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Daily and seasonal changes in soil amino acid composition in a semiarid grassland exposed to elevated CO_2 and warming

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Abstract Soil amino acids are often an important source of nitrogen (N) for plants, and anticipated global changes, including climate warming and rising atmospheric CO_2 levels, have the potential to alter plant and microbial production and consumption of this N source in soils. We determined soil amino acid composition over a 1-year period at diurnal and seasonal time scales in a multi-factor global change experiment with elevated CO_2 and warming in native semiarid grassland. Soil amino acids were collected in April, May and June of 2011 and April 2012 using a soil water perfusion and extraction method that minimized soil disturbance. This was a particular advantage when taking diurnal measurements. The extracts were analyzed by ultra performance liquid chromatography. We detected 16 different soil amino acids throughout the study, and glutamine/glutamate (glu-x), arginine, serine and asparagine/aspartate (asp-x) were consistently at highest relative concentrations, comprising 3–41, 6–20, 2–22 and 7–24 % of total amino acids, respectively. No direct effects of experimental warming or elevated CO_2 on soil amino acid composition were observed. However, the relative abundance of individual soil amino acids shifted diurnally and seasonally with changes in soil temperature and soil moisture. Glu-x and arginine increased and serine decreased with higher temperature, while asp-x and serine increased and arginine decreased with higher moisture. Overall, the relative abundances of soil amino acids responded more strongly to both diurnal and seasonal changes in temperature and soil moisture than to elevated atmospheric CO_2 and experimental warming.

Keywords Organic nitrogen cycle · Soil amino acids · Grasslands · Nitrogen · Global change

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Introduction

Understanding effects of elevated CO_2 and warming on nitrogen (N) availability is important for predicting

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the extent to which ecosystem structure and function may respond to global change (West 1991; Rustad et al. 2001; Reich et al. 2006). Amino acid N has been observed in soils at concentrations comparable to or greater than that of inorganic N and may serve as an important N source for plants (Chapin III et al. 1993; Kielland 1994; Raab et al. 1996; Schimel and Bennett 2004; Inselsbacher and Näsholm 2012). Despite the important role that soil amino acids can have as a plant N source, we know little about how amino acid composition varies across environmental conditions and over time, or the mechanisms controlling soil amino acid composition. For example, exudation, cell lysis and decomposition of plant and microbial biomass can release and elevate the soil concentration of specific amino acids, like glutamine/glutamate and asparagine/aspartate, corresponding to their higher relative composition in parent material (Chapin III et al. 1986; Rozycki and Strzelczyk 1986; Näsholm et al. 1994; Ohlson et al. 1995; Diez and Alvarez 2001). Soil amino acid composition also is altered by selective uptake by plants and soil microorganisms, and differences in soil mobility related to molecular size, polarity, and charge (Jones and Kielland 2002; Weigelt et al. 2005; Näsholm et al. 2009; Inselsbacher et al. 2014). These processes operate rapidly and at varying rates, causing soil amino acid composition to be highly dynamic in space and time (Schimel and Bennett 2004; Raab et al. 1999; Amelung et al. 2006; Werdin-Pfisterer et al. 2009).

Rates of production and consumption of soil amino acids are responsive to changes in temperature and moisture (DeLuca et al. 1992; Lipson and Monson 1998; Conant et al. 2011), and are likely affected by variation in other environmental factors. However, effects of environmental change on soil amino acid concentrations and composition are not well documented or understood. In addition, few studies have investigated how this potentially important N source varies temporally, and only one study has investigated how soil amino acids can vary over short, diurnal time scales (Inselsbacher et al. 2014). Such information is important for predicting the forms and quantities of soil amino acids available for plant uptake and how amino acid availability mediates plant responses to global change (West 1991; Williams and Miller 2001; Luo et al. 2004).

Anticipated climate changes are likely to influence availability of N for plants. Atmospheric CO_2 concentration is projected to reach between 790 and 1,140 ppm by the end of the current century and drive increases in global temperatures by 1.9–3.7 °C (IPCC 2013). As atmospheric CO_2 rises, N is predicted to become more limiting to primary production due to increased biotic immobilization (Luo et al. 2004). Experimental studies confirm that soil inorganic N pools often decrease in terrestrial ecosystems in response to elevated atmospheric CO_2 (Zak et al. 2000; Hungate et al. 1999; Dijkstra et al. 2010). As N becomes less available in soils, plants increasingly compete with microbes for organic N (Schimel and Bennett 2004), thus reducing soil amino acid pools. Indeed, increases in biotic soil amino acid uptake and decreases in amino acid pools under experimentally elevated atmospheric CO_2 have been observed in some ecosystems (Andresen et al. 2009; Jin and Evans 2010), but not in others (Hofmockel et al. 2007). In contrast, soil warming may stimulate decomposition of N containing compounds and increase soil amino acid pools (Sowden et al. 1977; Melillo et al. 2002), though concurrent increases in plant and microbial consumption of amino acids with warming can have the opposite effect (Jones 1999; Andresen et al. 2009; Jin and Evans 2010). Elevated CO_2 and warming also directly and indirectly affect soil moisture (Dai et al. 2004; Morgan et al. 2004), which can alter amino acid production and consumption rates (e.g., Lipson and Monson 1998, 2001). Determining how soil amino acid composition will be affected by global change is important because plant uptake of amino acids is dependent upon amino acid form as well as plant species specific uptake capacity (Raab et al. 1999). Changes in soil amino acid composition in the future can thereby alter N availability and, consequentially, plant productivity and composition.

