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Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland

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Summary

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- Nitrogen (N) and phosphorus (P) are essential nutrients for primary producers and decomposers in terrestrial ecosystems. Although climate change affects terrestrial N cycling with important feedbacks to plant productivity and carbon sequestration, the impacts of climate change on the relative availability of N with respect to P remain highly uncertain.
- In a semiarid grassland in Wyoming, USA, we studied the effects of atmospheric CO_2 enrichment (to 600 ppmv) and warming (1.5/3.0°C above ambient temperature during the day/night) on plant, microbial and available soil pools of N and P.
- Elevated CO_2 increased P availability to plants and microbes relative to that of N, whereas warming reduced P availability relative to N. Across years and treatments, plant N : P ratios varied between 5 and 18 and were inversely related to soil moisture.
- Our results indicate that soil moisture is important in controlling P supply from inorganic sources, causing reduced P relative to N availability during dry periods. Both wetter soil conditions under elevated CO_2 and drier conditions with warming can further alter N : P. Although warming may alleviate N constraints under elevated CO_2, warming and drought can exacerbate P constraints on plant growth and microbial activity in this semiarid grassland.

Introduction

Ongoing increases in atmospheric CO_2 and temperature have large effects on carbon (C) and nutrient cycles in terrestrial ecosystems, with potentially strong feedbacks to the global climate system (Heimann & Reichstein, 2008). Much attention has been given to CO_2 and temperature effects on the nitrogen (N) cycle because of its importance for plant growth, C sequestration and biodiversity (An *et al.*, 2005; Reich, 2009; Niu *et al.*, 2010; Norby *et al.*, 2010). Elevated CO_2 often results in reduced N availability to plants, as increased plant growth stimulates plant N uptake and immobilization in long-lived plant biomass, and increased soil C stimulates microbial N immobilization in the soil (Luo *et al.*, 2004). Reduced soil N availability may eventually dampen the stimulatory effect of elevated CO_2 on plant growth (Luo *et al.*, 2004; Norby *et al.*, 2010). An increase in temperature, however, often increases N availability to plants, because increased microbial activity in the soil releases N from organic matter (Rustad *et al.*, 2001; Melillo *et al.*, 2002), which, in turn, can offset the immobilization effect of elevated CO_2 (Hovenden *et al.*, 2008).

Plant growth and microbial activity in terrestrial ecosystems are also often affected by phosphorus (P) availability (Güsewell, 2004; Cleveland & Liptzin, 2007; Elser *et al.*, 2007). In most soils, N is

supplied through biological N fixation and atmospheric deposition, whereas the primary source of P is rock weathering. Both nutrients are recycled through soil organic matter decomposition. N limitation often occurs in young soils with limited accumulation of atmospheric N input (Vitousek & Farrington, 1997), or in systems with high N loss through nitrification and denitrification processes, fires and leaching (Vitousek & Howarth, 1991). P limitation often occurs in old soils in which P is no longer supplied through weathering, or in soils with high P sorption/precipitation capacity (Walker & Syers, 1976; Vitousek & Howarth, 1991). Primary productivity in terrestrial ecosystems is therefore frequently limited by both N and P in soils of intermediate age (Vitousek & Farrington, 1997) or when the availability of both nutrients is low (Harpole *et al.*, 2011). Indeed, the limitation of both N and P on primary productivity is widespread in terrestrial ecosystems (Güsewell, 2004; Elser *et al.*, 2007; Craine *et al.*, 2008; Craine & Jackson, 2010; Harpole *et al.*, 2011).

The availability of P as well as N may be altered by elevated CO_2 and warming, potentially causing shifts in N and P supplied to plants. Although the release of both nutrients is biologically controlled through the mineralization of soil organic matter by microbes, P availability is also controlled geochemically through sorption/desorption and dissolution/precipitation reactions (Achat

et al., 2009; Yang & Post, 2011). Elevated CO_2 and warming effects on these biotic and abiotic processes could alter the relative supply of N and P, and eventually cause changes in primary productivity, soil organic matter decomposition and biodiversity (Güsewell, 2004; Wassen et al., 2005; Güsewell & Gessner, 2009; Liu et al., 2010).

Plants and microbes require N and P in specific ratios for optimal growth and have limited flexibility to take up N and P under conditions of variable and often unbalanced N and P availability. Perturbations affecting the relative availability of N and P may have different impacts on plant growth and microbial activity because of their dissimilar requirements for N and P. For instance, microbial N : P ratios tend to be lower than plant N : P ratios (Cleveland & Liptzin, 2007), suggesting that the demand for P relative to N is greater for microbes than for plants. Furthermore, plant species differ in their stoichiometric homeostasis in the N : P ratio, or in their ability to maintain a constant N : P ratio under variable supply of both nutrients (Sterner & Elser, 2002; Güsewell, 2004). The maintenance of stoichiometric homeostasis in environments with variable nutrient supply is energetically expensive for plants (Sterner & Elser, 2002). Thus, environments with temporally variable nutrient supply may favour species that are flexible in their N : P stoichiometry (Sardans et al., 2012). The importance of stoichiometric N : P flexibility in plant growth responses to climate change is unknown.

In this study, we examine how elevated CO_2 and warming affect N and P cycling in a semiarid grassland in Wyoming, USA with low N and P availability. We include an irrigation treatment to study the direct effects of soil water availability on N and P cycling. Previously, we have demonstrated that elevated CO_2 increases significantly soil moisture as a result of reductions in plant stomatal conductance, and that warming decreases significantly soil moisture as a result of desiccation effects at this site (Morgan et al., 2011). Consequently, warming induces more extreme soil moisture conditions (i.e. faster and more severe soil drying after rainfall events), whereas elevated CO_2 dampens soil moisture extremes. The C3 grasses at this site respond most strongly to elevated CO_2, whereas the C4 grasses also respond to warming. Further, we have shown that soil N availability decreases with elevated CO_2 and increases with warming (Dijkstra et al., 2010; Carrillo et al., 2012). Here, we build on this work by examining P and N : P in plants, microbes and soils. The objectives of this study were to examine how elevated CO_2 and warming affect plant, microbial and available pools of N and P in the soil; how N and P dynamics are related to soil moisture; and how stoichiometric flexibility in plant N : P relates to changes in plant growth among individual species in response to elevated CO_2 and warming.

