Dear S. Sleutel

We have accommodated the typesetting according to your advice.

Kind regards

L.C. Andresen

The reference list still needs to be adjusted to SOIL's style: periods after initials, abbreviated journal names, commas between journal name, volume number, page numbers and year of publication, colons after author names. No '&' but 'and'
The reference list is modified following the instructions.

Add periods after author initials in the 'Author contributions' section.

This has been corrected n the author contributions.

Fig. 2C: y-axis: °C instead of C, replace 'mai' by 'May', Fig2B: a unit is missing for moisture index (-)
The 2C axis' are corrected.

The 2B: the index is a relative index without any unit. This is now denoted -

Tables: Seperate 'N' from the units by a space: 'mg N kg-1', not 'mgN kg-1'
Done

Equations: 'Equations: These should be numbered sequentially with Arabic numerals in parentheses on the right-hand side, i.e. (1), (2), etc. If too long, split them accordingly. If there are chemical formulae included, i.e. reactions, please number them (R1), (R2), etc. When using WORD, the equation editor and not the graphic mode should be used under all circumstances.

Arabic numbers are used now.

Amino acid and N mineralization dynamics in heathland soil after long-term warming and repetitive drought

L.C. Andresen[1*], S. Bode[2], A. Tietema[3], P. Boeckx[2] and T. Rütting[1]

1 Department of Earth Sciences, University of Gothenburg, Box 460, 405 30 Göteborg, Sweden

2 Isotope Bioscience Laboratory - ISOFYS, Ghent University, Coupure Links 653, 9000 Gent, Belgium

3 Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, Box 94240, 1090 GE Amsterdam, The Netherlands.

*) *Corresponding author*: email: louise.andresen@bot2.bio.uni-giessen.de; *current address*: Dr. Louise C. Andresen; Department of Plant Ecology; Justus-Liebig-University Giessen; Heinrich-Buff-Ring 26; 35392 Gießen; Germany.

Abstract

Monomeric organic nitrogen (N) compounds such as free amino acids (FAA) are an important resource for both plants and soil microorganisms and a source of ammonium (NH_4^+) via microbial FAA mineralization. We compared gross FAA dynamics with gross N mineralization in a Dutch heathland soil using a ^{15}N tracing technique. A special focus was made on the effects of climate change factors warming and drought, followed by rewetting. Our aims were to: 1) compare FAA mineralization (NH_4^+ production from FAAs) with gross N mineralization, 2) assess gross FAA production rate (depolymerization) and turnover time relative to gross N mineralization rate, and 3) assess the effects of a 14 years warming and drought treatment on these rates.

The turnover of FAA in the soil was ca. 3 hours, which is almost two orders of magnitude faster than that of NH_4^+ (i.e. ca. 4 days). This suggests that FAAs is an extensively used resource by soil microorganisms. In control soil (i.e. no climatic treatment), the gross N mineralization rate (10 ± 2.9 µg N $g^{-1}day^{-1}$) was eight-times smaller than the total gross FAA production rate of five AAs (alanine, valine, leucine, isoleucine, proline: 127.4 to 25.0 µg N g^{-1} day^{-1}). Gross FAA mineralization (3.4 ± 0.2 µg N g^{-1} day^{-1}) contributed by 34 % to the gross N mineralization rate and is therefore an important component of N mineralization. In the drought treatment, a 6-29% reduction in annual precipitation, caused a decrease of gross FAA production by 65% and of gross FAA mineralization by 41%, compared to control. On the other hand, gross N mineralization was unaffected by drought, indicating an increased mineralization of other soil organic nitrogen (SON) components. A 0.5-1.5°C warming did not significantly affect N transformations, even though gross FAA production declined.

Overall our results suggest that in heathland soil exposed to droughts a different type of SON pools are mineralized. Furthermore, compared to agricultural soils, FAA

mineralization was relatively less important in the investigated heathland. This indicates more complex mineralization dynamics in semi-natural ecosystems.

Keywords: depolymerization, ammonification, N cycle, ^{15}N pool dilution, amino acid mineralization, mirror experiment

1. Introduction

Heathlands are protected under the European Union Habitats Directive (Directive (92/43/EEC); EUR-Lex) as this ecosystem type has declined throughout Europe (Fagundez, 2013). In the Netherlands, the heathland area has declined by 95% since the year 1900 (Fagundez, 2013). Sustaining this characteristic ecosystem type requires management of the vegetation (Webb, 1998; von Oheimb, 2009; Garcia et al., 2013) to mitigate the effects of the major present-day threats; climate change and increased nitrogen (N) deposition (Aerts et al., 1995; Fagundez, 2013). Most heathlands and shrublands are developed on nutrient poor soil, hence available N is a limited resource. Plant and microbial use of both inorganic N (IN; mainly ammonium (NH_4^+) and nitrate (NO_3^-)) and organic N (ON; *e.g.* free amino acids, FAA) is dependent on the availability and production of the different N moieties (Nordin et al., 2004; Jones and Kielland, 2012). However, knowledge of the relative importance of IN and FAAs for plants and microbes in heathlands is fragmented and variable (Nordin et al., 2004; Andresen et al., 2005, 2011; Clemmensen et al., 2008), and we need to understand how the interplay of available N and global change factors threaten this ecosystem type.

Ammonium (NH_4^+) is produced during mineralization of ON. Gross mineralization is depending on the availability of FAAs, because FAA mineralization is the main pathway of ammonium production (Barraclough 1997; Stange and Döhling 2005; Geisseler et al. 2012), hence, gross N mineralization depends on the FAA production rate. However, FAAs are not the only source of gross N mineralization. FAAs are produced in the soil during depolymerization of peptides, proteins and other components of detritus and litter (Weintraub and Schimel, 2005; Wanek et al., 2010; Mooshammer et al., 2012). The quantification of FAA production and FAA mineralization is until now poorly investigated and is one of the major knowledge gaps in soil N cycle (Gärdenäs et al., 2011). Methodologies using ^{14}C to study FAA turnover have revealed that the transformation of N from proteins to ammonium

was much slower than from amino acid to ammonium, which suggest that the depolymerization rate is the main important constraining factor of N availability in forest ecosystems (Jones and Kielland, 2002; 2012). Carbon (C) to N ratio of amino acids is not a good predictor of FAA mineralization rates (Roberts et al., 2009; Rothstein, 2010), because microbial assimilation of FAAs differs between small C-poor and large C-rich amino acids (Knowles et al., 2010; Mooshammer et al., 2014). Recent developments of a ^{15}N-AA pool dilution assay (Wanek et al., 2010; Wild et al., 2013) now enables us to study simultaneously gross FAA production rates (depolymerization rates), gross FAA mineralization and gross N mineralization rates.

