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EXOTIC INVASIVE PLANTS DRIVE DIFFERENT ECOSYSTEM PROCESSES
THAN NATIVES IN MONTANA GRASSLANDS

By

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Exotic invasive plants drive different ecosystem processes than natives in Montana grasslands.

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Invasion is associated with unexpected increases in aboveground net primary productivity and altered ecosystem function, including increased nitrogen availability and cycling. These shifts are well documented, however many previous studies have been observational, focused on a single plant species, or have not examined belowground microbial communities. I combined field and experimental techniques to examine changes in productivity and ecosystem function, and the abundance of ammonia-oxidizing bacteria (AOB) for the exotic invaders *Bromus tectorum*, *Centaurea stoebe*, *Euphorbia esula*, and *Potentilla recta*. To quantify effects of these invasive species on N cycling and AOB abundance we compared soil from invaded and native communities in the field and in an experimental garden. AOB are bacteria responsible for a rate-limiting step in nitrification. We found that invasion was associated with increased abundance of AOB across all species of invader. For other variables, the magnitude of response to invasion varied by species, but we found in general invasion was associated with increased aboveground net primary productivity and soil nitrogen cycling. In addition results from the experimental garden suggest some species of invader may drive increases observed in the field. Finally we report on a novel relationship between aboveground net primary productivity and soil $\text{NO}^3\text{-N}$ indicating that invaders may drive ecosystem processes in ways different from native communities.

INTRODUCTION

Exotic plant invasions can dramatically alter the composition and functioning of invaded ecosystems (Vitousek *et al.* 1990, Vilà *et al.* 2011) and are often associated with local decreases in native plant abundance and diversity. Somewhat counter-intuitively, these decreases in diversity after invasion usually correspond with substantial increases in annual rates of aboveground net primary productivity (ANPP) and concomitant increases in plant-available nitrogen (N) pools and fluxes (Ehrenfeld 2003, Liao *et al.* 2008, Rout & Callaway 2009). In a 2008 meta-analysis, Liao *et al.* (2008) found that invasion was associated with increases of 83% in ANPP, 17% in soil NO₃-N, and 53% in nitrification rates across ecosystems and invader life histories. However, several important gaps remain in current understanding of the relationship between invasion and ecosystem function. First, little is known about concomitant shifts in the belowground soil microbial community that may help to us understand links between increased productivity and altered ecosystem function. Many studies have focused solely on aboveground mechanisms to explain increased productivity, such as plant life history, traits, and litter decomposition rates (Liao *et al.* 2008). Second, most, studies of relationships among invasion, ANPP, and N cycling are correlative and thus have the potential to be confounded if invaders preferentially colonize nutrient-rich sites (but see Maron and Marler 2008, Zavaleta *et al.*, Maron *et al.* *in press*,). Third, as emphasized by Liao *et al.* (2008), there are limitations to the interpretation of syntheses from many studies done in different ways over different times, and the timescale of how invaders affect ecosystem properties is unknown.

If invasion is a *cause* of increased ANPP and N cycling rates, there are several potential mechanisms by which abundant invaders could boost productivity and rates of N cycling. First, invasive species may experience less herbivory than native species (Keane and Crawley 2002, Agrawal *et al.* 2005, Tallamy 2010, Schaffner *et al.* 2011), resulting in higher observed ANPP simply because less biomass is removed. Second, fundamental differences in the traits of invaders and the traits of natives (Baker 1965, Liang *et al.* 2006, Castro-Diez *et al.* 2013) may result in increased productivity due to species-specific differences in timing, quality, or amount of energetically rich inputs to the soil. Third, an increased supply of easily mineralizable organic matter due to a change in litter quality could result in short-term dramatic turnover of soil microbial biomass resulting in a pulse of elevated available N in the soil (Kuzyakov *et al.* 2000). However, these mechanisms fail incorporate the contribution of soil N-cycling bacteria to rapidly occurring but long lasting increases in ANPP and N cycling within established invaded patches. There are examples of increases in nitrogen fixation following invasion (Vitousek *et al.* 1987, Musil 1993; Rout *et al.* 2013) but these studies tend to focus on the introduction of novel species that harbor N-fixing bacteria rather than species which rely on free-living N-fixers or inorganic N cycled from organic N.

Here we explore another possible link between increased ANPP and N cycling associated with invaders. Invaders might drive generally consistent shifts in the microbial community resulting in increased N availability, particularly increased abundance of in the functional group responsible for a rate-limiting step in the N cycle, ammonia-oxidizing bacteria (AOB). Nitrifiers, including AOB, are affected by access to $\text{NH}_4\text{-N}$ (Carney *et al.* 2004), soil moisture, soil pH (Stephen *et al.* 1998), and soil

temperature (Avrahami *et al.* 2003). In addition to these environmental factors, AOB are responsive to processes such as competition and available niche space. Invasion is associated with shifts in other components of the soil microbial community including arbuscular mycorrhizal fungi (Lekberg *et al.* 2013) and pathogens (Morris *et al.* 2007, Kulmatiski *et al.* 2008). Such shifts in abundance of other components of the microbial community could potentially free up resources allowing AOB to increase in abundance.

The role of soil bacterial functional groups in N cycling (van der Heijden *et al.* 2008) and the mechanics of this cycling (Hart *et al.* 1994, Cabello *et al.* 2009) are well understood. However we know very little about how invasion might change the composition, abundance, and function of these groups (Rout & Callaway 2009). In particular, ammonia-oxidizing archaea (AOA) and bacteria (AOB) perform a rate-limiting step in nitrification, part of the process by which organic matter is made available for uptake by plants and microbes. While AOA numerically dominate soils, AOB tend to drive nitrification in relatively nutrient rich systems such as grasslands (Di *et al.* 2009). Other studies have shown that exotic invasions can be associated with increases in AOB, which has the potential to affect gross nitrification (Hawkes *et al.*, 2008, Booth *et al.*, 2003, but see Evans *et al.*, 2009). Thus the abundance and activity of AOA and AOB have the potential to play an important and largely unexplored role in ecosystem functioning in response to invasion.

Here we asked if invasion drives increases in NPP, N pools, and N cycling, and hypothesized that increases in productivity and ecosystem function following invasion would be associated with increased abundance of AOB. To this end we compared these processes in 1) paired patches of adjacent invaders and natives in the field, 2) in

experimentally established plots of monocultures of invaders and established plots of native mixtures of species, and 3) by measuring the abundances of AOB in invaded and native patches.