Here we evaluated effects of anticipated global change on soil amino acid composition in a temperate semiarid grassland ecosystem. Understanding changes in soil amino acid availability in temperate grasslands in response to elevated CO_2 and warming is important because this biome covers 11 % of the terrestrial surface, accounts for 9 % of global terrestrial net primary production and plays an important role in grazing of domestic livestock (Mitchell et al. 1990; Saugier et al. 2001). We investigated variation in soil amino acid composition over diurnal and seasonal time scales. Until recently, the monitoring of soil amino acid composition over a diurnal period has not

been practical because of lengthy extraction times and the destructive nature of multiple samplings. However, using a recently developed soil perfusion and extraction method (Chen and Williams 2013), we were able to rapidly extract soil amino acids in this experiment with minimal disturbance to soil. This method of soil amino acid extraction enables the relative abundances of amino acids to be determined, but cannot be used to measure absolute abundances. We predicted that soil amino acid composition would vary diurnally due to rapidly shifting rates of microbial and plant activity (Christensen 1983; Rayment and Jarvis 2006; Yuste et al. 2007).

Amino acid composition in soil pore water was determined in a semiarid grassland field experiment in Wyoming, USA. The complete factorial experiment included elevated CO_2, warming and elevated CO_2 plus warming treatments. The effect of these treatments on soil amino acid composition was analyzed on daily and seasonal timescales. As N becomes more limiting under elevated atmospheric CO_2, the demand for and consumption of soil amino acid N should increase and some amino acids should become more depleted compared to others under elevated CO_2. Specifically, we predicted that amino acids that are commonly used to transfer N to other compounds in plants and microorganisms, such as glutamine/glutamate and asparagine/aspartate, are likely in higher demand and should decrease in abundance relative to other soil amino acids with plant and microbial uptake. We also predicted that warming would further increase plant and microbial consumption of amino acids, such as glutamine/glutamate and asparagine/aspartate, faster than they can be supplied by increased rates of decomposition thus depleting pools of these amino acids.

Methods

Study site

Our work was conducted within the prairie heating and CO_2 enrichment (PHACE) experiment located at the US Department of Agriculture Agricultural Research Service (USDA-ARS) High Plains Grasslands Research Station in southeastern Wyoming, USA (latitude 41°11′ N, longitude 104°54′ W). This northern mixed-grass prairie is dominated by the C3 grasses *Pascopyrum smithii* (Rydb.) A. Love and *Hesperostipa comata* Trin and Rupr. and a warm season C4 grass, *Bouteloua gracilis* (H.B.K.) Lag. and an assortment of less dominant forbs, sedges and other grasses. N fixing plants are a minor component of the experimental plots, and plant productivity is limited by N (Blumenthal 2009). The growing season extends from April through September, with most precipitation falling at the beginning of the growing season between March and May (Lauenroth and Milchunas 1992; Sala et al. 1992). Mean annual precipitation is 384 mm with a little over 70 % of precipitation occurring during the growing season (1973–2005; HPGRS data). The soil is a fine-loamy, mixed, mesic Aridic Arigiustoll with a pH range of 6.5–7.0 that is consistent across PHACE treatments, likely due to buffering by high levels of calcium carbonate. Organic soil carbon content is 1.9 % (SD = 0.3) from 0 to 5 cm. No treatment effects were observed on C content (Carrillo et al. 2011). Mean air temperatures in January and July are −2.5 and 17.5 °C, respectively.

Experimental design

Twenty 3.4-m diameter plots were established for experimental manipulation of CO_2 and temperature using free air CO_2 enrichment (FACE) technology (Miglietta et al. 2001) and infrared heaters (Kimball et al. 2008). Controlled release of pure CO_2 at the periphery maintained an atmospheric CO_2 concentration of 600 ± 40 ppm during the growing season in FACE plots (initiated in April 2006). Ceramic infrared heaters placed 1.5 m above each plot (1,000 W; Mor Electric Heating Assoc., Inc., Comstock Park, MI, USA) warmed treated plots by 1.5 °C above ambient temperature during the day and 3 °C during the night, year round (initiated in April 2007). The four plot treatments consisted of either ambient CO_2 and temperature (ct), ambient CO_2 and warmed temperature (cT), elevated CO_2 and ambient temperature (Ct) or elevated CO_2 and warmed temperature (CT), with five replications each. Plots were divided into north and south blocks within the PHACE experimental site.