Materials and Methods

The Prairie Heating And CO_2 Enrichment (PHACE) experiment is located at the US Department of Agriculture Agricultural Research Service High Plains Grasslands Research Station, Wyoming, USA (latitude 41°11′N, longitude 104°54′W). The ecosystem is a northern mixed-grass prairie dominated by the cool-season C3 grasses Pascopyrum smithii (Rydb.) A. Love and

Hesperostipa comata Trin and Rupr., and the warm-season C4 grass Bouteloua gracilis (H.B.K) Lag. Other species include the sedge Carex eleocharis L. Bailey and the forb Sphaeralcea coccinea (Nutt.) Rydb. These five species comprise between 80 and 99% of the total aboveground biomass. The mean annual precipitation is 384 mm and the mean air temperatures are 17.5°C in July and −2.5°C in January. The soil is a fine loamy, mixed, mesic Aridic Argiustoll with a pH of 7.0, bulk density of 1.2 g cm^{-3}, 62% sand, 23% silt and 15% clay. The calcareous soil contains substantial amounts of solid inorganic P (calcium phosphates), forming 42% of total soil P, whereas almost all soil N is in organic form (Table 1).

The core experiment consists of 20 circular plots (each 3.4 m in diameter) with two atmospheric CO_2 concentration levels (ambient and 600 ppmv) and two temperature levels (ambient and 1.5/3.0°C above ambient temperature during the day/night) in a full factorial design (five replicates for each treatment combination). The experiment uses Free-Air CO_2 Enrichment, or FACE, technology to raise the CO_2 concentration, and ceramic infrared heaters with a proportional-integral-derivative feed-back loop to raise the canopy temperature. The CO_2 treatment began in April 2006 and the warming treatment in April 2007. The experiment includes five extra plots that are kept under ambient CO_2 and temperature conditions, but with 60 mm yr^{-1} of irrigation applied in three to four events during the growing season. This amount of added water increased the soil moisture content to levels approximating those observed in plots exposed to elevated CO_2. The volumetric soil moisture content was continuously monitored in all plots at a soil depth of 10 cm (Sentek Envirosmart sensors, Sentek Sensor Technologies, Stepney, SA, Australia). Detailed information about the site and experiment has been reported elsewhere (Dijkstra et al., 2010; Morgan et al., 2011).

Aboveground plant biomass was clipped in mid-July of 2007, 2008 and 2009 at peak biomass. A metal grid containing 24 quadrats (25 cm × 25 cm; total of 1.5 m^2) was placed inside the plots, and vegetation in every other quadrat (12 in total) was clipped to the crown. Aboveground biomass was separated by green and senesced biomass. Green biomass was further separated by species (senesced biomass could not be identified by species). We analysed N and P on senesced plant tissue and green tissue of the five species mentioned above. Samples were dried (60°C), weighed and ground, and then analysed for N concentration on an elemental analyser (Carlo Erba, Haake Buchler Instruments, Saddle Brook, NJ, USA). Subsamples were ashed before colorimetric P analysis using the ammonium molybdate–vanadate reagent (Jackson, 1958) on a spectrophotometer (Bausch and Lomb, Rochester, NY, USA). In 2007 and 2009, Plant Root Simulator (PRS) resin

Table 1 Soil C, N and P pools at a soil depth of 0–15 cm in the Prairie Heating And CO_2 Enrichment (PHACE) experiment in the control plots (± SE)

Pool	Total g m^{-2}	Organic form g m^{-2}	% of total	Inorganic form g m^{-2}	% of total
C	2989 (168)	2945 (167)	98.5	44 (19)	1.5
N	256 (14)	256 (14)	99.8	0.4 (0.03)	0.2
P	64.4 (2.2)	38.3 (2.1)	59	26.1 (0.6)	41

probes (Western Ag Innovations, Saskatoon, CA, USA) were used to measure the availability of N and P in the soil (at 2–7.6 cm soil depth) during the growing season (May–October). After retrieval, probes were washed with deionized water and sent to Western Ag Innovations for analyses. Probes were extracted with 0.5 N HCl solution and the extractant was analysed for NH_4^+ and NO_3^- colorimetrically on a flow injection analyser (Seal Analytical, Mequon, WI, USA) and for P using inductively coupled plasma emission spectroscopy (Perkin Elmer, Inc., Waltham, MA, USA). These probes provide a nondestructive integrative index of available N and P to plants during the period for which they are in the soil (Johnson *et al.*, 2007). Because only inorganic, and not organic, forms of N and P (nitrate, ammonium and inorganic phosphate) were measured, available N and P may have been underestimated. Although plants are capable of taking up organic N as amino acids that are present in many soils (Hofmockel *et al.*, 2010), it remains unclear how important organic N uptake is for plant nutrition (Näsholm *et al.*, 2009). Even less clear is the importance of organic P uptake (Macklon *et al.*, 1994). In 2005, before experimental treatments started, soils were sampled in each plot from 0 to 5 and 5 to 15 cm soil depth, and analysed for bulk density and inorganic C as carbonates (Sherrod *et al.*, 2002).

In 2009, soils were sampled 1 wk after the aboveground biomass sampling in each plot at the same depths as in 2005. After inorganic C had been removed with 1 M H_3PO_4, soils were analysed for total organic C and N on an elemental analyser. Total organic and inorganic P were analysed using the ignition $- 0.2$ N H_2SO_4 extraction method (Saunders & Williams, 1955) with subsequent colorimetric measurement of P in the extracts using the ammonium molybdate–vanadate reagent. Soil subsamples were extracted with 0.05 M K_2SO_4 and analysed for inorganic N (NH_4^+ and NO_3^-) on a flow injection analyser (Lachat Instruments, Loveland, CO, USA). Microbial C, N and P were measured using the fumigation–extraction method (Brookes *et al.*, 1982, 1985). Soil subsamples were fumigated with chloroform for 5 d and, together with nonfumigated subsamples, were extracted with 0.05 M K_2SO_4 for C and N analyses on a total organic C analyser (Shimadzu Scientific Instruments, Wood Dale, IL, USA) and with 0.5 M $NaHCO_3$ for colorimetric P analysis using the ammonium molybdate–ascorbic acid reagent (Olsen & Sommers, 1982). To correct for P sorption during fumigation and extraction, five off-plot samples were spiked with 100 µg P and similarly analysed for P (Brookes *et al.*, 1982). We used 0.45, 0.54 and 0.4 extraction efficiency corrections for C, N and P, respectively, to calculate microbial C, N and P. Although these sorption and extraction efficiency correction factors are soil type dependent (e.g. soil texture), we do not expect them to be affected by the treatments of our experiment. All soil measurements were expressed per unit area using the bulk density measurements conducted in 2005 and by combining the two soil depths. Detailed methods for plant, PRS probe and soil N analyses are provided elsewhere (Dijkstra *et al.*, 2010; Morgan *et al.*, 2011).