METHODS

Focal invasive species of field and experimental garden studies

We selected four invasive plant species that represented different plant families, life history strategies, and distributions. First, *Bromus tectorum* (cheatgrass) is a widespread, highly invasive annual distributed throughout the American West. *Bromus tectorum* has been associated with increased AOB and increased N cycling rates and pool sizes (Svejcar & Sheley 2001, Sperry et al. 2006). Second, *Centaurea stoebe* (spotted knapweed) is an abundant, highly invasive, perennial forb throughout much of the Rocky Mountain West. Third, *Euphorbia esula* (leafy spurge) is a deep-rooted, perennial invasive forb, which occurs throughout grasslands of the Northern Great Plains and Rocky Mountains. Finally, *Potentilla recta* (sulfur cinquefoil) is a perennial forb distributed throughout the Northern United States with heavy infestations from the Mountain West to the Great Lakes region (Rice 1999). Invasion by each of these species has been correlated with decreases in native species richness (Ortega & Pearson, 2005).

Field site information and sampling design

We established field sites within grasslands in the Missoula and Bitterroot valleys of western Montana. Mean annual precipitation is 36 cm, with the majority falling as

rain in May and June. The mean annual temperature is 43°F. Soils consisted of fine to gravelly, loamy, Argixerolls and Haploxerolls.

We utilized a paired plot design to examine differences between invaded and native patches both within and among target invasive species. For each of the 4 target invasive species, plots were established in 8 densely invaded naturally occurring mono-dominant patches. Patches ranged in size from 5x5 meters to greater than 25x25 meters. Each of these 32 invaded patches was then paired with an adjacent patch that was dominated by native species and in which none of the invasive species occurred. Patch pairs were matched for elevation, aspect, slope, and landscape position to minimize difference in environmental factors. The maximum distance between invaded and native plot centers was 15 m and we avoided obviously disturbed areas for any sampling. Soil and biomass samples were taken in early June 2012 within three 0.25 m² plots located within each patch: two m from the center at the cardinal directions N, E, S.

Field Soil Sampling and Biogeochemical Analysis

Eight soil cores were taken in a systematic fashion using an Oakfield probe (0-10cm depth) from each plot. Soil cores were pooled within each of the three plots within each invaded or native patch and these composited sub samples were immediately frozen and stored at -80°C until extraction to determine abundance of ammonia oxidizing archaea (AOA) and bacteria (AOB). Remaining soil was stored at 4°C for up to 4 days preceding biogeochemical analysis. .

Each pooled soil sample was subdivided for the following analyses: inorganic N pools, net and potential nitrification, microbial biomass, pH, total carbon and N.

Gravimetric water content was determined for all soil samples, and raw data have been corrected and are reported on a $\mu\text{g/g}$ oven dry soil basis for each nutrient. To determine inorganic N pools, extractions of inorganic N were performed in the lab using 2M KCl (Hart et al. 1994). Fifteen mL of soil was added to 100mL of 2M KCl, shaken on an orbital shaker for 1 hour, allowed to settle overnight, filtered, and frozen until analysis. Determination of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ from filtrates were analyzed colorimetrically using a Synergy 2 Microplate Reader (BioTek, USA) after Weatherburn and Doane(1967) and Horwath(2003) for $\text{NH}_4\text{-N}$ and $\text{NO}_3\text{-N}$, respectively.

We determined net nitrification using the buried bag field method (Hart et al. 1994). During initial soil sampling an additional intact soil core was collected, placed inside of a breathable polyethylene bag, loosely closed using a twist tie to allow gas but not water exchange, and reburied. After 28 days we collected bagged soil cores from the field. Pre- and post-incubation samples were extracted with 2M KCl and analyzed as described above.

We determined nitrification potential for each plot using the shaken slurry method (Hart et al. 1994). Nitrification potential is a net measurement the conversion of ammonium ($\text{NH}_4\text{-N}$) into nitrate ($\text{NO}_3\text{-N}$) by the soil nitrifier community less inhibitory properties of the soil and microbial ammonium uptake. We chose to include this measurement as net nitrification is affected by the abundance of nitrifiers, microbial uptake, and inhibitors, but also by highly variable abiotic factors including: water availability, pH, $\text{NH}_4\text{-N}$ availability, uptake of $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ by plants and microbes, and such variability can mask differences among sites and treatments. Nitrification potential, in contrast, normalizes all abiotic factors, allowing a more precise estimate of

capacity of the microbial community. A composite sample was formed from the 3 soil samples from each patch. We added 15g of this composite to 100 mL of 1 mM phosphate buffer solution (pH 7.2) and 1.5 mM $(\text{NH}_4)_2\text{SO}_4$ in 250 mL flask covered with perforated parafilm which was shaken on an orbital shaker. From this aliquots were sampled, centrifuged, and supernatant frozen at 2, 4, 22, and 24 hours. Determination of $\text{NO}_3\text{-N}$ from aliquots was performed using micro-plate analytical technique described above.

Microbial biomass was determined by extracting 2 subsamples from each pooled sample taken in each patch. One 15mL subsample was extracted by adding 50 mL 0.5 M K_2SO_4 , shaking for 1 hour on an orbital shaker, settling overnight, filtered, and frozen until analysis. A second subsample was fumigated for 5 days with ethanol-free chloroform (Horwath & Paul 1994) and then extracted in the same manner as above. Determination of non-purgable organic carbon (NPOC) and total nitrogen (TN) were determined using a Shimadzu TOC-V TN Analyzer (Shimadzu Corporation, Kyoto, Japan). Un-fumigated sample TN and NPOC values were subtracted from post fumigation TN and NPOC values to determine microbial biomass nitrogen and carbon for each sample.

The soil remaining from each sample was air-dried and then ground to a fine powder using mortar and pestle. We measured pH on this air-dried soil in 0.01 M CaCl_2 , using a 1:1 (w/v) soil: liquid ratio (Accumet Dual Channel pH/Ion/Conductivity Meter). Total Carbon and Nitrogen of a subset of soil samples were determined using a CE Elantech elemental analyzer (Eager Xperience Ver. 1.1 September, 2009).

In early July 2012 we collected intact cores for modified acetylene reduction analysis of the free-living N fixer soil community from each of the sites following the

method of Reed *et al.* (2007). Briefly, we collected 3 intact cores from each patch by driving a 55 mL, 2.54 cm diameter acrylic tube into the soil. We capped the bottoms of the tubes with stoppers and transported them to the lab. There, we stimulated the microbial community by placing the tubes under lights for 12-hour cycles and misting with DI water. After 48 hours we fitted all experimental units with a one-hole stopper fitted with a septa, and injected acetylene to 10% headspace by volume and incubated for 12 hours. The ethylene concentration of 1mL gas sub samples was analyzed using a (GC name and information).