Soil moisture and temperature

Soil moisture probes (EnviroSMART probe; Sentek Sensor Technologies, Stepney, Australia) were installed within each plot at 10, 20, 40, 60 and

80 cm depths, and thermocouples were installed 10 cm above the soil surface and at 3 and 10 cm depths for determination of volumetric soil moisture content and soil temperature at hourly time intervals.

Soil sampling and amino acid extraction

Amino acids were collected from soils in the early afternoon on a single day in 22 April, 23 May and 16 June of 2011 and 20 April of 2012. Extractions were limited to early and mid growing season when amino acids are present in soils, as they were undetectable in the late growing season. Soil amino acids were collected also on 23 May 2011 three times during a 24-h period in the morning (9 am; also used for evaluation of seasonal variation), late afternoon (4 pm), and night (3 am) to characterize the diurnal change in amino acid composition. Soil was sampled for each event at five points across a 2 m transect arc 60 cm inside of each plot circumference. Sampling at five points within each plot was considered sufficient to capture whole plot soil amino acid composition, as sampling within plant microsites did not demonstrate spatial variation in soil extracts (Chen and Williams 2013). At each point, a small cavity was created to a depth of 5 cm, 5 mL deionized water was slowly injected into the cavity, and soil extracts were collected using a soil water perfusion and extraction technique described by Chen and Williams (2013). The process was repeated for all five points within the plot with cumulative collection of soil water to create a single plot sample. The retrieved water was filtered immediately through a 0.2 μm surfactant-free cellulose acetate Nalgene syringe filter (Thermo Scientific, Inc., USA) designed for low protein binding and low extractables prior to storage on ice in 1.5 mL polypropylene centrifuge tubes. Samples were transported to the laboratory within several hours and then stored at −20 °C until analysis.

Soil amino acid chemical analysis

All samples were analyzed within 1 year of collection. Samples initially were concentrated 50 times by lyophilization and re-suspension in HPLC grade water in a sonicating bath for 5 min to increase their detection. Total amino acid concentrations in solutions were then determined by ultra performance liquid chromatography (UPLC) using a Waters acquity ultra performance LC analyzer and standard protocol provided in the UPLC amino acid analysis solution system guide (Waters Corp., MA, USA). Peak separation was achieved using a Column-AccQ-Tag^{TM} Ultra$^{®}$ C18 1.7 μm column (2.1 × 100 mm) (Waters Corp., MA, USA). Amino acid concentrations that were determined by UPLC were then recalculated to determine original concentrations prior to lyophilization.

Sixteen primary amino acids and ammonium were identified by UPLC. However, due to ammonium contamination of soil extracts by the cellulose acetate filter, results for ammonium concentration were omitted from this study. Cysteine and tryptophan were not resolved and glutamate and glutamine and aspartate and asparagine are reported only as glu-x and asp-x molecules, respectively, because of no peak separation and standard limitation of the Waters UPLC AccQ-Tag derivatization reaction. All peaks were eluted within 8 min and peak areas were determined using Waters Empower 2 software with automatic baseline integration. A maximum number of 32 samples were run between standards and initial and final run standards were compared to correct for drift. Amino acid quantification was performed by comparing peak areas of samples to calibration curves of known amino acid standards that were analyzed at the beginning and end of each sequence (Pierce Amino Acid Standard H, Thermo Scientific, Inc., USA). The range in concentration of amino acid standards bracketed the measured sample values.

Data analysis

Because of variations in soil extract recovery, soil sampling volume and soil moisture content, comparison of soil amino acids between treatments and dates was performed based on the relative abundance of amino acids. Contrary to previous studies that reported relative abundances of soil amino acid N by calculating the ratios of individual amino acid N to the total amount of amino acid N detected (Werdin-Pfisterer et al. 2009; Chen and Williams 2013), we compared the relative abundance of amino acids by using the molar ratio of N from the α-amino group in individual amino acids to the total amount of N from the α-amino group in all detected amino acids (AA:TAA). Because N from the α-amino group is present in a 1:1 molar ratio with its amino acid, AA:TAA reflects the relative

abundance of each soil amino acid without bias from amino acids with different total molecular N. This is important when considering amino acids such as arginine, histidine and lysine, which contain more than one N atom. Different methods of reporting the abundance of amino acids yield different comparative values of soil amino acids and selection of the approach for reporting these abundances should depend on the study goal.