The degree of homeostasis, or the H factor, was calculated by plotting the log-transformed values of green plant N : P and PRS probe N : P from each of the 20 core plots measured in 2007 and 2009, where the H factor is the inverse of the slope (Sterner & Elser, 2002):

$$\log(\text{green plant N : P}) = a + (1/H) \log(\text{PRS probe N : P})$$

We used ANOVA to test for the main effects of CO_2 and warming and their interactive effect on plant, PRS probe and microbial N, P and N : P in each year using the 20 core plots. To test whether the five species differed in their stoichiometric flexibility (log(green plant N : P)) to relative changes in available N and P in the soil (log(PRS probe N : P), we used ANCOVA with species as a main effect, log(PRS probe N : P) as a covariate and their interaction. We used ANOVA to test for irrigation effects on plant, PRS probe and microbial N, P and N : P in each year, comparing the five ambient CO_2 and temperature plots with the five irrigated plots. We used linear and nonlinear regressions to test for relationships between soil moisture and plant N, P and N : P ratios, and among N : P ratios in soil organic matter, microbes, plants and PRS probes. In some cases, data were log transformed to improve the assumptions of normality and homoscedasticity. All statistical analyses were performed with JMP (version 4.0.4; SAS Institute, Cary, NC, USA).

Results

The climate change treatments at PHACE altered the balance of available nutrients in soil and in plant and microbial biomass pools (Fig. 1). In general, elevated CO_2 decreased N : P ratios in aboveground plant biomass (Fig. 1a,b). Warming increased N : P ratios, particularly in 2008 and 2009, although the warming effect was not as strong as the elevated CO_2 effect. Elevated CO_2 and warming had similar effects on the N : P ratios of the plant species *B. gracilis*, *H. comata* and *P. smithii*, but did not alter significantly the N : P ratios of the less abundant species *C. eleocharis* and *S. coccinea* (Supporting Information Table S1). The N : P ratios measured in green plant biomass were always smaller than in senesced plant biomass, indicating that the resorption of P was greater relative to that of N. Warming increased microbial N : P in 2009, particularly under ambient CO_2 (Fig. 1c). The N : P ratio of available N and P in the soil, measured with the PRS resin probes for the 2007 and 2009 growing seasons, decreased with elevated CO_2 (Fig. 1d).

Treatment effects on N : P stoichiometry were sometimes caused by changes in N, sometimes by changes in P, and were sometimes only expressed by simultaneous changes in N and P (Tables S2–S4). For example, although treatment effects on green plant N : P were caused by changes in N concentration in 2007 and 2008, they were caused by changes in P concentration in 2009 (Table S2). Warming increased the green plant N pool for the five dominant species in all 3 yr ($P < 0.05$ in 2008 and $P < 0.1$ in 2007 and 2009), as reported previously for all species combined (Dijkstra *et al.*, 2010; Carrillo *et al.*, 2012), but had no effect on the green plant P pool (Table S3). Elevated CO_2 had no effect on the green plant N and P pools of the five dominant species. However, the highest P amounts were observed in the CT treatment (see Fig. 1 for definition), causing a marginally significant $CO_2 \times$ temperature interactive effect in 2009. Elevated CO_2 decreased the available N measured by the PRS probes in both 2007 and 2009, but had no effect on available P (Table S4). Warming increased available N
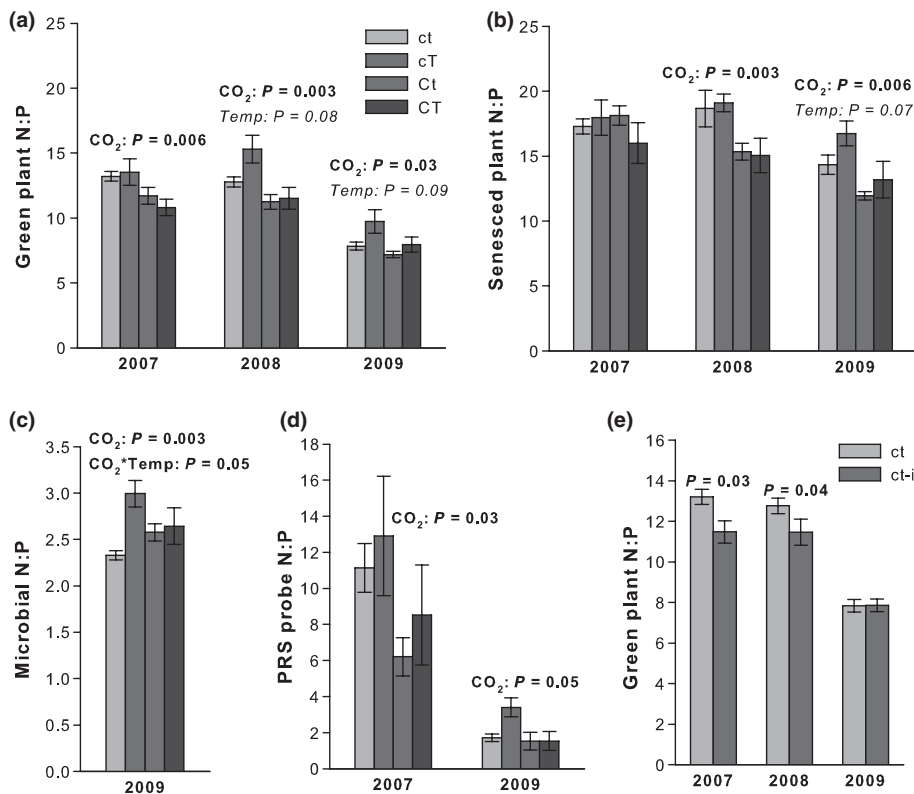
Fig. 1 N : P ratios in green (a, e) and senesced (b) plant tissue, microbes (c) and on Plant Root Simulator resin probes (PRS probes) (d) in response to elevated CO_2, warming and irrigation (ct, ambient CO_2 and temperature; cT, ambient CO_2 and 1.5/3°C day/night warming; Ct, 600 ppmv CO_2 and ambient temperature; CT, 600 ppmv CO_2 and 1.5/3°C day/night warming; ct-i, ambient CO_2 and temperature with 60 mm yr^{-1} irrigation). Green plant N : P ratios are species-weighted averages. Error bars represent 1SE. ANOVA P values are reported when $P < 0.05$ (in bold) or $P < 0.1$ (in italics).

in both years, and also increased available P in 2007, but not to the same extent as N. Treatment effects on microbial N : P were caused by simultaneous changes in microbial N and P (Table S4). Elevated CO_2 caused a marginally significant increase in the microbial N : C ratio (reflecting an increase in microbial N concentration), whereas warming reduced both the microbial P : C ratio (or microbial P concentration) and P amount.