For NW Europe (including the Netherlands) it is expected that the future climate will be characterized by longer dry periods during summer and 1 to 2 °C warmer air temperatures (IPCC 2013). Changes in soil N dynamics occurring in response to these conditions diverge for the two factors warming and drought. At experiments using field scale future climate change scenarios, net production of IN increased in response to warming (Emmett et al., 2004; Andresen et al., 2010; Bai et al., 2013). Furthermore, results studying *Calluna* litter mineralization rates suggested a positive correlation of IN production with temperature and moisture (van Meeteren et al., 2007). Likewise, in response to warming, gross mineralization rate increased in a *Calluna* - *Deschampsia* dominated heathland (Björsne et al., 2014). Contrastingly, drought events decreased gross mineralization rates (Björsne et al., 2014) and net mineralization rates (Emmett et al., 2004; Andresen et al., 2010). However, in the event of re-wetting following drought stress, gross N mineralization may rapidly increase to compensate the drought response (Pulleman and Tietema 1999; Chen et al., 2011). Overall, effects from changes in microclimate may increase (warming treatment) or decrease (drought treatment) enzymatic activity (Sardans et al., 2008; Vranova et al., 2013).

By experimental manipulation of rainfall and temperature at heathlands and shrublands across Europe, the field site 'Oldebroek' took part in investigating the research question: 'Are heathlands vulnerable or resilient to climate change'. The effect of drought during growing season and passive night-time warming was followed since 1999 (van Meeteren et al., 2007; Kopittke et al., 2012). The present study aimed to investigate gross N dynamics in the heathland soil, especially, FAA mineralization, total N mineralization, and FAA production, and how it is affected by climate change. We hypothesized that: i) drought would decrease gross rates and ii) warming would increase gross rates of all investigated N transformations.

2. Methods and Calculations

2.1 Field site

The study was conducted at the experimental site Oldebroek (52°24'N 5°55'E), which is part of the Oldebroekse heide, a large native heathland c. 25 m above sea level. The vegetation is dominated by the evergreen shrub *Calluna vulgaris*, the grass *Molinia caerulea* and mosses (mainly *Hypnum cupressiforme*). The *Calluna v.* plants were 28 year old (in 2012), which is at the end of their lifespan (Gimmingham, 1972). The soil is a Haplic Podzol and the parent material cover sand, a fluvioglacial deposit from the Saalien. Soil pH is 4.3. Nitrogen deposition is 23 kg N ha^{-1} yr^{-1} and N leaching is 29 kg N ha^{-1} yr^{-1}. Annual rainfall was 1072 mm and the annual average temperature 10.1˚C (Kopittke et al., 2012).

Climate change manipulations are conducted since 1999, including: 1) continuous passive night time warming by automated curtains and 2) sequential growing season drought by precipitation removal (Beier et al., 2004). Each plot (three per treatment) is 5 × 4 m with a 0.5 m buffer strip at the margin. Light galvanized steel tube scaffold structures were constructed over all the plots. The warming curtains are IR reflecting and they are pulled over the plots after sunset when light intensity is lower than 200 lux, and removed at sunrise when the light

intensity increased and also during night rain-events. During heavy winds (wind speeds over 10 m s^{-1}) or frost and snowfall the curtains were not active. Throughout the 14 years, the warming treatments increased the top soil temperature by 0.5 to 1.5 °C. The drought treatment was applied each year in early growing season (April to July). Precipitation was excluded for two to three months which reduced precipitation by a PVC curtain that was automatically drawn over the vegetation during rain events. Throughout the 14 years, the treatment reduced precipitation by 6-29% annually (Kopittke et al., 2012). The recent drought period in 2013 started on April 15 and ended the day before soil sampling (June 22). Rainfall was recorded in all plots by funnels (75 mm diameter at 1 m height). Soil moisture and soil temperature (at a soil depth of 4-7 cm) was recorded in each plot by Decagon sensors. Soil moisture index was calculated for each probe relative to an average obtained from the wettest month (December 2012), where no drought treatment was active and all soils were water saturated.

2.2 Soil sampling and soil handling

Soil sampling was conducted on the 23rd of June 2013. Vegetation and loose litter were pushed gently aside. From three locations within each plot, three soil cores were sampled with a corer of 4.5 cm diameter to a depth of 5 cm. The 9 soil cores from each plot were mixed to a composite sample and stored until further processing within 48 hours. Roots were discarded and the remaining soil homogenized by hand. Gravimetric soil moisture content was determined by drying 10 g soil for 24 hours at 100 °C. Because of the extreme dryness of the drought soil (partly hydrophobic) the drought soils were adjusted to the same water content as the control plots, 12 hours before the isotope labels were amended. This enabled homogenous mixing of the isotope label solution with the soil. Soil organic matter was determined on 2 g of dried soil samples by loss of ignition (4 hours at 500° C). Total N and C was determined with an elemental analyzer (ANCA SerCon, Crew, UK).

2.3 ^{15}N pool dilution method

The set-up consisted of two isotope (^{15}N) dilution experiments conducted with the 'mirror approach' (Barraclough, 1997; Rütting et al., 2011; Figure 1). FAA production rate and FAA mineralization rate were determined by adding a ^{15}N labelled amino acid mixture (^{15}N-AA mix; 'Cell Free' amino acid mix (20 AA) U-^{15}N 96-98%, Cambridge Isotope laboratories, USA) and gross N mineralization rate was determined by adding ^{15}N labelled $(^{15}NH_4)_2SO_4$ (98% ^{15}N). Both isotope experiments received both N moieties, in which one was labelled, the other unlabelled. In total 9.1 µg AA-N g^{-1}dry soil and 60.4 µg NH_4-N g^{-1}dry soil was added. For the ^{15}N-AA labelling experiment 4 mL of the label solution was added to 40 g wet soil and stirred with a clean glass rod. Immediately after labelling the soil was evenly divided in two bottles for two parallel extractions after incubation. For the ^{15}N-NH_4 labelling experiment, 2 mL of the label solution was added to 20 g of this soil. Incubation took place at room temperature (18-20˚C).