Plant Biomass Analysis – Field plots

We estimated aboveground net primary productivity (ANPP) at our sites by measuring peak standing biomass. We clipped all plant material at ground level from all plots in at peak biomass production in July 2012. This time period captures biomass produced by dominant native grasses and perennial invasive forbs in our system, but may miss a relatively small proportion of biomass produced by early season ephemeral forbs. Biomass was dried at 70°C for 72 hours and then weighed. We harvested live biomass produced in the current year, so that our samples approximated net aboveground primary production for that year. A subset of each biomass sample was ground and analyzed for total Carbon and Nitrogen using a CE Elantech elemental analyzer (Eager Xperience Ver. 1.1 September, 2009).

Abundance of AOB and AOA – Field plots

We explored potential changes in the microbial community coupled with N cycling and increases in available NO₃-N by quantifying the abundances of AOB and AOA in soils from the invaded and native paired field plots. The three plots within each invaded and native field patch were combined to form a composite sample. Genomic DNA was extracted from approximately 0.25 g of soil from each composite sample using the Mo Bio Powersoil kit (Carlsbad, CA, USA). Extracted DNA suspended in 100 µL of sterile elution buffer was stored at -80°C, packed in dry ice, and transported to the French National Institute for Agricultural Research, Dijon France for qPCR analysis of the abundance of functional marker gene *amoA* in AOB and AOA.

Experimental garden plots

To determine if invaders caused shifts in productivity and ecosystem function observed in field studies we also collected soil and plant biomass from an experimental common garden located at the MPG Ranch in Florence, MT (lat: 46°40'48.92"N, long: 114° 1'40.73"W). This common garden was located near our field sites and was surrounded by a similar native community to that in our native field plots. This experiment was established in spring, 2011 and consisted of 25, 2x2 m plots planted with monocultures of 64 seedlings each of 4 invasive species discussed above or mixtures of natives (n=5 for each treatment). The native mix consisted of nine common dominant grasses and forbs (additional information on the site information, species, and establishment is in Supplementary Attachment 1). The experimental garden received supplemental water during the first year but no water was added thereafter. In mid-June

2012, in each of the 25 plots, we randomly located 2 0.25 m² subplots and collected soil and estimated ANPP by collecting aboveground plant peak biomass following the protocol described above for the field study. Soil samples were subsampled for the following analyses; inorganic N pools, net nitrification and microbial biomass. However, we did not include an estimate of ANPP for *E. esula* due to substantial herbivory by *Apthona lacertosa* and *A. nigriscutis*, two biological control insects.

Statistical Analyses

For field data we analyzed ANPP, NO₃-N, NH₄-N, and potential nitrification with generalized linear mixed model with invaded species as a fixed factor and Site and Site x Pair as random factors. This model accounts for potential spatial autocorrelation of patches, as well as the paired nature of our study. We then used contrasts to compare invaded and native patches for each species of invader. Distribution of means were visually inspected and checked for normality using Generalized Chi-Square / DF fit test. Data were log transformed when necessary to satisfy assumptions of approximate normal distribution of means and homoscedasticity.

For the regression of ANPP and soil NO₃-N we performed a stepwise multiple regression ANPP was the sole significant factor in the invaded treatment, Site and Species were both not significant factors. We then ran ANOVA on both native and invaded observation groups, and ran additional ANOVA on models test for significant differences in intercept and slope among invaded and native groups. We also reported R² values for each group.

For experimental garden data, a one-way ANOVA with species as a fixed factor was used to compare differences in ANPP, NO₃-N, NH₄-N, and net nitrification across 5 plant communities (4 invaded monocultures and mixed native community). Tukey's post hoc analysis was used to determine differences among species ($\alpha=0.05$).

All means and standard deviations are reported as raw data rather than transformed data to facilitate comparison with other published studies. Significance is defined as ($P<0.05$). Statistical analyses were performed using SAS (GLIMMIX module, SAS ver. 9.1) and R (version 1.40, 2011).

RESULTS

Field Study

Aboveground net primary productivity (ANPP) was 74% higher in invaded patches compared to native patches (Fig. 1a; $F_{\text{INVADED}}=33.47$, $df=1,28$, $P<0.0001$). At the individual species level, *B. tectorum*-dominated patches produced 70.9% more biomass ($t=3.57$, $df=46.66$, $P=0.003$), *C. stoebe* produced 63.4% more ($t=3.90$, $df=46.49$, $P=0.001$), and *E. esula* produced 200.4% more ($t=6.66$, $df=46.51$, $P<0.0001$), as compared to native patches. Biomass in *P. recta*-invaded patches was not higher than native patches ($t=-0.23$, $df=42.25$, $P=0.82$).

Extractable soil NO₃-N was 103% higher in invaded patches compared to native patches (Fig. 1b; $F_{\text{INVADED}}=34.23$, $df=1,53$, $P<0.001$). At the individual species level *B. tectorum*-dominated patches were associated with a 42.3% increase in NO₃-N ($t=4.00$, $df=56$, $P=0.0008$), *C. stoebe* with a 124.2% increase ($t=3.96$, $df=57$, $P=0.0008$), and *E. esula* with a 193.2% increase ($t=5.81$, $df=57$, $P<0.0001$), as compared to native patches.

NO₃-N concentrations in soil beneath *P. recta* did not differ from those in paired native soil samples ($t=1.03$, $df=54.01$, $P=0.31$).

The ratio of inorganic N to total N in invaded patches was 183% that of native patches (Supplemental Table 1). At the species level, *B. tectorum* was associated with a 89.3% increase N:TN ($t=436$, $df=8.12$, $P=0.04$), *C. stoebe* with a 125.2% increase ($t=429$, $df=10.9$, $P=0.03$), and *E. esula* with a 51.4% increase ($t=468$, $df=6.8$, $P<0.04$). The ratio of N:TN in soil beneath *P. recta* did not differ from that in paired native soil.

We did not find significant differences for more than one species of invader for any other measured variables (Supplemental Table 1).