The irrigation treatment decreased plant N : P ratios in 2007 and 2008, similar to that observed with elevated CO_2, but not during the relatively wet year of 2009 when annual precipitation was 17% higher than the 132-yr mean for this site (Fig. 1e). In that relatively wet year, plant and PRS probe N : P ratios were lower than in the two previous years when precipitation was within 7% of the long-term mean. The PRS probe N : P ratios varied between 0.1 and 25, whereas the green plant N : P ratios varied between 5 and 18 across the CO_2 and warming treatments and years. Further, PRS probe and green plant N : P ratios were negatively related to mean early-season soil moisture content measured at a soil depth of 10 cm (Fig. 2a,b). For the PRS probes, this negative relationship was caused by a decrease in N with increased soil moisture, whereas, for plants, this was caused by both a decrease in plant N concentration and an increase in plant P concentration with increased soil moisture (Fig. 2c–f). Green plant N content was not related to soil moisture, but green plant P content increased significantly with increased soil moisture (Fig. 2g,h).

We observed a significant positive relationship between microbial and green plant N : P and between microbial and PRS probe N : P measured in 2009 (Fig. 3). Microbial N : P ratios were much smaller than green plant N : P ratios, suggesting that P requirements relative to N were larger for microbes than for plants. However, the

PRS probe N : P ratios measured in 2009 were similar to the microbial N : P ratios. Microbial N : P ratios were not related to total soil organic matter N : P ratios ($P > 0.1$, data not shown).

The N : P ratios of individual plant species were responsive to changes in the relative availability of N and P in the soil measured in 2007 and 2009 in the 20 core plots (Fig. 4). The five species measured showed a variable degree of stoichiometric flexibility or homeostasis, with the C4 grass *B. gracilis* showing the greatest flexibility (least homeostatic) to relative changes in available N and P in the soil, and the C3 grass *P. smithii* showing the least flexibility (most homeostatic). The slopes of the relationships shown in Fig. 3 between soil-available and plant N : P ratios were significantly different among the five species (interactive effect of species × log (PRS probe N : P) in ANCOVA, $P = 0.03$), and the H values, a measure of the degree of homeostasis (Sterner & Elser, 2002), ranged between 4.3 and 9.6. Despite this range, we did not observe consistent relationships between species-specific H values and plant growth responses to elevated CO_2 and warming.

Discussion

In general, soil-available, plant and microbial N : P ratios decreased under elevated CO_2 and increased with warming. We found strong evidence that the opposing effects of elevated CO_2 and warming on available N : P to plants and microbes were driven by variations in soil moisture. Elevated CO_2 increased soil moisture as a result of reductions in plant stomatal conductance, whereas the desiccating effect of warming decreased soil moisture (Morgan *et al.*, 2011). Soil moisture control on soil-available and plant N : P ratios was supported by lower N : P ratios with irrigation, lower N : P ratios in
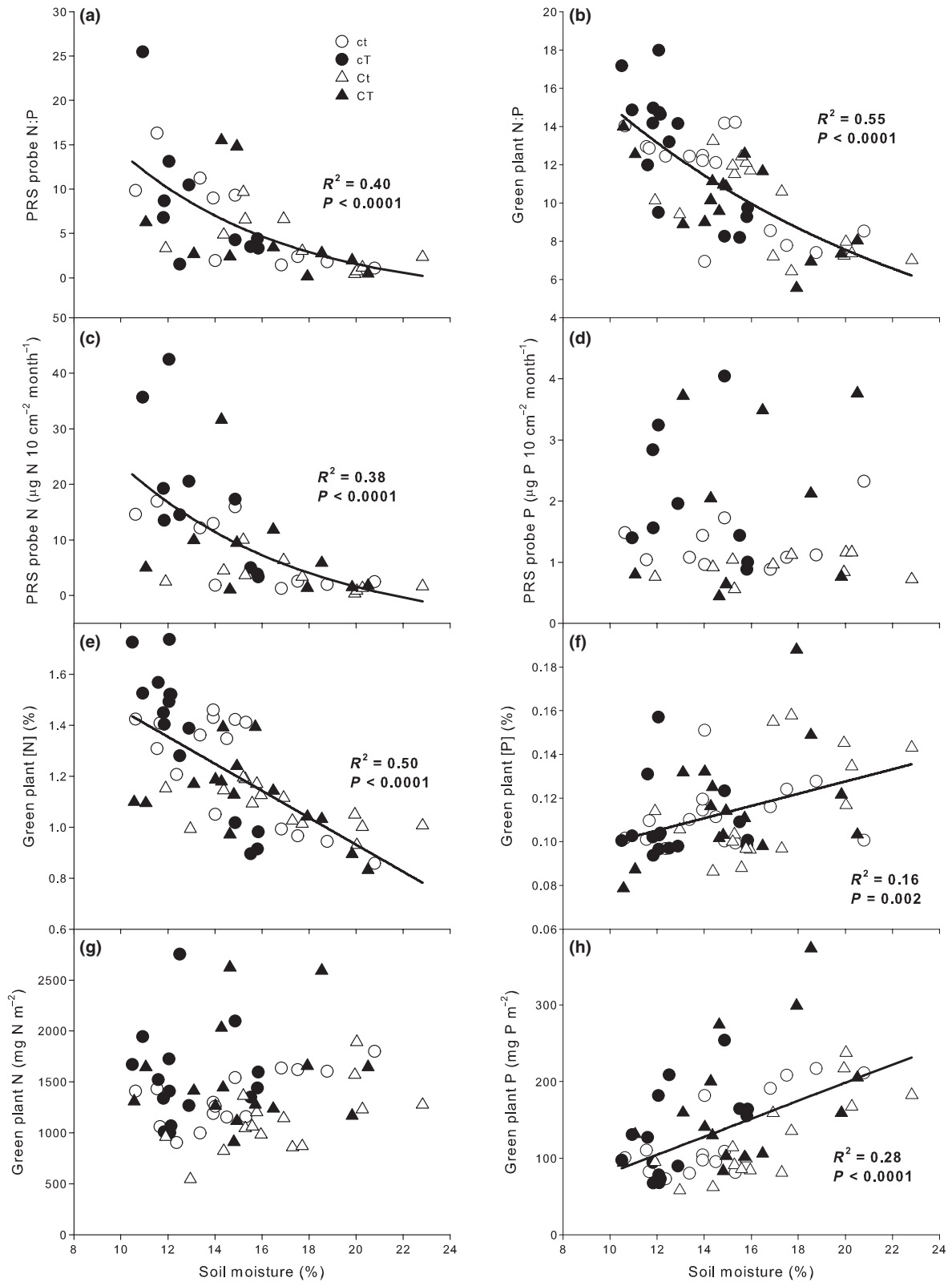
Fig. 2 Relationships between soil moisture and N : P ratios on the Plant Root Simulator (PRS) probes (a) and in green plants (b), N (c) and P (d) contents on PRS probes, N (e) and P (f) concentrations in green plant tissue, and green plant N (g) and P (h) pools. Relationships are shown for the species-weighted average and volumetric soil moisture at a soil depth of 10 cm, averaged over days of year (DOY) 100–200. Each point represents a specific plot measured in 2007, 2008 and 2009 (2007 and 2009 for PRS probes). Treatments: ct, ambient CO_2 and temperature; cT, ambient CO_2 and 1.5/3°C day/night warming; Ct, 600 ppmv CO_2 and ambient temperature; CT, 600 ppmv CO_2 and 1.5/3°C day/night warming.
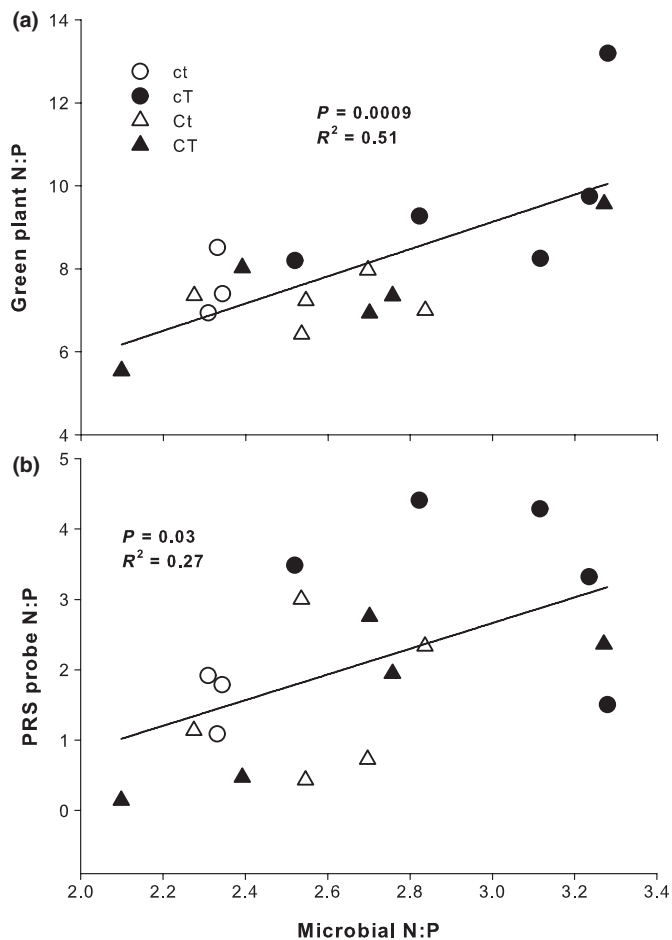
Fig. 3 Green plant N : P (a) and Plant Root Simulator (PRS) probe (b) N : P ratios in relation to microbial N : P ratios. Each point represents a specific plot measured in 2009. Treatment abbreviations: ct, ambient CO_2 and temperature; cT, ambient CO_2 and 1.5/3°C day/night warming; Ct, 600 ppmv CO_2 and ambient temperature; CT, 600 ppmv CO_2 and 1.5/3°C day/night warming.