The soil was extracted after 10 min, 30 min and 7 hours of incubation. A sub-sample of the ^{15}N-AA labelled soil was extracted with 10 mM $CaSO_4$ containing 3.4% formaldehyde to stop the microbial activity. The subsample was hand-shaken, sonicated by ultra sound (20 mW cm^{-3} by Elma S 100 H) during 30 sec, and then shaken for 30 min at 100 rpm and centrifuged for 10 min at 3500 rpm. Finally the supernatant was filtrated (0.45 µm). Samples for ^{15}N-AA analysis were purified using cation-exchange cartridges (OnGuard II H, 1 cc, Dionex), conditioned with ultrapure water (> 18.2 MΩ), 3M NH_3 and 1M HCl. After loading the extract on the cation-exchange resin, the cartridge was washed with 10 mL of water and amino acids were eluted with 30 mL 3 M NH_3. The purified sample was dried under reduced pressure at 35°C, and finally derivatized using ethanol-pyridine and ethylchloroformate (Wanek et al., 2010). The other sub-sample (20 g wet soil) of the ^{15}N-AA label, as well as

the $^{15}NH_4^+$ labelled soil were extracted with 40 mL 1M KCl, then shaken for 30 min at 100 rpm, centrifuged for 10 min at 3500 rpm and finally filtrated.

2.4 ^{15}N Amino acid analysis

The internal standard added to samples during purification was a mixture of two non-biological amino acids: nor-valine and nor-leucine. The method described by Wanek et al., 2010 was developed further for our instrumentation at ISOFYS, Ghent University. Concentration and ^{15}N enrichment were determined using gas chromatography - mass spectrometry (GC - MS, Trace GC - DSQ, Thermo Fisher). Separation was done on a VF 5-MS column (30m x 0.25mm ID x 0.25µm film). We focused on five detectable amino acids (alanine mz: 116/117, valine mz: 144/145, leucine mz: 158/159, isoleucine mz: 158/159, and proline mz: 142/143).

2.5 ^{15}N-NH_4 determination

The ^{15}N enrichment of NH_4^+ in the KCl soil extracts was determined, using an ANCATGII Automated Nitrogen Carbon (Trace Gas) Analyzer (PDZ Europa, UK) coupled to a 20-20 Isotope Ratio Mass Spectrometer (IRMS; SerCon, UK), after conversion to nitrous oxide (Hauck, 1982; Saghir et al., 1993). For this ammonia (NH_3) was liberated from the sample extracts by adding magnesium oxide (MgO), and absorbed by an acid solution. Nitrous oxide is produced by reaction with sodium hypobromite (NaOBr).

2.6 Data analysis and calculations

Gross mineralization and gross FAA production rates (for each individual AA) were estimated by using time steps 10 min and 7 h or 10 and 30 min, respectively, using analytical equations (Kirkham and Bartholomew, 1954).

$$m = \frac{N_t - N_0}{t} * \frac{\ln(a'_0/a'_t)}{\ln(N_t/N_0)} \quad [\mu g\ N\ g^{-1}\ day^{-1}] \qquad (1)$$

For the few cases with (nearly) constant NH_4^+ concentration throughout the incubation time of 7 h, the gross N mineralization was calculated as follows (Kirkham and Bartholomew, 1954):

$$m = \frac{N_{av}}{t} * \ln\left(\frac{a'_0 * N_0}{a'_t * N_t}\right) \quad [\mu g\ N\ g^{-1}\ day^{-1}] \qquad (2)$$

N_0 and N_t are the concentrations of the respective N pool (i.e. NH_4^+ or AA) at time 0 and t, respectively; N_{av} is the average of N_t and N_0.

a'_0 and a'_t are the excess ^{15}N abundances at time 0 and t, respectively. Gross N mineralization had three replicates per treatment analyzed at each time step.

Total FAA production rate was equal to the sum of the individual FAA production rate. Some treatments had only two replicates successfully analyzed at each time step, both numbers are reported in addition to the average. The turnover time (mean residence time) was calculated as N_0 / m.

The fraction of mineralization derived from FAA mineralization (α) from the ^{15}N-AA mixture was obtained by measuring ^{15}N-NH_4^+ production in three replicates per treatment at the time steps 10 min and 7 hours, and was calculated according to Watkins and Barraclough, 1996:

$$\alpha = \frac{a'_t * (N_t / N_0)^{\frac{m}{\theta}} - a'_0}{a'_{aa} * (N_t / N_0)^{\frac{m}{\theta}} - a'_{aa}} \qquad (3)$$

Hereafter FAA mineralization was calculated as

$$m_{AA} = \alpha * m\ [\mu g\ N\ g^{-1}\ day^{-1}] \qquad (4)$$

a'_{aa} is the excess ^{15}N abundance of AA calculated for the total AA pool, averaged for the two time steps;

a'_0 and a'_t are the excess ^{15}N abundances of the NH_4^+ pool at time 0 and t, respectively

$\theta = (N_t - N_0) / t$, where N refers to NH_4^+ concentration.

m is the gross NH_4^+ production (gross mineralization) calculated from equation 1 or 2

213

214 Statistical analysis was conducted using SigmaPlot 11; t-test, by comparing drought (D) or
215 warming (T) to control (C).

216 3. Results

217 3.1 Climate and soil properties

218 The efficiency of the climatic treatments varied between years since 1999 when the
219 manipulations started. The drought treatment imposed via precipitation reduction was within
220 6-29 % of annual precipitation from 1999 till 2011 (Kopittke et al., 2012). The precipitation
221 exclusion in 2013 (from April 15 - June 28) prior to soil sampling reduced the annual
222 accumulated precipitation till June 28 by 43% (Figure 2a). Furthermore, soil moisture index
223 (average volumetric moisture content at 4-7 soil depth, relative to the wet month December
224 2012), decreased most during early summer in drought treatment (Figure 2b). The gravimetric
225 soil moisture of the sampled soil was significantly reduced in the drought treatment ($P = 0.007$; Table 1). Soil temperature in the top layer (0-7 cm) was enhanced during the 14 years
226
227 by 0.5 °C in the warming treatment compared to control (Figure 2c).