Nitrogen cycling – field study

In the field, the nitrification potential of native soil patches was 59% higher compared to native patches (Fig. 4; $F_{\text{INVADED}}=13.48$, $df=1,30$, $P<0.001$). Nitrification potential was 52.2% greater in *C. stoebe*-dominated patches ($t=1.93$, $df=47.31$, $P=0.0592$), patches of *E. esula* produced 88.0% greater nitrification potential ($t=3.63$, $df=47.44$, $P=0.0007$), and *P. recta* 93.7% greater ($t=2.86$, $df=43.43$, $P=0.0065$) compared to native patches. The nitrification potential in soil beneath *B. tectorum* did not differ from that in native soil ($t=0.28$, $df=47.77$, $P=0.78$).

NPP, soil NO₃-N, and invasion in the field

In addition to significant increases in ANPP, N pools, and nitrification potential, the relationship between biomass production and N pools differed among invaders and native patches ($F=4.5$, $df=1,59$, $P=0.038$). In invaded soil, ANPP was positively

correlated with soil NO₃-N pool and there was no significant effect of invader identity (Fig. 3; $F_{\text{INVADDED}}=30.71$, $df=1,29$, $P<0.001$, $R^2 = 0.51$). In contrast, in soil occupied by native species there was no significant relationship between ANPP and with soil NO₃-N pool (Fig. 3; $F_{\text{NATIVE}}=1.56$, $df=1,30$, $P=0.221$, $R^2 = 0.05$).

Characterization of amoA AOB and AOA Field

Abundance of functional marker gene *amoA* in AOB in invaded patches was 4.69 times higher compared to native patches (Fig. 5a; $F_{\text{INVADDED}}=66.79$, $df=1,28$, $P<0.0001$) At the species level, *B. tectorum*-dominated patches demonstrated an increase of AOB of 620% ($t=5.80$, $df=44.21$, $P<0.0001$), *C. stoebe* 330% ($t=5.55$, $df=44.16$, $P<0.0001$), *E. esula* 282% ($t=4.46$, $df=47.43$, $P<0.0001$), and *P. recta* 225% ($t=3.54$, $df=41.17$, $P<0.001$) as compared to native patches. For *P. recta* there was a strong trend for increased AOA abundance (Fig. 5b; $t=1.92$, $df=43.85$, $P=0.061$).

Experimental garden

Aboveground net primary productivity of planted native plots averaged 727.3 ± 148.5 g/m², much higher than average ANPP of native patches in the field (Fig 2a; see Fig. 1a for comparison). There was a 57% increase in ANPP in invaded relative native plots in the common garden, but this varied significantly among species and this general shift was driven solely by *C. stoebe* (Fig. 2a; $F_{\text{species}}=53.286$; $df=3,16$; $P<0.001$). *Centaurea stoebe* had higher ANPP than natives (Fig. 2a; $t=6.75$, $df=4.95$, $P=0.001$), whereas *B. tectorum* had lower ANPP than native plots (Fig. 2a; $t=6.98$, $df=4.98$, $P<0.001$). *Potentilla recta*

did not significantly increase ANPP and *E. esula* biomass values are not reported because an introduced biological control beetle, *Aphthona sp.* heavily grazed these plots.

Extractable NO₃-N in soil from native plots in the garden averaged 8.5±2.7 μg g⁻¹. This was 2.3 times the extractable NO₃-N measured in native patches in the field. The amount of increase in NO₃-N in invaded plots varied significantly by species and was driven by *E. esula* (Fig. 2b; t=4.77, df=4.19, P=0.008). *Centaurea stoebe* trended towards significantly higher amounts of NO₃-N (Fig. 2b; t=2.23, df=4.12, P=0.08), and *B. tectorum* and *P. recta* did not significantly alter NO₃-N.

DISCUSSION

Many studies have reported positive correlations between exotic invasion and ecosystem productivity (see Liao *et al.* 2008) and here we found similar correlations in the field and experimental evidence that one invasive species caused increases in ANPP and available NO₃-N. Furthermore, our consistent field patterns of greater concentrations of soil N-cycling bacteria in soils associated with invaders suggests that shifts in belowground microbial communities may play a general role in increased ANPP and soil NO₃-N. Finally, we found a stronger and steeper positive regression relationship between ANPP and soil NO₃-N for invaders than for natives, indicating that invaders may affect ecosystem function in different ways than native communities. Our results contribute to a growing body of evidence that shows fundamental differences in how invaders and natives affect ecosystem processes (Hawkes *et al.* 2008, Liao *et al.* 2008, Lee *et al.* 2012).

Some of our results were general across species, such as reported in Liao *et al.* (2008), but caution is warranted in *over*-generalizing because the magnitude of invader-

native differences in the field differed substantially among species, varying from no effect (*P. recta*) to a 200% increase in ANPP, (*E. esula*), a 28% (*P. recta*) to 193% (*E. esula*) increase in NO₃-N, no effect (*B. tectorum*) to a 91% (*P. recta*) increase in nitrification potentials, and a 266% (*P. recta*) to 620% (*B. tectorum*) increase in the abundance of AOB. Our field results correspond well with the increases in ANPP reported by Liao et al. (2008) for many species, but the increase in soil NO₃-N we measured associated with invasion was greater than that reported in their meta-analysis.

Our experimental findings demonstrated that several invasive species caused substantial increases in ANPP and soil NO₃-N, and importantly that these changes occurred before the end of only a second growing season. Native ANPP and soil NO₃-N were much greater in the experimental garden than in the field; however, the effect of invasion on ANPP and soil NO₃-N were similar. The effects of individual species differed to some degree between the field sampling and the common garden experiment. *Centaurea stoebe* had consistent positive effects on ANPP and NO₃-N in both scenarios, but *B. tectorum* had strong effects in the field on ANPP but not in the experimental garden. Conversely, *P. recta* had no effect on ANPP in the field but significant effects in the experimental garden. We excluded the value of ANPP for *E. esula* because of substantial destruction by *Apthona lacertosa* and *nigriscutis*, two species of biological control insects. Interestingly, NO₃-N increased dramatically under *E. esula* in the field and in the experiment, but this increase may have been affected by heavy grazing of this species. The effects of *B. tectorum* and *P. recta* on NO₃-N were generally consistent in both scenarios. Our findings are consistent with previously published studies showing increases of NO₃-N associated with invasive grasses and shrubs (Hawkes et al. 2006,

Kourtev *et al.* 2002, Evans *et al.* 2001), but variation between the field results and garden results for the same species suggests conditionality in these ecosystem effects that in turn warrants substantially broader investigation. In this context, Thorpe & Callaway (2011) *C. stoebe* tended to depress soil NO₃-N flux (as measured by resin capsules) and nitrification potential. Their results highlight the importance of both temporal dynamics influencing patterns observed in natural systems as well as differences between field, greenhouse, and experimental garden results. Our results are consistent with experimental studies that reported increased ecosystem productivity (Maron & Marler 2008), and potential nitrification (Lee *et al.* 2011) associated with invasion.