the relatively wet year of 2009 and a strong negative relationship between mean early-season soil moisture content and plant N : P. A consistent increase in soil moisture with elevated CO_2 and decrease with warming could therefore have important implications for the functioning of this ecosystem in the long term.

Reduced available N : P to both plants and microbes with increased moisture may have occurred through a greater proportional loss of N than P, or a greater proportional supply of P than N. Gaseous N loss through nitrification/denitrification or the loss of NO_3^- through leaching could decrease the relative availability of N, and thus simultaneously decrease the N : P ratio of plants and microbes. Both gaseous N loss and leaching may increase in wetter conditions (Austin *et al.*, 2004), contributing to the reduced N : P in plants and microbes observed with increased soil moisture (e.g. under elevated CO_2). However, because N losses are small in these semiarid grasslands (Mosier *et al.*, 2002; Burke *et al.*, 2008), we expect that differential loss of N and P played a minor role in causing variable plant and microbial N : P ratios. Further, the increase in green plant P concentration and the green plant P pool (Fig. 2f,h) with increased soil moisture cannot be explained by increased N loss.

Changes in soil moisture caused by elevated CO_2 or warming could also affect the proportional supply of P and N. Desorption and dissolution of inorganic P in the soil can function as an important source of P for plants and microbes, particularly in calcareous soils (Lajtha & Bloomer, 1988; Tunesi *et al.*, 1999). The desorption and dissolution of inorganic P depend on inorganic equilibria with the P concentration in soil solution. An increase in soil moisture increases the diffusivity of P, thereby enhancing the uptake by plants and microbes (Lambers *et al.*, 2006). Increased plant and microbial uptake of P would deplete soluble P rather rapidly if soluble P was not buffered by desorption and dissolution reactions (Fitter & Hay, 2002). An increase in soil moisture may then have increased the supply of P to plants and microbes through enhanced desorption and dissolution. At our site, a large fraction of the total P pool in the soil was in inorganic form (Table 1), suggesting that P desorption and dissolution may be especially important for P supply in this system. Increased P desorption and dissolution with increased soil moisture could explain why both green plant P concentrations and pools increased with increased soil moisture (Fig. 2f,h). However, the decrease in green plant N concentration with increased soil moisture (Fig. 2e) was most probably a result of a dilution effect caused by the increase in plant productivity, as the green plant N pool was unaffected by soil moisture (Fig. 2g). This also suggests that the decrease in available N with increased soil moisture (Fig. 2c) was caused by an increase in microbial N immobilization, as opposed to an increase in plant uptake. Previously, we have shown that irrigation and elevated CO_2-induced increases in soil moisture also increase microbial N immobilization (Dijkstra *et al.*, 2010; Carrillo *et al.*, 2012). Here, we propose that the soil moisture-induced increases in microbial N immobilization may also have been stimulated by increased microbial P supply through desorption and dissolution.

Further indirect support for the importance of inorganic reactions in controlling P supply at our site comes from the relationship between microbial and plant N : P. If plant N and P were predominantly supplied through organic matter decomposition, ecological stoichiometry theory would predict that the relationship between plant and microbial N : P ratios should be negative (Sterner & Elser, 2002). When microbes depend solely on soil organic matter as their source of N and P, a shift to greater uptake and storage of N relative to P during soil organic matter decomposition should result in greater release of P relative to N. In contrast, available and plant N : P ratios were both positively related to microbial N : P ratios in the soil (Fig. 3). This suggests that the availability of P was not primarily controlled by soil organic matter decomposition. It also suggests a decoupling of the supply of N (released mostly through mineralization) and P (mostly through desorption/dissolution) in this system. Similarly, decoupling of N and P supply from organic matter may also occur because N is released through biological mineralization (or oxidation of organic matter), whereas P can also be released through biochemical mineralization (release from P esters with the help of extracellular enzymes; McGill & Cole, 1981).