Comparison of the relative abundance of individual amino acids over time and among treatments was performed using a completely randomized one-way ANOVA analysis by GLM procedures with $n = 5$ (SAS Institute Ver 9.3). Identification of relationships between AA:TAA and temperature and soil moisture also was performed using CORR procedures in SAS. To detect effects of experimental treatments and time on the soil amino acid community composition, we complimented the above univariate analyses with a multivariate analysis approach. We used a permutational multivariate analysis of variance (PERMANOVA) test on a Bray–Curtis similarity index generated from square-root transformed soil amino acid abundance data. Elevated CO_2, temperature and date or time of day were the main effects. To test for the effect of spatial variation on soil amino acid composition, experimental block was included as a random effect in all PERMANOVA models. The PERMANOVA analysis was combined with a similarity percentage analysis to determine the relative contribution of individual soil amino acids to the observed compositional dissimilarities among plots, with a 10 % contribution cut-off for both similarity and dissimilarity analyses. To examine the relationship between soil amino acid community composition and the associated environmental variables volumetric soil water content and soil temperature measured at 3 cm depth measured concurrently with amino acids, we used the RELATE function. The RELATE function compares the rank correlation of the actual data with results from samples randomly permuted 999 times using a Mantel test and reports a Rho value and associated statistical significance. Data visualization using a principal coordinates ordination (PCO) analysis was performed separately for each date to assess differences among treatments. To examine diurnal and seasonal shifts in soil amino acid composition, we used a distance-based redundancy analysis (dbRDA), based on the principal coordinates analysis output. PERMANOVA, dbRDA, RELATE, and PCO analyses were performed in PRIMER (PRIMER-E, version 6, Clarke and Gorley 2006).

Results

Site temperature and precipitation

Mean daily air temperatures on sample dates in April, May and June of 2011 and April 2012 were 3.8, 9.4, 17.6 and 8.8 °C, respectively. Mean daily soil temperatures on sample dates in April, May and June of 2011 and April 2012 were 5.4, 11.2, 20.0 and 10.7 °C, respectively. Mean air temperatures of the sampling periods for morning, afternoon and night on the May 2011 extraction were 8.1, 14.1 and 5.2 °C, respectively. Mean soil temperatures from 0 to 3 cm depth during sampling periods for morning, afternoon and night on the May 2011 extraction were 11.4, 17.6 and 12.7 °C, respectively. Precipitation at the site between January and August was 395 mm in 2011, close to the long-term annual mean of 384 mm. Precipitation over the same period in 2012 was 160 mm. Soil volumetric water content from 0 to 10 cm depth (prior to extraction, averaged across treatments) averaged 19.1, 19.6, 13.4 and 11.6 % on the April, May and June 2011 and April 2012 sampling dates.

PHACE treatment effects

Amino acid composition

Among the 16 detectable amino acids, serine, arginine, glu-x and asp-x were consistently observed in highest relative amounts (Fig. 1) accounting for 6–20, 2–22, 7–24 and 3–41 % of the total amino acids, respectively. These amino acids vary in charge, size, polarity, molecular complexity and N composition. Histidine, lysine, tyrosine, methionine, isoleucine, leucine and phenylalanine were consistently detected at the lowest relative levels, all constituting less than 6.5 % of the total amino acid pools (Fig. 1).

When AA:TAA was compared for individual soil amino acids, there was only a significant effect of PHACE treatments on glycine in May 2011 and April 2012 ($P = 0.0415$ and 0.0215, respectively). In May 2011, glycine was proportionally lower in elevated CO_2 plus warming treated plots compared to all other