To our knowledge our comparison of the relationship between native and invaded ANPP and soil NO₃-N (Fig. 3) is novel. ANPP accounted for 51.4% of the variation in NO₃-N in invaded soils. The mechanisms responsible for this correlation remain unclear; however, there are several potential mechanisms. First, it is possible that the differences in ANPP reported here and elsewhere are due to disproportional avoidance of invasive species by native generalist herbivores. However, it is not clear why an increase in ANPP via escape from consumers would correspond with a rapid increase in soil NO₃-N and AOB abundance such as we found in the common garden experiment. Second, invasiveness may correlate with common traits among invaders that promote either greater acquisition or more rapid cycling of N. For example, it is possible that invaders in general have tissue stoichiometry that promotes rapid N cycling and thus promotes increased AOB and soil N. In this context, Sardans *et al.* (2010) compared 35 native and 38 exotic (whether invasive or not was not reported) species in Hawaii and found that in general exotic species had greater photosynthetic capacities and N content, and lower leaf

mass per area than natives. Finally, the consistent increase in AOB associated with each of the four invasive species suggests the possibility of belowground processes that drive changes to N cycling and a reorganization of the soil microbial community.

Although it is not entirely clear why invasive species from different plant families with different life history strategies would have such generally consistent effects on soil biogeochemistry, several possible mechanisms are possible. Plants can affect soil bacteria by altering temperature, moisture, and pH (Wardle *et al.* 2004). For example, increases in the pH of wild and agricultural soils correspond with increased rates of net N mineralization, potential nitrification, and overall N availability (Carney *et al.* 2004, Curtin *et al.* 1998, De Boer & Kovalchuk 2001). If shared traits of invaders cause consistent and rapid changes in soil moisture or temperature it is possible that this would drive consistent changes in the abundance of AOB. Escape from above and belowground consumers in general may make a larger portion of plant fixed carbon available for uptake by microbial community. Alternately, invasive species often experience much weaker feedbacks from soil biota than native species (Kulmatiski *et al.*, 2008), and this combined with escape from soil pathogens (Maron *et al.*, *in press*), decreased (Broz *et al.* 2007) or increased (Lekberg *et al.*, 2013) diversity or abundance of beneficial AMF, or promotion of other free living microbial groups (Batten *et al.*, 2004), could result in an overall shift in balance of competition within the soil microbial community (Morris *et al.*, 2007). In addition, changes in the composition of the AOB community itself may result in altered efficacy or function of that community (Carney *et al.*, 2004). In general, the mechanism may be a combination of a decrease in pathogenic or antagonistic components of the soil community and an increase in beneficial organisms, such as AOB.

The composition of the soil microbial community and abundance of free-living microbes affect plants in natural systems through mineralization of and competition for nutrients (van der Heijden *et al.*, 2008). To be clear, we do not know the mechanism behind consistent increases in the abundance of AOB in our study, but increased abundances of this group and resulting alterations to ecosystem function may contribute to invasive success in our study system.

A better understanding the effect of increases in abundance of beneficial soil microbes, rather than just lack of antagonistic soil microbes is needed to fully understand the role of soil communities in plant invasions (Reinhart & Callaway 2006). The soil microbial community is dynamic and may vary in response to competition and resource limitation in ways that are similar to responses in plant communities. Shifts in the microbial community can in turn influence the abundance of particular plant species or groups of plants in ways that appear to differ between invaders and natives (see also Kulmatiski *et al.*, 2008). Our results contribute to a growing understanding of how plants affect ecosystem functioning through tight links with beneficial N-cycling soil bacteria, nitrogen cycling, and ecosystem productivity.

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Figure Legends

Figure 1. Net primary productivity (a) and soil extractable NO₃-N (b) of *Bromus tectorum*, *Centaurea stoebe*, *Euphorbia esula*, and *Potentilla recta* and respective native paired plots in the field. Error bars represent ± 1 SE, and asterisks denote significant difference in mean between the invaded plot and native paired plot.

Figure 2. Net primary productivity (a) and soil extractable NO₃-N (b) of *Bromus tectorum*, *Centaurea stoebe*, *Euphorbia esula*, and *Potentilla recta* native plots in the experimental garden. Error bars represent ± 1 SE and different letters represent significant differences in *post hoc* comparisons ($\alpha=0.05$).

Figure 3. Relationships of soil extractable NO₃-N and aboveground net primary productivity (ANPP), in the field. In a stepwise multiple regression ANPP was the sole significant factor in the invaded treatment. Not significant in native treatment. In the invaded ANPP explained 51% of variation in soil extractable NO₃-N.

Figure 4. Nitrification potential of *Bromus tectorum*, *Centaurea stoebe*, *Euphorbia esula*, and *Potentilla recta* and respective native paired plots in the field. Error bars represent ± 1 SE, and asterisks denote significant difference in mean between the invaded plot and native paired plot.

Figure 5. Relative abundance of amoA genes from ammonia oxidizing bacteria (AOB) (a) and ammonia oxidizing archea (AOA) (b) in soils under patches of *Bromus tectorum*, *Centaurea stoebe*, *Euphorbia esula*, *Potentilla recta*, and paired native plots for each invader in the field. Error bars represent ± 1 SE, and asterisks denote significant difference in mean between the invaded plot and native paired plot.

Supplemental Information Attachment 1. Details regarding experimental garden plot establishment and species identity.