Under dry conditions, when geochemical reactions of P are limited by P diffusivity, the warming effect on N and P availability may have been largely driven by changes in soil organic matter
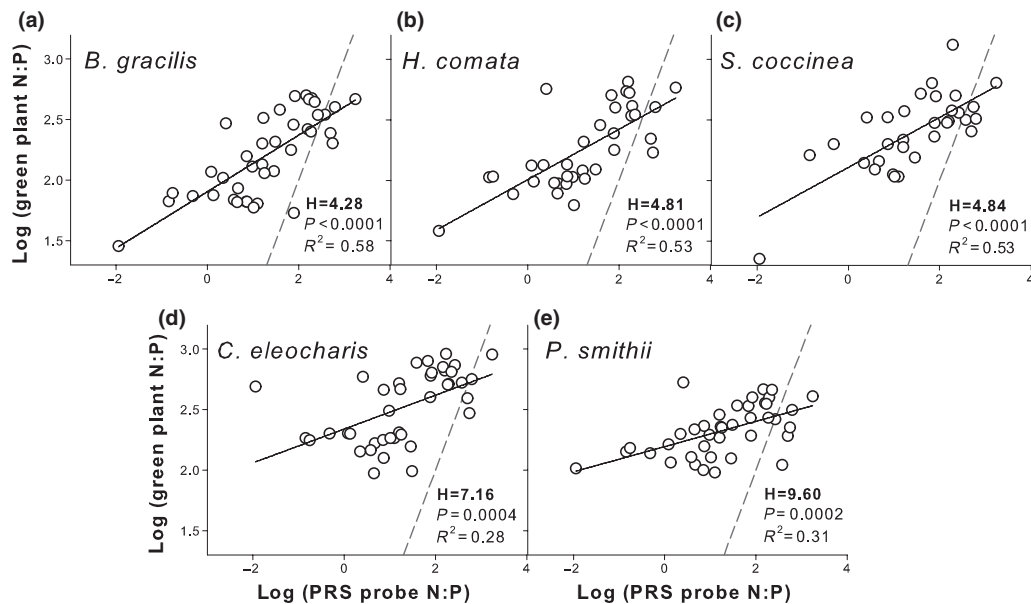
Fig. 4 Relationship between N : P ratios in green plant tissue of five dominant grasses and N : P ratios on Plant Root Simulator (PRS) probes. Relationships are shown for the C4 grass *Bouteloua gracilis* (a), the C3 grass *Hesperostipa comata* (b), the forb *Sphaeralcea coccinea* (c), the sedge *Carex eleocharis* (d) and the C3 grass *Pascopyrum smithii* (e). Each point represents the N : P ratios measured in a specific plot for a specific year (2007 and 2009). For each species, the H factor is reported, which quantifies the degree of stoichiometric flexibility or homeostasis: a larger H (slope of the regression deviating more strongly from the dashed 1 : 1 line) indicates a smaller degree of flexibility or greater degree of homeostasis (see the Materials and Methods section for details).

decomposition. For instance, available P measured with the PRS probes increased with warming in the relatively dry year of 2007 (Table S4), despite a potentially lower P supply from geochemical reactions as a result of warming-induced soil drying. This suggests that warming increased P mineralization in that year. Available N and plant N pools also increased with warming, further suggesting enhanced N mineralization with warming (Dijkstra *et al.*, 2010). In contrast, the green plant P pool was not affected by warming in 2007 (Table S3), possibly because, under dry soil conditions, plant uptake of P was limited by the diffusion of P to the root, more so than N (Fitter & Hay, 2002). A direct warming effect on P mineralization may also help to explain why the available P did not relate to soil moisture (Fig. 2d). Overall, the supply of P at our site is likely to be controlled by a suite of mechanisms that are active under different environmental conditions, because of the nearly co-dominant inorganic and organic P sources.

We have no direct evidence for N and P limitation at our site. However, there are several reasons why we believe that both N and P were important for plant growth at our study site. First, soil available N and P concentrations at our site were low, increasing the likelihood that both nutrients affected plant growth (Harpole *et al.*, 2011). The low N and P conditions are illustrated by the extremely low losses of N as N_2O at this site (< 2 mg N m^{-2} yr^{-1}, F. A. Dijkstra *et al.*, unpublished results) and the low levels of available P (4–7 mg P kg^{-1} soil, measured in nonfumigated soil extracts) relative to those in 98 North American grassland soils (2–100 mg P kg^{-1} soil; Craine & Jackson, 2010). Second, species-weighted green plant N : P ratios in 2007 and 2008 were between 10 and 20, and, although critical plant N : P ratios for nutrient limitation are not always unambiguous, values between 10 and 20 may indicate limitation of both N and P for plant growth at

the ecosystem level (Güsewell, 2004). In the relatively wet year 2009, when green plant N : P ratios were often below 10, plant growth may have become more N limited. Third, we found indirect evidence that N made available to plants through microbial mineralization may be mediated by P supply to microbes that have a relatively high P requirement. Indeed, the dependence of plant productivity on both N and P may be widespread in grasslands because of the nature of the interaction between P and N availability (Craine & Jackson, 2010).

Although the aboveground biomass of C3 and C4 grasses responded differently to elevated CO_2 and warming (Morgan *et al.*, 2011) and the N : P ratio differed among plant species (Table S1), elevated CO_2 and warming effects on overall plant N : P ratios were most probably not caused by differential species responses and shifts in vegetation. The N : P ratios of the three dominant plant species *B. gracilis*, *H. comata*, and *P. smithii* all showed similar responses to elevated CO_2 and warming. In contrast, the N : P ratios of *C. eleocharis* and *S. coccinea* did not respond to elevated CO_2 and warming, but their contribution to the total aboveground biomass was relatively small (on average 10% for *C. eleocharis* and 3% for *S. coccinea*). All plant biomass samples were taken at peak biomass in mid-July, when plants were at a similar late stage of development, indicating that potential differences in plant N : P among species and years caused by developmental differences were most probably small.