228 Total soil N was decreased by the drought treatment ($P = 0.012$; Table 1) and soil organic
229 matter content tended to be reduced by drought, while both factors were unaffected by
230 warming (Table 1). The soil C content and C to N ratio was unaffected by climatic treatments
231 (Table 1). The initial soil concentration of the sum of the five considered AAs was 0.0024 ±
232 0.0006 µg N g^{-1} and total AA and individual AA concentrations were not significantly
233 affected by climate manipulation (Figure 3).

234 3.2 N transformations

235 In control soil, gross FAA production was 76.2 µg AA-N g^{-1} day^{-1}, which was ca. eight-fold
236 larger than the gross N mineralization rate of 10.0 µg N g^{-1} day^{-1} (Table 2). FAA

mineralization (NH_4-N production rate directly from FAAs) was 3.4 µg N g^{-1} day^{-1} in control, representing 34% of the total gross N mineralization (Table 2). In drought treatment FAA mineralization was reduced (P = 0.006; Table 2), and gross FAA production declined with drought and warming (Table 2) though, due to limited amount of replicates this could not be tested statistically. AA turnover time ranged between 1 h (valine, C) and 32 h (leucine, T), while turnover time for NH_4^+ was 4.3 days in control (Table 2).

4. Discussion

In heathland ecosystems, FAAs are only sparsely studied, but previous research suggested a wide concentration range in soil of 0.02 to 36 µg N g^{-1} (Abuarghub and Read, 1988a; 1988b; Kielland, 1995; Finzi and Berthrong, 2005; Andresen et al., 2008; 2011). These studies showed that the FAA-N pool was in general smaller than the NH_4^+-N pool. The turnover time of FAAs in our study indicate a longer residence time compared with results from ^{15}N labelling studies in forest litter and agricultural soils (0.5 to 1.5 h in Wanek et al., 2010; 3.5 h in Geisseler et al., 2012). Moreover, studies using ^{14}C methodologies suggest a maximum turnover of 12 h (Jones et al., 2009; Farrell et al., 2014; Wilkinson et al., 2014).

The gross FAA production rates quantified in the current study can be considered as an indicator for total depolymerization, as the rates are only based on five AAs. Nevertheless, total FAA production rate was ca. 8 times larger than gross N mineralization (control plots), which is in line with observations by Wanek et al., (2010) and Wild et al., (2013).

FAA mineralization, which was calculated based on the addition of 20 ^{15}N-labelled AAs, was an important component of gross N mineralization. However, due to the addition of a large amount of AA-N, the FAA mineralization rates are potentially overestimated. Nevertheless, the contribution of FAA mineralization to total gross N mineralization of 18-41% was smaller than what has been found in other ‘mirror ^{15}N experiments’ (39-100%;

261 Table 3) in agricultural systems. This indicates more complex mineralization dynamics in
262 semi-natural ecosystems.

263 Warming had, unlike our hypotheses, no significant effect on any of the measured N rates,
264 which may also be related to the warming of 0.5 °C, which was possibly too low to have a
265 significant impact, even though it was conducted over 14 years. In contrast, at a Danish
266 heathland, using the same technique of passive warming and precipitation removal, drought
267 evidently reduced and warming increased gross N mineralization and nitrification rates
268 (Larsen et al., 2011; Björsne et al., 2014). The weak responses of rates in the present study
269 were obscured by the low number of replicates and the observed large variability. Moreover,
270 for the drought treatment a rewetting of the soil was necessary prior to ^{15}N label addition.
271 Consequently, the findings for that treatment reflect the effect at the moment of re-wetting
272 after severe drought rather than a direct drought effect.

273 The relative contribution from FAA mineralization to gross N mineralization rate was
274 differently affected by drought and warming treatments. While under drought FAA
275 mineralization became relatively less important for total N mineralization, its importance was
276 unchanged by warming. This implies that the various proteolytic enzymes involved in N
277 mineralization are inhibited in the drought affected soil, and as the observed gross
278 mineralization was not affected, other sources for ammonium became relatively more
279 important than the amino acids. This together with the fact that drought treated soils had
280 markedly smaller amount of total soil N percentage, reflected the many years of soil
281 disturbance by severe droughts (Sowerby et al., 2008), combined with smaller organic matter
282 input to the soils from the drought-inhibited vegetation. Down-regulation of N dynamics in
283 drought treated ecosystems can be a temporary phenomenon, which is alleviated by peak rain
284 events (Pulleman and Tietema, 1999; Chen et al., 2011) or by simultaneous warming (Björsne

et al., 2014). However, changes in the N availability, as observed in this study, is a potential realistic effect during droughts, followed by sudden rain events in the main growing season.

Overall, we conclude that N transformation processes in response to drought will shift towards a dominance of inorganic N rather than organic N (*e.g.* FAA) production. We suggest further analysis of seasonal effects on these production rates, and a look into the combination of drought and warming treatments.

Author contributions

A. T. set up and maintained the long term field experiment; L. C. A. carried out the fieldwork together with T. R.; L. C. A. carried out the ^{15}N labelling laboratory experiment; S. B. and L. C. A. set up the GC-MS method under supervision of P. B.; calculations by T. R., P. B. and L. C. A.; L. C. A. wrote the first draft of the paper, while all authors contributed to writing and interpretation of results.

Acknowledgements

Special thanks to Jan Vermeulen and Katja van Nieuland from Isofys and to the Royal Netherlands Army (Koninklijke Landmacht) for access to the field site. Financial support from: Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (Formas) and Strategic Research Area Biodiversity and Ecosystem Services in a Changing Climate (BECC). This study was carried out with a Transnational Access grant within the INCREASE research infrastructure (INCREASE FP7-INFRASTRUCTURE-2008-1, Grant Agreement no. 227628).

References

Abuarghub, S. M. and Read, D. J.: The biology of mycorrhiza in the Ericaceae XI. The distribution of nitrogen in soil of a typical upland *Callunetum* with special reference to the 'free' amino acids, New Phytol., 108, 425-431, 1988a.

