showed that bacterial alpha diversity had negative relationships with soil moisture, soil organic carbon (SOC), total nitrogen (TN), and total phosphorus (TP). Bacterial community structure was significantly associated with NDVI, the change rate of NDVI, moisture, pH, SOC, TN, as well as soil C:N and C:P ratios. Different bacterial phyla had different relationships with these environmental variables. For example, Proteobacteria were positively correlated with soil moisture, SOC, TN, and soil C:N and N:P ratios while Chloroflexi were negatively correlated with those variables. These results indicate that grassland degradation shifts bacterial diversity and community structure in soil (Figure 7.1e).

In Chapter 6, we investigated the bacterial communities in stream biofilms in sub-basins with different grassland status. The results showed that Proteobacteria, Bacteroidetes, Cyanobacteria, and Verrucomicrobia were the dominant bacterial phyla. 7050 OTUs were detected in total, within which, 19 were abundant taxa and 6922 were rare taxa. Bacterial alpha diversity was positively correlated with carbon (C), nitrogen (N), and phosphorus (P) in biofilms *per se*. The variation of bacterial communities in stream biofilms was closely associated with rate of change in NDVI, pH, conductivity, as well as C, N, P contents and C:N ratio in biofilms *per se*. Abundant subcommunities were more influenced by environmental variables relative to the whole community and rare subcommunities. The results suggest that the history of grassland degradation (indicated as the rate of change in NDVI) had the potential to influence bacterial communities in stream biofilms (Figure 7.1j).

Grassland degradation has long been of considerable research interest in terrestrial ecology. Many of the research efforts have focused on quantifying and modeling grassland status as well as its influences on terrestrial ecosystem processes (Wu and Tiessen, 2002; Liu et al, 2004; Bird et al, 2007; Dlamini et al, 2014). In such studies, impacts on aquatic ecosystems are not often considered. Thus, it is hard to propose a comprehensive stratagem for grassland management or watershed management. Overall, my dissertation helps to fill this gap by demonstrating impacts of grassland degradation on nutrients and microbial communities in coupled terrestrial-aquatic ecosystems in the Qinghai Lake watershed. Contrasting responses of soil and stream ecosystems to grassland degradation were also revealed. Potential consequences of grassland degradation were inferred from the associations of biological and abiotic properties with spatial patterns of NDVI. This study provides data that might help to motivate and guide the protection and management of the fragile grassland ecosystems on the QTP in the rapidly changing world.

References

Bird SB, Herrick JE, Wander MM, Murray L. 2007. Multi-scale variability in soil aggregate stability: Implications for understanding and predicting semi-arid grassland degradation. Geoderma 140: 106-118.

Dlamini P, Chivenge P, Manson A, Chaplot V. 2014. Land degradation impact on soil organic carbon and nitrogen stocks of sub-tropical humid grasslands in South Africa. Geoderma 235-236: 372-381.

Liu Y, Zha Y, Gao J, Ni S. 2004. Assessment of grassland degradation near Lake Qinghai, West China, using Landsat TM and *in situ* reflectance spectra data. International Journal of Remote Sensing 25: 4177-4189.

Wu R, Tiessen H. 2002. Effect of land use on soil degradation in alpine grassland soil, China. Soil Science Society of America Journal 66: 1648-1655.