The biomass N : P ratios of individual plant species were positively related to soil-available N : P ratios (none were strongly homeostatic), but species varied in their stoichiometric flexibility in response to changes in soil-available N : P (Fig. 4). It has been suggested that environments with a temporally variable nutrient supply may favour species that are flexible in their N : P

stoichiometry (Sardans *et al.*, 2012), as the maintenance of stoichiometric homeostasis under these conditions is energetically expensive for plants (Sterner & Elser, 2002). In semiarid grasslands, flexible or nonhomeostatic nutrient uptake rates may also be advantageous for plants, so that N uptake is not impeded by low P availability during the long dry periods, and vice versa, and so that P uptake is not hindered by the relatively low N availability during the short wet periods. We found partial support for this. Warming induced more extreme soil moisture conditions (i.e. faster and more severe soil drying after rainfall events) and increased the above-ground biomass of *B. gracilis* (Morgan *et al.*, 2011), which also showed the greatest flexibility in N : P. Similarly, water savings under elevated CO_2 reduced the extremes in soil moisture at our site, which could be relatively more advantageous to species with low N : P flexibility. Indeed, *P. smithii* showed the lowest N : P flexibility and was one of the C3 grasses that responded most strongly to elevated CO_2 in 2007 and 2008. In contrast, *H. comata* and *S. coccinea*, which showed a similar high flexibility in N : P to *B. gracilis*, did not respond to warming or elevated CO_2. Possibly, differences in photosynthetic pathways and rooting depths (LeCain *et al.* 2006) confound the clear relationships between species-specific N : P flexibility and their responses to climate change. Stoichiometric homeostasis has also been linked to plant dominance in a similar semiarid grassland in Inner Mongolia (Yu *et al.*, 2010), but we found no strong support for this. At our site, *B. gracilis* and *P. smithii* were two of the three dominant species, but showed the most and least N : P flexibility, respectively.

It has been argued that plant productivity may become increasingly constrained by N under elevated CO_2 alone (Luo *et al.*, 2004; Norby *et al.*, 2010). Our results suggest that an increase in P availability relative to N under elevated CO_2 may further exacerbate N constraints. However, warming could alleviate N constraints on plant growth under elevated CO_2, and may increase P constraints, particularly under dry conditions. As soils dried out more rapidly with warming, P became progressively less available to plants relative to N. It is unclear whether similar effects would occur in wetter environments and in noncalcareous soils that contain less inorganic P, and where P supply is largely controlled by organic matter decomposition. Enhanced precipitation increased plant P uptake, but elevated CO_2 and warming had no effect on plant P or N : P in an annual grassland in California (Menge & Field, 2007) or in other mesic ecosystems (Chapin *et al.*, 1995; Niklaus *et al.*, 1998; Finzi *et al.*, 2001). Our results suggest that primary productivity and microbial activity in semiarid grasslands that occur widely on calcareous soils may become more reliant on P availability in a warmer and drier world. The enormous increase in anthropogenic N fixation and resultant increase in atmospheric N deposition (Galloway *et al.*, 2008) could further exacerbate the increased P constraints on plant growth in a warmer and drier environment. Thus, the dynamics of P relative to N will be critical in predicting responses of semiarid grasslands to climate change.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1 Average green plant N : P ratios by species

Table S2 Average green and senesced plant N and P concentrations (%) averaged across all five species

Table S3 Average N and P pools in green plant biomass of the five species combined

Table S4 Average N and P pools on Plant Root Simulator (PRS) probes (2007 and 2009) and N : C, P : C and N and P pools in microbial biomass (2009)

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Climate change alters stoichiometry of phosphorus and nitrogen in a semiarid grassland

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pools in microbial biomass (2009)

Table S1 Average green plant N:P ratios by species

Treatment	Species					
	B. gracilis N:P			*C. eleocharis* N:P		
	2007	2008	2009	2007	2008	2009
ct	13.3 ± 0.6	12.6 ± 0.7	6.8 ± 0.4	16.9 ± 0.7	16.1 ± 0.7	8.5 ± 0.5
cT	14.2 ± 0.4	15.3 ± 0.7	9.2 ± 0.8	17.0 ± 0.7	16.8 ± 0.7	10.5 ± 1.5
Ct	10.3 ± 1.3	10.9 ± 0.8	6.3 ± 0.1	15.5 ± 0.7	15.6 ± 0.4	9.7 ± 0.1
CT	10.2 ± 0.7	11.8 ± 0.9	6.5 ± 0.8	14.0 ± 1.2	15.7 ± 1.7	11.5 ± 1.3
ct-i	11.1 ± 0.5	11.1 ± 0.7	6.8 ± 0.4	12.0 ± 0.6	15.9 ± 0.9	10.9 ± 0.4
ANOVA P-values						
CO_2	**0.0006**	**0.004**	**0.01**	**0.02**	0.44	0.28
Temp	0.63	**0.04**	**0.03**	0.40	0.74	*0.08*
CO_2×Temp	0.54	0.28	*0.08*	0.34	0.74	0.93
Irrigation	**0.02**	0.15	0.98	**0.0009**	0.86	**0.005**

Treatment	Species					
	H. comata N:P			*P. smithii* N:P		
	2007	2008	2009	2007	2008	2009
ct	14.8 ± 0.7	11.1 ± 0.6	7.6 ± 0.3	12.5 ± 0.4	12.5 ± 0.3	9.3 ± 0.4
cT	14.4 ± 0.8	15.5 ± 0.8	9.8 ± 2.0	12.7 ± 1.3	16.3 ± 2.1	11.3 ± 1.2
Ct	11.2 ± 0.7	10.3 ± 0.5	7.2 ± 0.2	11.0 ± 0.5	11.3 ± 0.5	7.9 ± 0.3
CT	10.5 ± 1.2	9.4 ± 0.8	6.8 ± 0.6	10.7 ± 0.5	11.7 ± 0.9	8.6 ± 0.7
ct-i	11.1 ± 0.8	10.3 ± 0.6	7.2 ± 0.5	12.0 ± 0.9	11.5 ± 0.9	9.3 ± 0.8
ANOVA P-values						
CO_2	**0.002**	**0.0002**	*0.09*	**0.03**	**0.03**	**0.009**
Temp	0.58	**0.02**	0.35	0.95	*0.09*	*0.08*
CO_2×Temp	0.88	**0.002**	0.21	0.74	0.18	0.38
Irrigation	**0.01**	0.38	0.48	0.61	0.31	0.93

Treatment	Species		
	S. coccinea N:P		
	2007	2008	2009
ct	12.4 ± 0.3	11.9 ± 0.9	8.9 ± 0.6
cT	14.1 ± 0.9	14.9 ± 0.8	10.4 ± 1.1
Ct	12.2 ± 0.9	12.3 ± 0.9	8.9 ± 0.7
CT	12.4 ± 1.4	12.9 ± 1.6	8.1 ± 1.8
ct-i	12.5 ± 0.5	13.2 ± 0.4	10.8 ± 0.2
ANOVA P-values			
CO_2	0.42	0.50	0.43
Temp	0.42	0.15	0.78
CO_2×Temp	0.52	0.31	0.41
Irrigation	0.32	0.23	**0.01**

ct: Ambient CO_2, ambient temperature; cT: ambient CO_2, elevated temperature; Ct: elevated CO_2, ambient temperature; CT: elevated CO_2, elevated temperature; ct-i: ambient CO_2, ambient temperature, irrigated.