Abuarghub, S. M. and Read, D. J.: The biology of mycorrhizae in the Ericaceae. XII Quantitative analysis of individual 'free' amino acids in relation to time and depth in the soil profile, New Phytol., 108, 433-441, 1988b.

Aerts, R., Huiszoon, A., van Oostrum, J. H. A., van De Vijver, C. A. D. M. and Willems, J. H.: The Potential for Heathland Restoration on Formerly Arable Land at a Site in Drenthe, J. Appl. Ecol., 32, 827-835, 1995.

Andresen, L. C., Jonasson, S., Ström, L. and Michelsen, A.: Uptake of pulse injected nitrogen by soil microbes and mycorrhizal and non-mycorrhizal plants in a species diverse subarctic heath ecosystem, Plant Soil, 313, 283–295, 2008.

Andresen, L. C. and Michelsen, A.: Off-season uptake of nitrogen in temperate heath vegetation, Oecologia, 144, 585-597, 2005.

Andresen, L. C., Michelsen, A., Jonasson, S., Schmidt, I. K., Mikkelsen, T. N., Ambus, P. and Beier, C.: Plant nutrient mobilization in temperate heathland responds to elevated CO_2, temperature and drought, Plant Soil, 328, 381-396, 2010.

Andresen, L. C., Michelsen, A., Jonasson, S. and Ström, L.: Seasonal changes in nitrogen availability, and root and microbial uptake of $^{15}N^{13}C_9$-phenylalanine and ^{15}N-ammonium *in situ* at a temperate heath, Appl. Soil Ecol., 51, 94-101, 2011.

Bai, E., Li, S., Xu, W., Li, W., Dai, W. and Jiang, P.: A meta-analysis of experimental warming effects on terrestrial nitrogen pools and dynamics, New Phytol., 199, 441-451, 2013.

Barraclough, D.: The direct or MIT route for nitrogen immobilization: a ^{15}N mirror image study with leucine and glycine, Soil Biol. Biochem., 29, 101-108, 1997.

Beier, C., Emmett, B., Gundersen, P., Tietema, A., Peñuelas, J., Estiare, M., Gordon, C., Gorissen, A., Llorens, L., Roda, F. and Williams, D.: Novel approaches to study climate change effects on terrestrial ecosystems in the field: drought and passive night-time warming, Ecosystems, 7, 583-597, 2004.

Björsne, A. K., Rütting, T. and Ambus, P.: Combined climate factors alleviate changes in gross soil nitrogen dynamics in Heathlands, Biogeochemistry, 120, 191-201, 2014.

Clemmensen, K. E., Sorensen, P. L., Michelsen, A., Jonasson, S. and Ström, L.: Site-dependent N uptake from N-form mixtures by arctic plants, soil microbes and ectomycorrhizal fungi, Oecologia, 155, 771-783, 2008.

Chen, Y. T., Bogner, C., Borken, W., Stange, C. F. and Matzner, E.: Minor response of gross N turnover and N leaching to drying, rewetting and irrigation in the topsoil of a Norway spruce forest, Eur. J. Soil Sci., 62, 709-717, 2011.

EUR-Lex Access to European Union law: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31992L0043:EN:NOT last access 13 November 2014

Emmett, B. A., Beier, C., Estiarte, M., Tietema, A., Kristensen, H. L., Williams, D., Penuelas, J., Schmidt, I. K., and Sowerby, A.: The Response of Soil Processes to Climate Change: Results from Manipulation Studies of Shrublands Across an Environmental Gradient, Ecosystems, 7, 625-637, 2004.

Fagundez, J.: Heathlands confronting global change: drivers of biodiversity loss from past to future scenarios, Ann. Bot. London, 111, 151-172, 2013.

Farrell, M., Macdonald, L. M., Hill, P. W., Wanniarachchi, S. D., Farrar, J., Bardgett, R. D. and Jones, D. L.: Amino acid dynamics across a grassland altitudinal gradient, Soil Biol. Biochem., 72, 75-83, 2014.

Finzi, A. C. and Berthrong, S. T.: The uptake of amino acids by microbes and trees in three cold-temperate forests, Ecology, 86, 3345–3533, 2005.

Garcıa, R. R., Fraser, M. D., Celaya, R., Miguel, L., Ferreira, M., Garcıa, U. and Osoro, K.: Grazing land management and biodiversity in the Atlantic European heathlands: a review, Agroforest. Syst., 87, 19-43, 2013.

Geisseler, D., Joergensen, R. G. and Ludwig, B.: Temporal effect of straw addition on amino acid utilization by soil microorganisms, Eur. J. Soil Biol., 53, 107-113, 2012.

Gimmingham, C. H.: Ecology of Heathlands, Chapman & Hall, London, 1972.

Gärdenäs, A. I., Ågren, G. I., Bird, J. A., Clarholm, M., Hallin, S., Ineson, P., Kätterer, T., Knicker, H., Nilsson, S. I., Näsholm, T., Ogle, S., Paustian, K., Persson, T., and Stendahl, J.: Knowledge gaps in soil carbon and nitrogen interactions - from molecular to global scale, Soil Biol. Biochem., 43,702-717, 2011.

Hadas, A., Sofer, M., Molina, J. A. E., Barak, P. and Clapp, C. E.: Assimilation of nitrogen by soil microbial population: NH_4 versus organic N, Soil Biol. Biochem., 24, 137-143, 1992.

Hauck, R. D.: Nitrogen Isotope Ratio Analysis, in: Methods of soil Analysis, 2nd ed part 2. Agron. Monogn. 9 ASA and SSSA, Madison, W I. 1982.

IPCC Intergovernmental Panel on Climate Change Working group I contribution to the IPCC fifth assessment report (AR5), Climate Change 2013: The Physical Science Basis, 2013.

Jones, D. L. and Kielland, K.: Soil amino acid turnover dominates the nitrogen flux in permafrost-dominated taiga forest soil, Soil Biol. Biochem., 34, 209-219, 2002.

Jones, D. L. and Kielland, K.: Amino acid, peptide and protein mineralization dynamics in a taiga forest soil, Soil Biol. Biochem., 55, 60-69, 2012.