Supplemental Table 1. Means (SE) of all measured variables for all invaded and native field plots. Bold text denotes significant differences among invaded and native means for that species

Figure 1

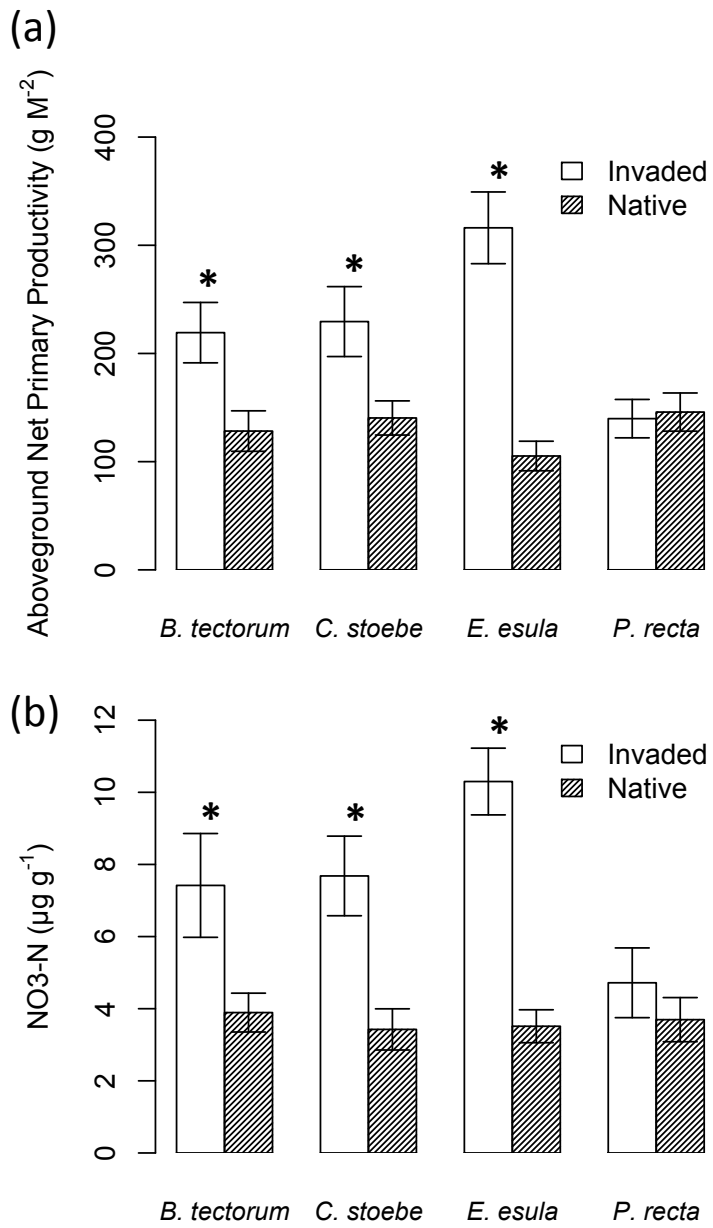


Figure 2.

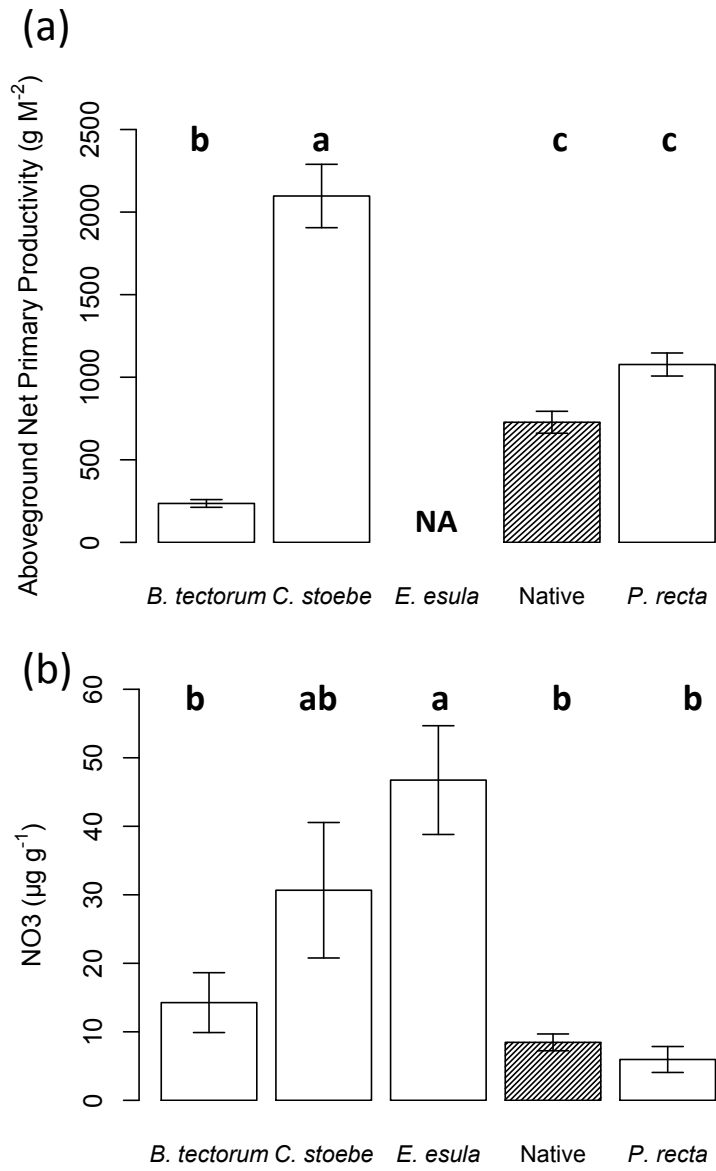


Figure 3.