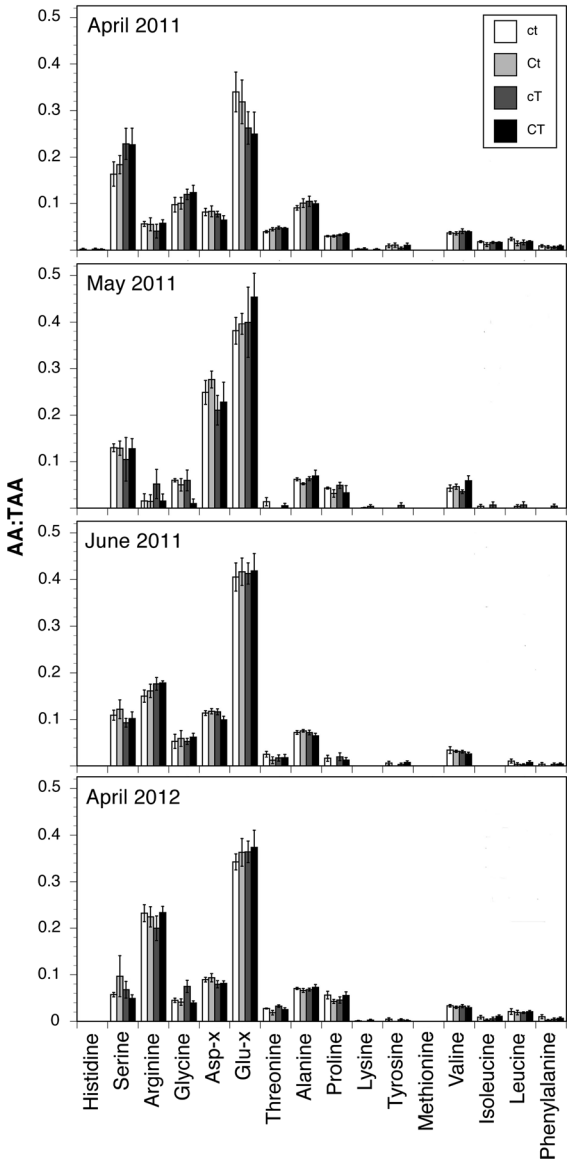

Fig. 1 Ratio of individual molar α-amino N to total α-amino N of soil amino extracts in the experimental site in *April*, *May* and *June 2011* and *April 2012*. *Asp-x* and *glu-x* are a combination of asparagine and aspartate and glutamine and glutamate, respectively. Soil extracts collected from plots with ambient CO_2 are labeled with 'c' and elevated CO_2 with 'C', ambient temperature with 't' and warming with 'T'. Each *bar* represents an average of five treatment replicates. *Error bars* indicate SE

PHACE treatments, and in April 2012, glycine was proportionally higher in warmed plots compared to all other PHACE treatments. Inconsistent PHACE treatment effects on glycine across time are difficult to interpret and we suspect that these results are statistically anomalous due to the chance of random significance when analyzing a high number of amino acids and time periods. There were also no consistent effects of PHACE treatments on soil amino acid composition across dates (PERMANOVA Pseudo-$F = 2.2$, $P = 0.14$ and Pseudo-$F = 0.5$, $P = 0.6$ for the effects of elevated CO_2 and temperature, respectively). The soil amino acid compositional similarity among plots was high across treatments within each sampling date (April 2011 = 91.1 %, May 2011 = 84 %, June 2011 = 87.4 %, and April 2012 = 90.7 %). There was no relationship between soil amino acid composition and soil temperature or volumetric water content (RELATE Rho = 0.25, $P = 0.1$). Additionally, there was no significant effect of block on soil amino acid composition ($P > 0.05$) in all PERMANOVA models that tested for the effect of the PHACE treatments across dates, indicating no significant spatial variation in amino acid composition.

Temporal dynamics

Seasonal soil amino acid composition

Because there was no detectable effect of CO_2 or warming on soil amino acid composition (except for glycine abundance, but treatment effects were not consistent), replicate measurements within each treatment were combined to perform a comparison of soil amino acid abundance over time. Dominant soil amino acids detected in PHACE treatment soils were selected to investigate temporal variation for amino acid relative abundance. Glu-x remained the dominant detectable soil amino acid between months and years, being present in the highest relative amount in May and June 2011 (Fig. 2). Contrary to glu-x, arginine increased in relative abundance in June 2011 and April 2012 compared to April and May 2011. Asp-x was present in its highest relative amounts in May 2011 and serine was highest in April 2011. While temperature increased, soil moisture decreased over the growing season (Fig. 2). Relationships of AA:TAA with temperature and moisture differed among soil amino acids. Relative abundance of glu-x was positively correlated with temperature ($r = 0.425$, $P = 0.0001$) and that of asp-x was positively correlated with soil moisture ($r = 0.420$, $P = 0.0001$). Arginine relative abundance displayed a positive

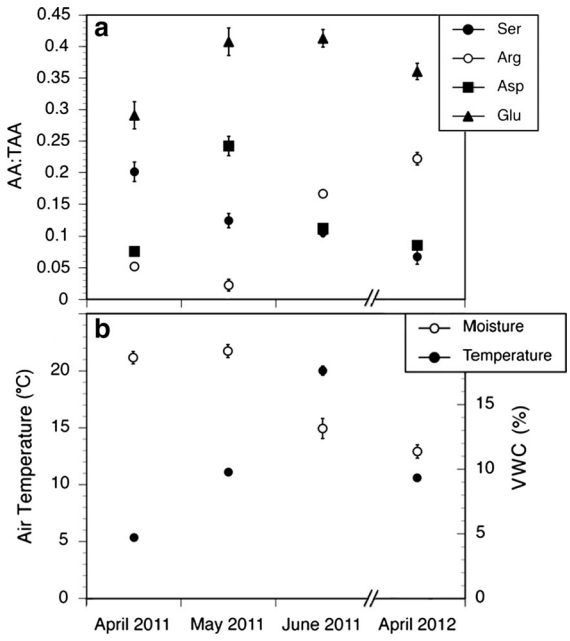

Fig. 2 Ratio of individual molar α-amino N to total α-amino N of soil amino extracts in the experimental site in *April*, *May* and *June* of *2011* and *April 2012* (**a**). Volumetric water content (VWC) of soil 10 cm below the soil surface and temperature of control plots to 3 cm below the soil surface (**b**). Serine, arginine, asparagine/aspartate and glutamine/glutamate were expressed as *Ser*, *Arg*, *Asp*, and *Glu*, respectively. Each *bar* represents an average of five treatment replicates. *Error bars* indicate SE