Jones, D. L., Kielland, K., Sinclair, F. L., Dahlgren, R. A., Newsham, K. K., Farrar, J. F. and Murphy, D. V.: Soil organic nitrogen mineralization across a global latitudinal gradient, Global Biogeochem. Cy., 23, GB1016, doi:10.1029/2008GB003250, 2009.

Kielland, K.: Landscape patterns of free amino acids in arctic tundra soils. Biogeochemistry, 31, 85-98, 1995.

Knowles, T. D. J., Chadwick, D. R., Bol, R. and Evershed, R. P.: Tracing the rate and extent of N and C flow from ^{13}C,^{15}N-glycine and glutamate into individual de novo synthesized soil amino acids, Org. Geochem. 41, 1259-1268, 2010.

Kirkham, D. and Bartholomew, W. V.: Equations for Following Nutrient Transformations in Soil, Utilizing Tracer Data, Soil Sci. Soc. Am. Pro., Division III Soil Microbiology, 33-34, 1954.

Kopittke, G. R., Tietema, A. and Verstraten, J. M.: Soil acidification occurs under ambient conditions but is retarded by repeated drought, Sci. Total Environ., 439, 332-342, 2012.

Larsen, K. S., Andresen, L. C., Beier, C., Jonasson, S., Albert, K. R., Ambus, P., Andersen, K. S., Arndal, M. F., Carter, M. S., Christensen, S., Holmstrup, M., Ibrom, A., Kongstad, J., van der Linden, L., Maraldo, K., Michelsen, A., Mikkelsen, T. N., Pilegaard, K., Priemé, A., Ro-Poulsen, H., Schmidt, I. K. and Selsted, M. B.: Reduced N cycling in response to elevated CO_2, warming and drought in a Danish heathland: Synthesizing results of the CLIMAITE project after two years of treatments, Glob. Change Biol., 17, 1884-1899, 2011.

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

Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schnecker, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K. M., Zechmeister-Boltenstern, S. and Richter, A.: Adjustment of microbial nitrogen use efficiency to carbon : nitrogen imbalances regulates soil nitrogen cycling, Nature Com., 5, e3694, doi:10.1038/ncomms4694, 2014.

Nordin, A., Schmidt, I. K. and Shaver, G. R.: Nitrogen uptake by arctic soil microbes and plants in relation to soil nitrogen supply, Ecology, 85, 955-962, 2004.

Pulleman, M. and Tietema, A.: Microbial C and N transformations during drying and rewetting of coniferous forest floor material, Soil Biol. Biochem., 31, 275-285, 1999.

Roberts, P., Stockdale, R., Khalid, M., Iqbal, Z. and Jones, D. L.: Carbon-to-nitrogen ratio is a poor predictor of low molecular weight organic nitrogen mineralization in soil, Soil Biol. Biochem., 41, 1750-1752, 2009.

Rothstein, D. E.: Effects of amino-acid chemistry and soil properties on the behavior of free amino acids in acidic forest soils, Soil Biol. Biochem., 42, 1743-1750, 2010.

Rütting, T., Huygens, D., Staelens, J., Müller, C. and Boeckx, P.: Advances in ^{15}N tracing experiments: new labelling and data analysis approaches, Biochem. Soc. T., 39, 279-283, 2011.

Sardans, J., Penuelas, J. and Estiarte, M.: Changes in soil enzymes related to C and N cycle and in soil C and N content under prolonged warming and drought in a Mediterranean shrubland, Appl. Soil Ecol., 39, 223-235, 2008.

Saghir, N. S., Mulvaney, R. L. and Azam, F.: Determination of nitrogen by micro diffusion in mason jars. 1. Inorganic nitrogen in soil extracts, Commun. Soil Sci. Plant Anal., 24, 1745-1762, 1993.

Schimel, J. P. and Bennett, J.: Nitrogen mineralization: challenges of a changing paradigm, Ecology, 85, 591-602, 2004.

Stange, F. and Döhling, F.: ^{15}N tracing model SimKIM to analyse the NO and N_2O production during autotrophic, heterotrophic nitrification, and denitrification in soils, Isoto. Environ. Health S., 41, 261-274, 2005.

Sowerby, A., Emmett, B. A., Tietema, A. and Beier, C.: Contrasting effects of repeated summer drought on soil carbon efflux in hydric and mesic heathland soils, Glob. Change Biol., 14, 2388-2404, 2008.

Wanek, W., Mooshammer, M., Blöchl, A., Hanreich, A. and Richter, A.: Determination of gross rates of amino acid production and immobilization in decomposing leaf litter by a novel ^{15}N isotope pool dilution technique, Soil Biol. Biochem., 42, 1293-1302, 2010.

Watkins, N. and Barraclough, D.: Gross rates of N mineralization associated with the decomposition of plant residues, Soil Biol. Biochem. 28, 169-175, 1996.

Weintraub, M. N. and Schimel, J. P.: Seasonal protein dynamics in Alaskan arctic tundra soils Soil Biol. Biochem., 37, 1469-1475, 2005.

Wild, B., Schnecker, J., Bárta, J., Capek, P., Guggenberger, G., Hofhansl, F., Kaiser, C., Lashchinsky, N., Mikutta, R., Mooshammer M., Santrucková, H., Shibistova, O., Urich, T., Zimov, S. A. and Richter, A.: Nitrogen dynamics in Turbic Cryosols from Siberia and Greenland, Soil Biol. Biochem., 67, 85-93, 2013.

Wilkinson, A., Hill, P. W., Farrar, J. F., Jones, D. L. and Bardgett, R. D.: Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient, Soil Biol. Biochem., 72, 75-83, 2014.

Webb, N. R.: The traditional management of European heathlands, J. Appl. Ecol., 35, 987-990, 1998.

van Meeteren, M. J. M., Tietema, A. and Westerveld, J. W.: Regulation of microbial carbon, nitrogen, and phosphorus transformations by temperature and moisture during decomposition of *Calluna vulgaris* litter, Biol. Fert. Soils, 44, 103-112, 2007.

Vranova, V., Rejsek, K. and Formanek, P.: Proteolytic activity in soil: A review, Appl. Soil Ecol., 70, 23-32, 2013.