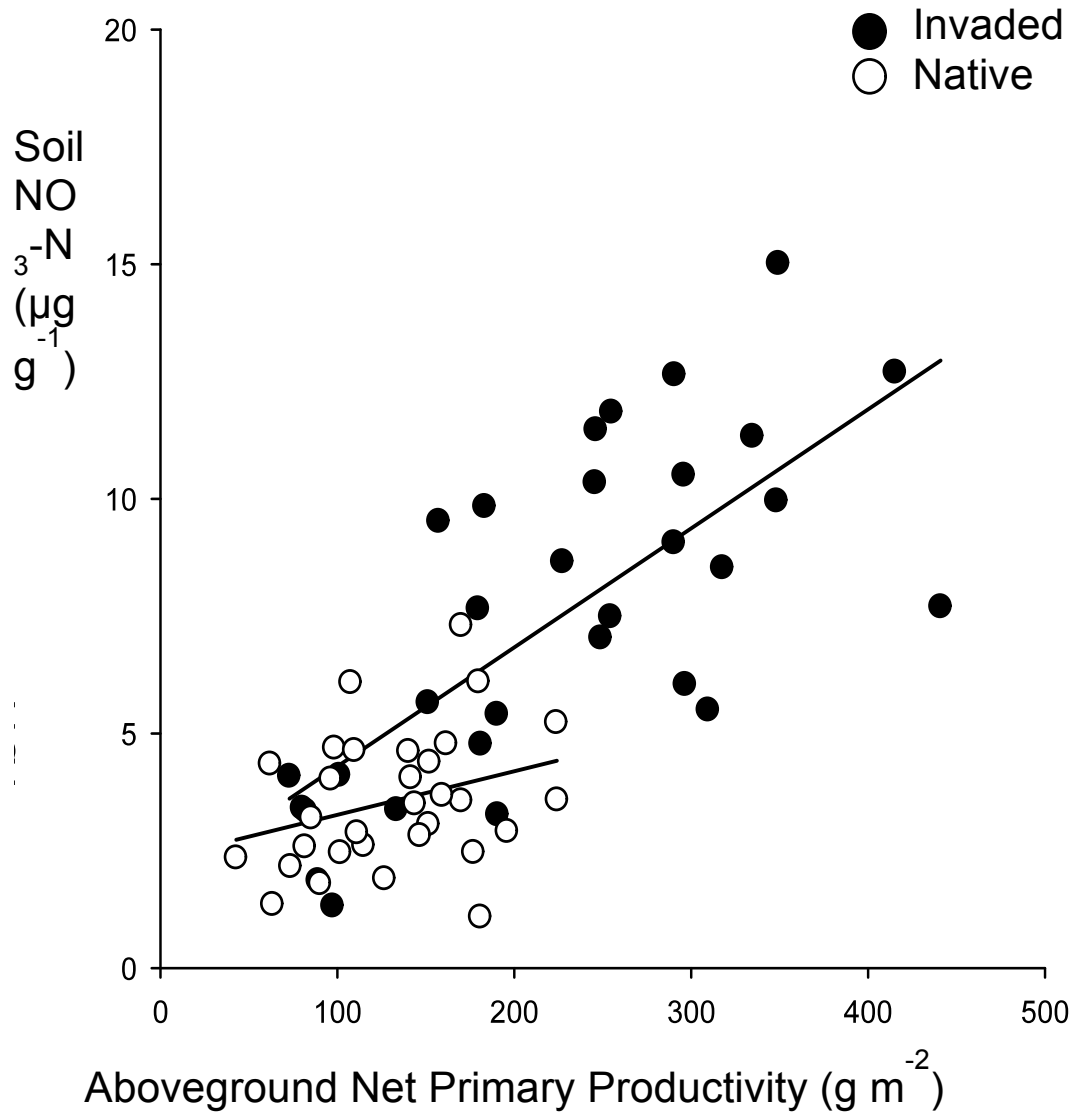


Figure 4

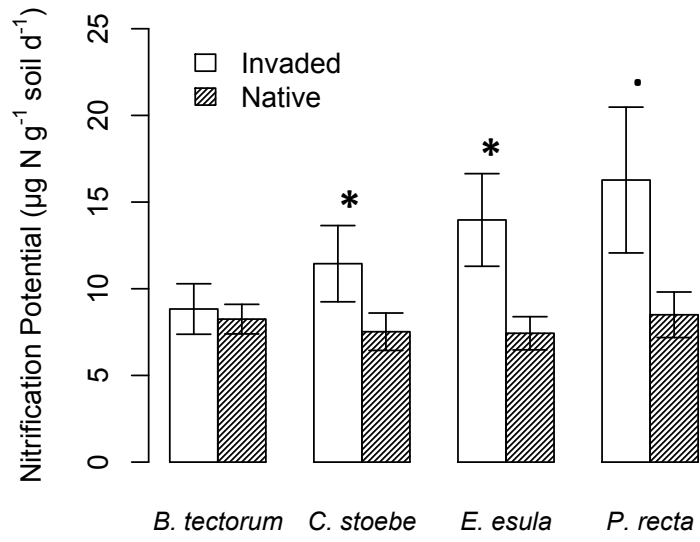
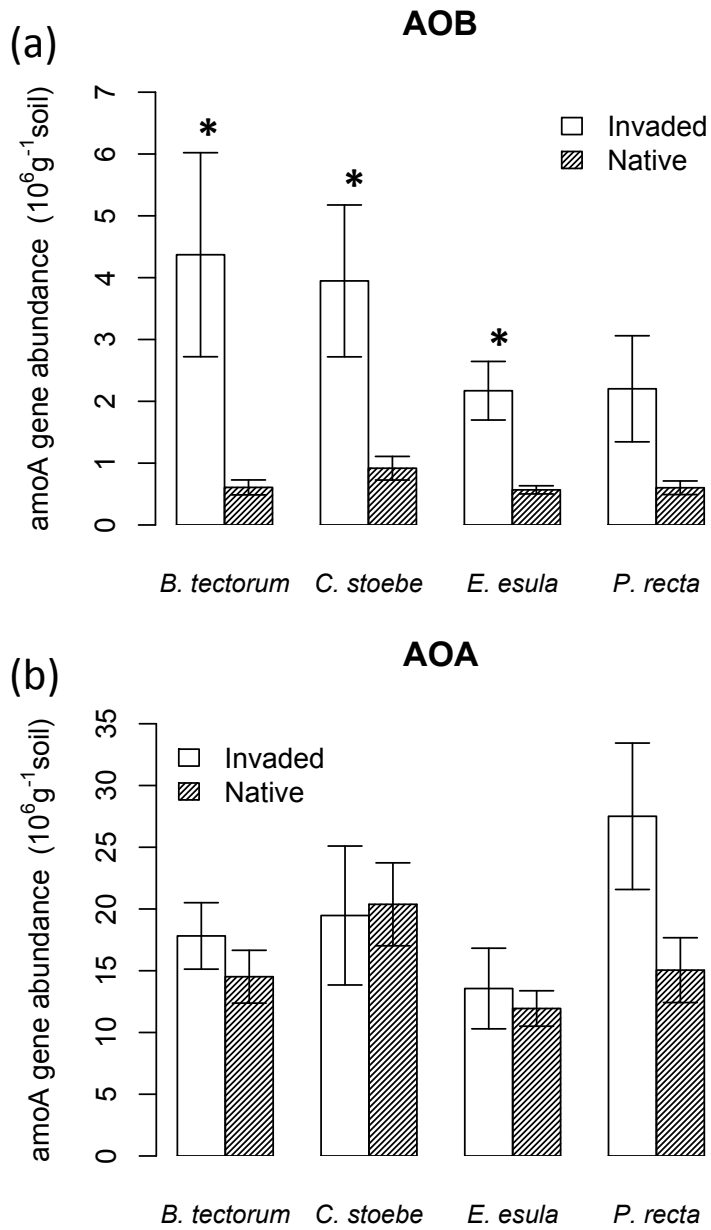


Figure 5.



Supplemental Information 1.