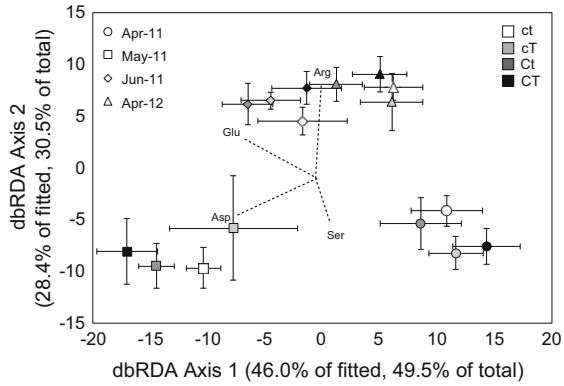

Fig. 3 Distance-based redundancy (*dbRDA*) plot illustrating the contribution of individual amino acids to the shift in soil amino acid composition across time. *dbRDA* axis scores were averaged across replicates of each treatment, within each sample date and the mean ± SE are plotted. Individual amino acids with largest contribution to the overall change in composition are represented (correlation >0.2). *Longer vector line* indicates larger influence on compositional shift. Vector direction along each axis indicates the direction of change in the relative abundance. Serine, arginine, asparagine/aspartate and glutamine/glutamate were expressed as *Ser*, *Arg*, *Asp*, and *Glu*, respectively

correlation with temperature ($r = 0.393$, $P = 0.0004$) and negative correlation with moisture ($r = -0.770$, $P < 0.0001$). Serine had a negative correlation with temperature ($r = -0.378$, $P = 0.0006$) and positive correlation with moisture ($r = 0.539$, $P < 0.0001$).

Soil amino acid composition also differed across time (PERMANOVA Pseudo-$F = 33.9$, $P = 0.001$; Fig. 3), with 20 % compositional dissimilarity between April and May 2011, largely driven by a decrease in the abundance of threonine, arginine, isoleucine, and leucine. Similarly, soil amino acid composition shifted by 22 % from May to June 2011, driven by increases in arginine, threonine, and glycine and a decrease in proline. Soil amino acid composition was relatively similar (14 % dissimilarity) during the same sampling month of April between 2011 and 2012 and several soil amino acids contributed to this small compositional shift. Arginine was more abundant in 2012 than in 2011, while isoleucine, tyrosine, phenylalanine, and serine decreased in relative abundance. The environmental variables average soil temperature and volumetric water content together explained 35.4 % of the seasonal variation in soil amino acid composition (data not illustrated in figures). Although it cannot be quantified, soil amino acid recovery was also significantly higher in April 2012 and correlates with significantly lower soil moisture (see Supplementary Information).

Diurnal soil amino acid composition

The amino acids with highest relative abundance determined in May 2011 also exhibited diurnal variation in AA:TAA (Fig. 4). There was a distinct shift in soil amino acid composition across the day (PERMANOVA Pseudo-$F = 16.9$, $P = 0.001$), but no differences in amino acid composition changes through the day were observed among treatments. The soil amino acid dissimilarity among all treatment plots was 23.5 % between morning and afternoon, largely driven by increases in relative abundance of arginine and threonine, and 15 % between afternoon and night as a result of increases in the relative abundance of threonine, glu-x, and arginine (Fig. 5). Among dominant detectable soil amino acids, only arginine and glu-x displayed a negative correlation with temperature changes over the day ($r = 0.319$, $P = 0.0155$ and

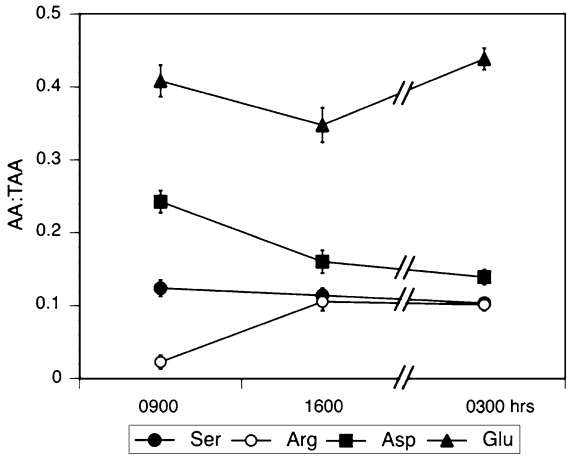

Fig. 4 Ratio of individual molar α-amino N to total α-amino N of soil amino extracts in the experimental site in May 2011 between morning (9 am–12 pm), afternoon (4–6 pm) and night (3 am). Serine, arginine, asparagine/aspartate and glutamate/glutamine were expressed as *Ser*, *Arg*, *Asp*, and *Glu*, respectively. Each *bar* represents an average of 20 treatment replicates. *Error bars* indicate SE