Table 1: Soil properties. Total soil nitrogen (N) and Carbon (C), soil organic matter (SOM) and gravimetric soil water content (GWC) in percentage of dry weight (%). Significant effect ($P < 0.05$) of treatment is indicated by asterisk (*) whereas ns is non-significant.

	Control	**Drought**	**Warming**	**stat.**
Total N (%)	0.38 ± 0.05	0.21 ± 0.03	0.43 ± 0.04	D: *; T: ns
SOM (%)	12.4 ± 2.8	7.6 ± 2.2	12.7 ± 2.1	D: ns; T: ns
Total C (%)	6.0 ± 1.0	3.9 ± 1.1	6.8 ± 1.0	D: ns; T: ns
C/N	19.3 ± 0.5	19.7 ± 0.6	19.2 ± 0.2	D: ns; T: ns
GWC (%)	5.4 ± 0.9	1.3 ± 0.4	6.5 ± 0.7	D: *; T: ns

Table 2: Nitrogen transformation rates and turnover times. Gross mineralization rate (*Eq. 1* & *2*), free amino acid (FAA) mineralization (*Eq. 4*), represented by average ± standard error. FAA production rate is the sum of the five AA, the two measurement points in square brackets, NH_4^+ turnover time (days) and turnover time of amino acids (hours). Significant effect ($P < 0.01$) of treatment is indicated by asterisk (**), ns. is non-significant, and nd. is non-determined.

	Control	**Drought**	**Warming**	**stat.**
FAA production rate	76.2 [127.4; 25.0]	27.0 [17.3; 36.6]	43.4 [34.6; 52.1]	nd.
(µg N g^{-1} day^{-1})				
Gross mineralization rate	10.0 ± 2.9	11.2 ± 1.6	9.3 ± 4.1	T: ns; D: ns
(µg N g^{-1} day^{-1})				
FAA mineralization	3.4 ± 0.2	2.0 ± 0.3	3.8 ± 0.2	D:**; T: ns
(µg N g^{-1} day^{-1})				
Turnover time NH_4	4.3 ± 2.2	3.2 ± 0.5	5.2 ± 3.1	T: ns; D: ns
(days)				
Turnover time FAA	2.9	11.5	7.2	nd.
(hours)				

Table 3: Mirror ^{15}N labelling experiments in literature recording proportion of gross N mineralization directly from amino acids. Reference to paper, ecosystem type, soil type, pH (all in H_2O), soil total C and ***N***, type of amino acid label (^{15}N-enriched) in the experiment, incubation time, free amino acid mineralization rate, gross mineralization rate and proportion (α) of N mineralization from free amino acids.

Reference	**Ecosystem**	**Soil (pH)**	**soil C and *N* (%)**	**^{15}N labelled amino acid**	**t (hour)**	**FAA min. rate (μg N g^{-1} h^{-1})**	**gross min. rate (μg N g^{-1} h^{-1})**	**α (%)**
Barraclough 1997	Agri. (wheat)	Sandy loam (6.0)	1.05 ***0.07***	Leucine	6	1.61	2.28	71
Barraclough 1997	Agri. (wheat)	Sandy loam (6.0)	1.05 ***0.07***	Glycine	6	6.24	8.94	70
Hadas et al.1992	Agri.	Chromoexert (7.8)	0.82 ***0.08***	Alanine	7	4.93	5.59	88
Hadas et al.1992	Agri.	Camborthid (8.1)	1.31 ***0.12***	Alanine	7	7.59	10.08	75
Stange& Döhling 2005	Agri.	Haplic Phaeozem	2.1 ***1.7***	Glycine	6	3.50	5.10	69
Geisseler et al. 2012	Pasture	Anthrosol (7.2)	1.44 ***0.15***	Gly&Leu	168	0.62	1.60	39
Geisseler et al. 2012	Pasture	Cambisol (8.0)	1.13 ***0.11***	Gly&Leu	168	0.42	0.90	47
Current study	Heathland	Haplic Podzol (3.9)	6.0 ***0.31***	AA mix	7	0.140	0.420	34

Figure Captions

Figure 1: Concept model of investigated N transformations in heathland soil by ^{15}N tracer techniques; 1) free amino acid (FAA) production (^{15}N-AA pool dilution), 2) FAA mineralization (^{15}N-NH^{+}_{4} production from ^{15}N-AA, ^{15}N-tracing), 3) mineralization from other soil organic matter (not measured directly), 4) gross N mineralization (^{15}N- NH^{+}_{4} pool dilution).

Figure 2: Climatic data for the treated plots control (black), drought (blue) and warming (red) **A**. precipitation (accumulated mm rainfall for the given month) **B**: soil moisture (index) for 0 to 5 cm depth; and **C**: temperature in the soil for 0 to 5 cm depth. This year drought treatment started April 15 and ended June 22 2013. Soil was sampled from all plots on June 23.

Figure 3: Initial amino acid concentrations for: a) alanine, b) valine, c) leucine, d) isoleucine and e) proline; in a heathland soil exposed to the climatic manipulations T = warming treatment, D = drought treatment and C = control.