Table S2 Average green and senesced plant N and P concentrations (%) averaged across all five species

Treatment	Green plant N concentration (%)			Green plant P concentration (%)		
	2007	2008	2009	2007	2008	2009
ct	1.39 ± 0.02	1.37 ± 0.04	0.96 ± 0.03	0.106 ± 0.003	0.107 ± 0.004	0.124 ± 0.008
cT	1.45 ± 0.03	1.61 ± 0.05	1.02 ± 0.07	0.111 ± 0.012	0.107 ± 0.006	0.106 ± 0.005
Ct	1.16 ± 0.01	1.08 ± 0.03	1.00 ± 0.02	0.101 ± 0.006	0.097 ± 0.003	0.139 ± 0.007
CT	1.16 ± 0.02	1.24 ± 0.06	0.95 ± 0.04	0.109 ± 0.008	0.110 ± 0.009	0.119 ±0.011
ct-i	1.41 ± 0.06	1.39 ± 0.05	1.07 ± 0.02	0.123 ± 0.005	0.122 ± 0.006	0.137 ± 0.006
ANOVA P-values						
CO_2	**<0.0001**	**<0.0001**	0.46	0.73	0.54	*0.09*
Temp	0.16	**0.0009**	0.91	0.41	0.31	**0.02**
CO_2×Temp	0.11	0.53	0.2	0.85	0.29	0.85
Irrigation	0.76	0.75	**0.02**	**0.02**	*0.07*	0.24

Treatment	Senesced plant N concentration (%)			Senesced plant P concentration (%)		
	2007	2008	2009	2007	2008	2009
ct	0.85 ± 0.03	0.79 ± 0.03	0.82 ± 0.03	0.049 ± 0.002	0.043 ± 0.004	0.058 ± 0.004
cT	0.87 ± 0.04	0.87 ± 0.04	0.88 ± 0.04	0.048 ± 0.006	0.046 ± 0.002	0.053 ± 0.003
Ct	0.73 ± 0.02	0.70 ± 0.03	0.80 ± 0.01	0.041 ± 0.002	0.046 ± 0.003	0.067 ± 0.002
CT	0.69 ± 0.04	0.76 ± 0.03	0.76 ± 0.04	0.045 ± 0.006	0.053 ± 0.006	0.060 ± 0.007
ct-i	0.91 ± 0.04	0.78 ± 0.04	0.82 ± 0.03	0.056 ± 0.006	0.051 ± 0.004	0.067 ± 0.004
ANOVA P-values						
CO_2	**0.0004**	**0.006**	**0.05**	0.18	0.26	*0.08*
Temp	0.73	**0.03**	0.79	0.72	0.25	0.21
CO_2×Temp	0.31	0.76	0.15	0.58	0.60	0.82
Irrigation	0.27	0.88	0.98	0.33	0.18	0.13

ct: Ambient CO_2, ambient temperature; cT: ambient CO_2, elevated temperature; Ct: elevated CO_2, ambient temperature; CT: elevated CO_2, elevated temperature; ct-i: ambient CO_2, ambient temperature, irrigated.

Table S3 Average N and P pools in green plant biomass of the five species combined

Treatment	Green plant N (mg m^{-2}) 2007	2008	2009	Green plant P (mg m^{-2}) 2007	2008	2009
ct	1336 ± 93	1095 ± 52	1585 ± 88	101 ± 5	86 ± 5	202 ± 7
cT	1457 ± 167	1336 ± 130	1848 ± 261	113 ± 20	89 ± 11	189 ± 19
Ct	1068 ± 91	931 ± 112	1368 ± 172	104 ± 16	82 ± 7	188 ± 18
CT	1489 ± 162	1242 ± 89	1937 ± 287	140 ± 18	110 ± 11	262 ± 37
ct-i	1606 ± 27	1533 ± 71	1638 ± 164	142 ± 8	136 ± 13	210 ± 21
ANOVA P-values						
CO_2	0.31	0.20	0.59	0.39	0.40	0.31
Temp	*0.06*	**0.02**	*0.08*	0.19	0.12	0.28
CO_2×Temp	0.21	0.52	0.41	0.36	0.15	*0.08*
Irrigation	**0.04**	**0.001**	0.86	**0.004**	**0.004**	0.88

Table S4 Average N and P pools on PRS-probes (2007 and 2009) and N:C, P:C and N and P pools in microbial biomass (2009)

Treatment	PRS-probes				Microbial biomass			
	N pool (μg 10 cm^{-2} month^{-1})		P pool (μg 10 cm^{-2} month^{-1})		N:C	P:C	N pool (g m^{-2})	P pool (g m^{-2})
	2007	2009	2007	2009	2009	2009	2009	2009
ct	14.5 ± 0.9	2.0 ± 0.2	1.35 ± 0.13	1.62 ± 0.28	0.094±0.001	0.040±0.001	8.31 ± 0.93	3.29 ± 0.45
cT	26.3 ± 5.5	8.8 ± 2.9	2.20 ± 0.36	1.13 ± 0.23	0.087±0.002	0.029±0.002	8.59 ± 0.84	2.86 ± 0.23
Ct	5.4 ± 1.3	1.5 ± 0.5	0.85 ± 0.09	1.24 ± 0.32	0.098±0.001	0.038±0.001	9.67 ± 0.94	3.75 ± 0.35
CT	13.6 ± 4.7	2.3 ± 0.9	2.14 ± 0.65	1.66 ± 0.30	0.098±0.003	0.038±0.003	8.72 ± 0.89	3.28 ± 0.21
ct-i	5.7 ± 1.9	1.9 ± 0.3	1.77 ± 0.42	1.49 ± 0.33	0.093±0.003	0.038±0.003	7.73 ± 0.67	3.15 ± 0.32
ANOVA P-values								
CO$_2$	**0.0009**	**0.04**	0.46	0.80	*0.06*	0.18	0.18	0.17
Temp	**0.009**	**0.03**	**0.01**	0.91	0.40	**0.02**	0.53	**0.04**
CO$_2$×Temp	0.63	*0.07*	0.57	0.13	0.29	**0.02**	0.26	0.77
Irrigation	**0.02**	0.75	0.36	0.77	0.85	0.59	0.63	0.80