All seeds except for *B. tectorum* were sown on 15-16 April, 2011, in a soil:peat:vermiculite:sand (1:1:1:2, v:v) mixture. The soil was collected from underneath *B. tectorum*, *C. stoebe*, *E. esula*, *P. recta* and remnant natives at three locations on MPG Ranch to ensure that microbes that normally associate with the target species were present in the media mixture. *Bromus tectorum* was planted in the same mixture on 6 April, 2011, placed in the refrigerator for one month to simulate winter and ensure flowering, and brought to the greenhouse on 6 May. All exotic seeds were collected on MPG Ranch in 2010 and all native seeds were purchased from local sources. Seedlings were grown under ambient light and 17-24 °C and fertilized two times with approximately 5 mL of half-strength Hoagland solution (Machlis and Torrey, 1956). We transplanted all seedlings into 2 x 2 m plowed plots on 2-3 June, 2011 using a replicated block design (n=5). *Bromus tectorum*, *C. stoebe*, *E. esula*, *P. recta* were planted in monocultures using 64 seedlings per plot, whereas the native plots received seven *Pseudoroegneria spicata*, *Elymus elymoides*, *Koeleria macrantha*, *Bouteloua gracilis*, *Penstemon strictus*, *Linum lewisii*, *Erigeron speciosus*, *Gaillardia aristata*, and *Achillea millefolium* each and one extra randomly selected seedling to make the total number the same as in the exotic plots. All plots were watered when needed in 2011 to enable good establishment, but not in 2012.

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Supplemental Table 1.

	<i>C. stoebe</i>		<i>B. tectorum</i>		<i>E. esula</i>		<i>P. recta</i>	
	Invaded	Native	Invaded	Native	Invaded	Native	Invaded	Native
ANPP (g M ⁻²)	229.5 (32.3)	140.3 (15.7)	219.2 (27.9)	128.2 (18.7)	316.1 (33.1)	105.2 (13.6)	139.7 (17.7)	145.8 (17.6)
NH ₄ -N (µg g ⁻¹ soil)	6.2 (0.9)	4.6 (0.4)	4.2 (0.5)	3.3 (0.3)	7.5 (1.2)	3.1 (0.3)	5.8 (1)	5.9 (1.1)
NO ₃ -N (µg g ⁻¹ soil)	7.7 (1.1)	3.4 (0.5)	8.3 (1.5)	3.8 (0.5)	10.2 (0.9)	4.3 (1.2)	4.7 (0.9)	3.6 (0.6)
Inorganic N:TN	0.003 (0.0004)	0.002 (0.0004)	0.004 (0.0008)	0.002 (0.0008)	0.003 (0.0005)	0.001 (0.0005)	0.001 (0.0001)	0.001 (0.0001)
MB NPOC (µg C g ⁻¹ soil)	153.9 (15.1)	127.1 (10.1)	112.5 (9.7)	120.3 (7.5)	165.9 (17.5)	112 (4.1)	169.5 (23.8)	162.3 (23.3)
MB TN (µg g ⁻¹ soil)	33.3 (4.3)	26.5 (3.1)	23.3 (2.7)	24.3 (2.2)	35.9 (4.4)	23.5 (1.3)	35.5 (5.6)	34.2 (6.5)
Soil TC (ppm)	45743 (3104)	46603 (9876)	31597 (2512)	29663 (3463)	54756 (6999)	31982 (2206)	73426 (18493)	53094 (26758)
Soil TN (ppm)	4206 (189)	4475 (959)	2818 (221)	2780 (320)	5052 (674)	3039 (290)	6840 (1816)	4852 (2415)
Net Nitrification (µg g ⁻¹ day ⁻¹)	1 (0.13)	0.76 (0.1)	0.76 (0.11)	0.6 (0.05)	0.89 (0.13)	1.05 (0.18)	1.23 (0.15)	0.78 (0.09)
Nitrification Potential (µg g ⁻¹ day ⁻¹)	11.4 (2.1)	7.5 (1)	8.8 (1.4)	8.2 (0.8)	13.9 (2.6)	7.4 (0.9)	16.2 (4.2)	8.4 (1.3)
ARA (µmol g ⁻¹ hour ⁻¹)	2.83 (0.5)	3.4 (0.8)	2.76 (0.26)	3.4 (0.7)	2.42 (0.26)	4.6 (2.3)	3.18 (0.5)	4.3 (1.1)
AOB (g ⁻¹ soil x 10 ⁶)	3.94 (0.36)	0.91 (0.19)	4.37 (0.27)	0.6 (0.12)	2.17 (0.26)	1.34 (0.78)	2.2 (0.48)	0.6 (0.1)
AOA (g ⁻¹ soil x 10 ⁶)	19.47 (5.62)	20.37 (3.35)	17.81 (2.69)	14.51 (2.13)	13.55 (3.26)	11.94 (1.43)	27.5 (5.92)	15.04 (2.62)
16S bact (g ⁻¹ soil x 10 ⁹)	37.57 (5.56)	33.67 (3.9)	31.51 (4.43)	23.98 (2.19)	31.62 (3.53)	20.79 (2.19)	52.53 (10.62)	30.33 (5.23)
16S arch (g ⁻¹ soil x 10 ⁸)	9.89 (2.35)	10.09 (1.36)	8.93 (1.26)	7.81 (0.87)	8.29 (1.66)	5.5 (0.46)	16.17 (3.5)	7.72 (1.48)
Plant TN (ppm)	14595 (1710)	8879 (426)	5996 (194)	7916 (539)	17709 (1942)	7644 (236)	9174 (410)	7775 (1159)
Plant TC (ppm)	448783 (2144)	425222 (4661)	432944 (5169)	423849 (2616)	451218 (1792)	426785 (3259)	443975 (1891)	427874 (3086)
MB C:N Ratio	4.8 (0.2)	4.9 (0.2)	5.1 (0.4)	5.1 (0.3)	4.7 (0.1)	4.9 (0.2)	4.9 (0.2)	5 (0.2)
Plant TC:TN	31.4 (3.1)	47.9 (1.7)	72.3 (1.8)	54 (3.6)	26.5 (2.4)	55.8 (1.3)	48.6 (2.1)	57.5 (8.6)
Soil TC:TN	10.8 (0.2)	10.4 (0.08)	11.2 (0.1)	10.6 (0.12)	10.9 (0.3)	10.6 (0.39)	10.8 (0.4)	10.8 (0.09)
Soil pH	5.96 (0.04)	5.9 (0.04)	6.02 (0.05)	6.13 (0.08)	6.15 (0.05)	5.95 (0.03)	6.07 (0.11)	5.92 (0.13)