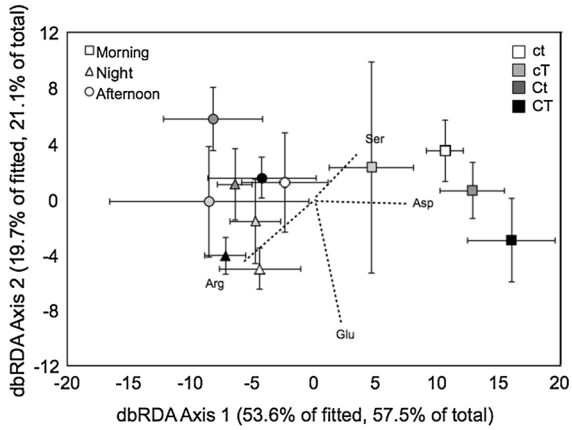

Fig. 5 Distance-based redundancy (*dbRDA*) plot illustrating the contribution of individual amino acids to the shift in soil amino acid composition throughout the day on 23 May 2011. *dbRDA* axis scores were averaged across replicates of each treatment, within each sample time point and the mean ± SE are plotted. Individual amino acids with largest contribution to the overall change in composition are represented (correlation >0.2). *Longer vector line* indicates larger influence on compositional shift. Vector direction along each axis indicates the direction of change in the relative abundance. Serine, arginine, asparagine/aspartate and glutamine/glutamate were expressed as *Ser*, *Arg*, *Asp*, and *Glu*, respectively

$r = -0.292$, $P = 0.028$, respectively). Because soil moisture does not typically vary over a diurnal period, correlation between AA:TAA and moisture was not determined. The negative correlation between glu-x and temperature over the diurnal period was opposite from its positive correlation with temperature observed over the season. Temperature explained 11 % of the variability in glu-x on a diurnal basis, but 20 % on a seasonal basis, illustrating a stronger relationship with temperature over a seasonal timescale.

Discussion

CO_2 and warming effects on soil amino acid composition

Contrary to our predictions, we observed no general effects of elevated CO_2 or warming on soil amino acid composition at our semiarid grassland site across diurnal or seasonal time scales. We also observed no effects on the relative abundance of individual amino acids, including glu-x and asp-x. Glu-x and asp-x, the dominant detectable soil amino acids in our study, also were dominant in other grasslands (Amelung et al. 2006). In contrast to findings from other grasslands (Amelung et al. 2006; Sauheitl et al. 2010), we did not observe high relative abundance of glycine, histidine and phenylalanine but did see high relative abundances of serine and arginine. Variation in soil amino acid composition across grassland studies may relate to extraction methods used, the timing of amino acid collection, or environmental variation (Schimel and Chapin III 1996; Raab et al. 1999; Chen and Williams 2013).

We know little about the mechanisms that drive availability of individual soil amino acids and why glu-x and asp-x amino acids were dominant in our semiarid grassland site. However, glu-x and asp-x and, to a lesser degree, serine and arginine are often the dominant soil-extracted amino acids and are likely such due to their reduced microbial and plant uptake as well as their increased inputs from microorganisms and plants. For example, glutamate is released by actinomycetes at the root zone (Rozycki and Strzelczyk 1986) and can be a large component of fungal amino acids along with serine (Wagner and Mutatkar 1968; Diez and Alvarez 2001). Glutamate and aspartate are also a large component of leaf litter (Top and

Filley 2014), and glutamine, asparagine and arginine are commonly stored in above and belowground plant tissues (Chapin III et al. 1986; Näsholm et al. 1994; Ohlson et al. 1995). All of these amino acids can be released into soils by decomposition, cell lysis, and exudation and serine can be exuded by plant roots (Phillips et al. 2004) into soil.

Prior work at the PHACE experiment demonstrated an increase in inorganic N pools with warming and decrease with elevated CO_2 (Dijkstra et al. 2010), along with an increase in dissolved organic N with warming (Carrillo et al. 2012), and contrasting effects of elevated CO_2 and warming on labile soil carbon pools between years (Carrillo et al. 2011). However, we found no associated changes in soil amino acid composition or relative abundances of individual amino acids. The lack of elevated CO_2 and warming treatment effects was surprising since a number of studies have reported strong effects of these global change factors on soil organic matter decomposition and soil amino acid consumption (Hungate et al. 2000; Melillo et al. 2002; Andresen et al. 2009; Jin and Evans 2010; Brzostek et al. 2010). Our field and extraction methods did not allow for quantification of total soil amino acid pools or their rapid and dynamic fluxes (Jones 1999) and measuring the relative abundance of individual amino acids may not have been adequate for detecting treatment effects on variations in amino acid availability in our study. Conversely, our amino acid extractions suggest that soil amino acid composition may not be highly responsive to these global change factors, perhaps due to unchanging rates of consumption and production or proportional shifts in these fluxes (Inselsbacher et al. 2014). Additional experiments are required to adequately detect soil amino acid availability and the effects of global change factors.

Although warming treatments did not have an effect on soil amino acid composition, the relative abundance of three out of four of the amino acids found at highest relative concentration (i.e., arginine, serine and asp-x) shifted from April to June 2011 as mean soil temperature from 0 to 3 cm soil depth increased from 5.4 to 20.0 °C. Amelung et al. (2006) also observed a convex parabolic relationship between soil amino acid concentrations and mean annual temperature supporting the idea that soil amino acids can respond to larger shifts in temperature. The temperature difference between ambient and warmed treatment plots in our study was substantially smaller than the temperature difference across the climate gradient reported in Amelung et al. (2006) and seasonal variations in temperature, which may account for the lack of any direct warming effects on soil amino acids in our study.

Lack of consistent or strong effects of warming or elevated CO_2 on soil amino acid composition suggests that amino acid composition may remain resilient to PHACE treatments for an extended period of time, and the relatively brief exposure to elevated CO_2 and warming in our study may not have been sufficient to stimulate measurable changes. If plots remained exposed to PHACE treatments for decades, soil amino acid composition might change as a result of a change in plant community composition. To investigate this hypothesis, we evaluated relationships between soil amino acid and plant community composition in the PHACE experimental plots, and despite large variations in plant community composition between plots, we found no significant effect of plant community structure on soil amino acid composition (data not shown). Therefore, we do not suspect that as community composition differences between PHACE treatments develop and diverge over a longer period of time, there will be a difference in soil amino acid composition. In fact, we found no differences in soil amino acid composition between C3 and C4 grass soil microsites in the PHACE experiment (Chen and Williams 2013). A previous study conducted in boreal forest also found no significant change in soil amino acid composition across forest successional stages, suggesting that amino acid composition is stable with changes in vegetation composition (Werdin-Pfisterer et al. 2009). The lack of soil microsite, plant community composition and block effects on amino acid composition also suggests minimal spatial variability within our grassland experimental site.

Temporal changes in soil amino acid composition

Despite a lack of overall significant effects of elevated CO_2 and warming on soil amino acid composition, we detected changes in amino acid composition through time at our semiarid grassland site. Changes in soil amino acid composition occurred over daily, monthly and annual timescales. Several soil amino acids remained dominant over the entire experiment, but their relative abundances varied. The significant shifts

in soil amino acid abundance and composition within a few hours have important implications for when and how amino acids should be sampled, reported and interpreted. Our results suggest that the timing of soil amino acid sampling is critical for accurate characterization of amino acid availability and comparison between studies. Both diurnal and seasonal shifts in soil amino acids can result from variation in soil moisture, temperature, decomposition and transpiration rates, and microbial and plant consumption that influence production and consumption (Schimel and Bennett 2004; Amelung et al. 2006; Cardon and Gage 2006; Lesuffleur et al. 2007).

In our study, soil temperature and moisture varied over time and were likely the drivers of soil amino acid compositional shifts that we observed over daily and seasonal time scales. Although it cannot be quantified, soil amino acid recovery also negatively correlated with soil moisture. These temporally variable environmental factors can have direct and indirect effects on soil amino acid availability by regulating rates of decomposition of N-containing compounds, amino acid mobility and consumption by plant and microbial organisms (Lipson and Monson 1998, 2001; Melillo et al. 2002). Among the dominant forms of amino acids detected within our grassland ecosystem, serine and glutamine are known to be taken up by various grassland plant species (Falkengren-Grerup et al. 2000; Harrison et al. 2007, 2008) but to our knowledge, grassland plant uptake of arginine, glutamate, aspartate and asparagine have not been investigated. The linear relationship between glu-x α-amino N and total α-amino N in our soil extracts in the afternoon and night suggest that there is strong immobilization of glu-x. Plant uptake of amino acids can also vary seasonally (Schimel and Chapin III 1996; Cardon and Gage 2006) although this has not been investigated in grassland ecosystems.

The seasonal patterns in soil amino acid abundance we report are similar to those observed in other studies. For example, a study conducted across a boreal forest successional sequence found an effect of season on relative soil amino acid N abundance, but not on dominant amino acid pool composition (Werdin-Pfisterer et al. 2009). Similar temporal variation of soil amino acid abundance also has been observed in other ecosystems, such as dry meadows, subalpine fens, and the shortgrass steppe (Raab et al. 1999). Ultimately, our study, along with others, suggests that in order to accurately characterize soil amino acid composition, samples must be taken over diurnal and seasonal time scales.

Conclusions

Contrary to our prediction, we did not observe a significant effect of elevated atmospheric CO_2 or warming on soil amino acid composition. This was consistent among all 16 detectable amino acids, regardless of their role in N transfer to other N containing compounds. However, we observed a distinct seasonal shift in soil amino acid composition, and more surprising, a diurnal shift in the abundance of several amino acids and overall soil amino acid soil composition. Further attention should not only be given to understanding the effects of elevated atmospheric CO_2 and warming on the drivers of soil amino acid availability, but to understanding the consequential effect on soil amino acid composition and availability itself over short and long time scales. Additionally, further attention should be given to understanding the effects of temporal shifts, even over short diurnal time scales.

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