Figure 1

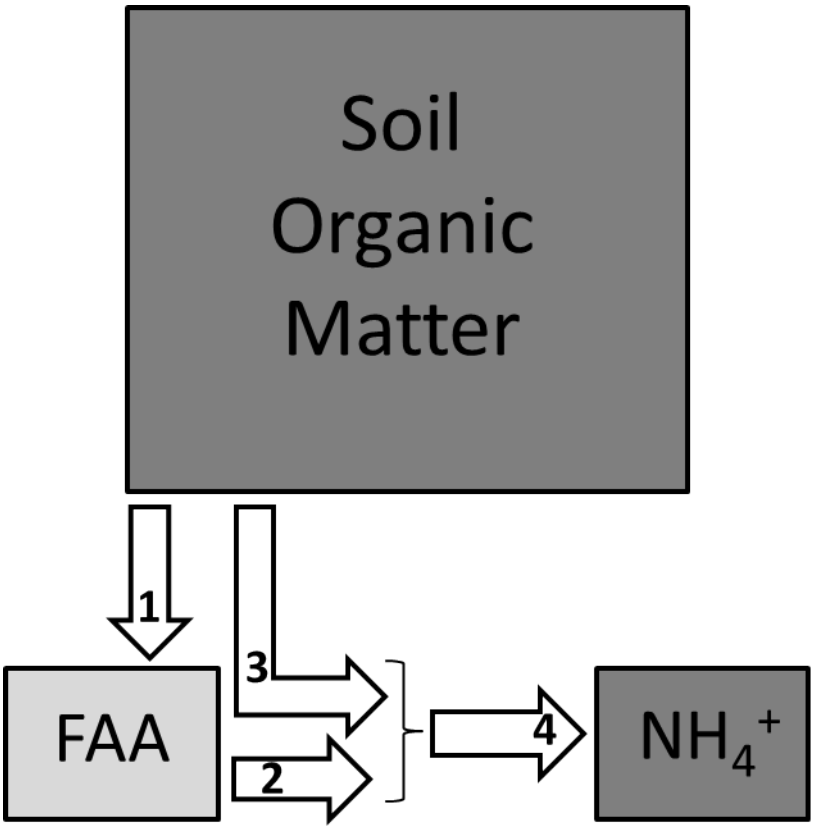
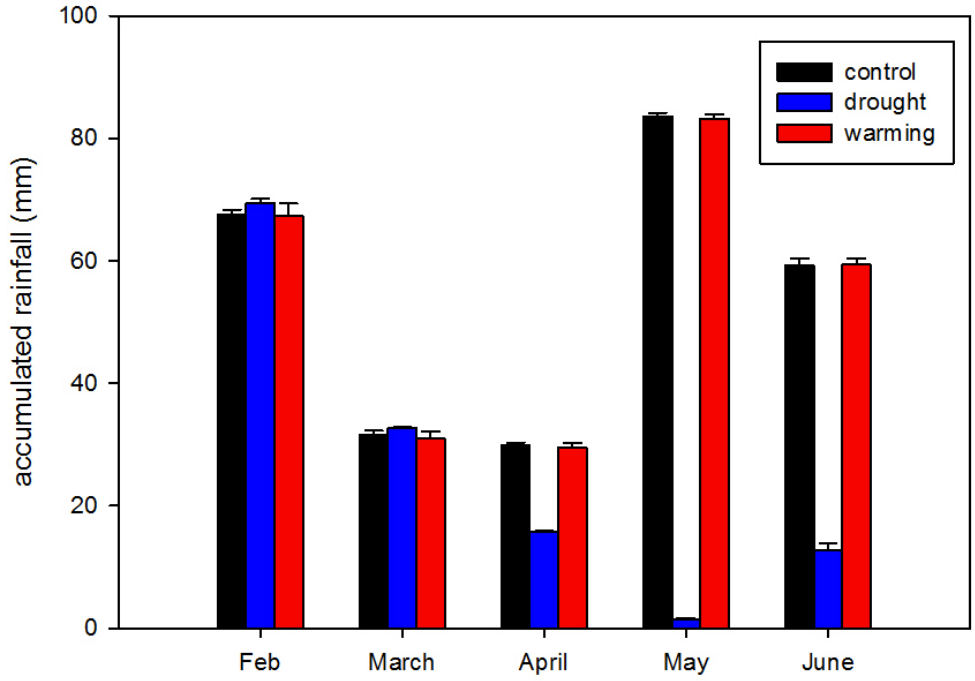
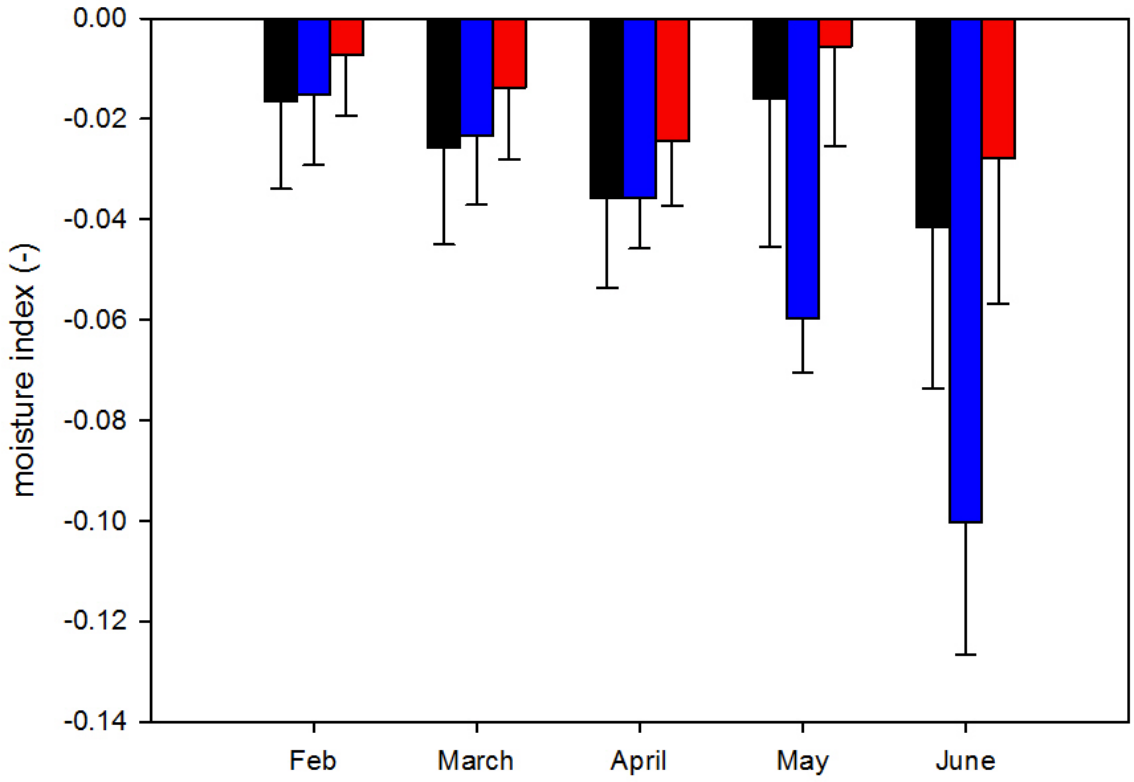


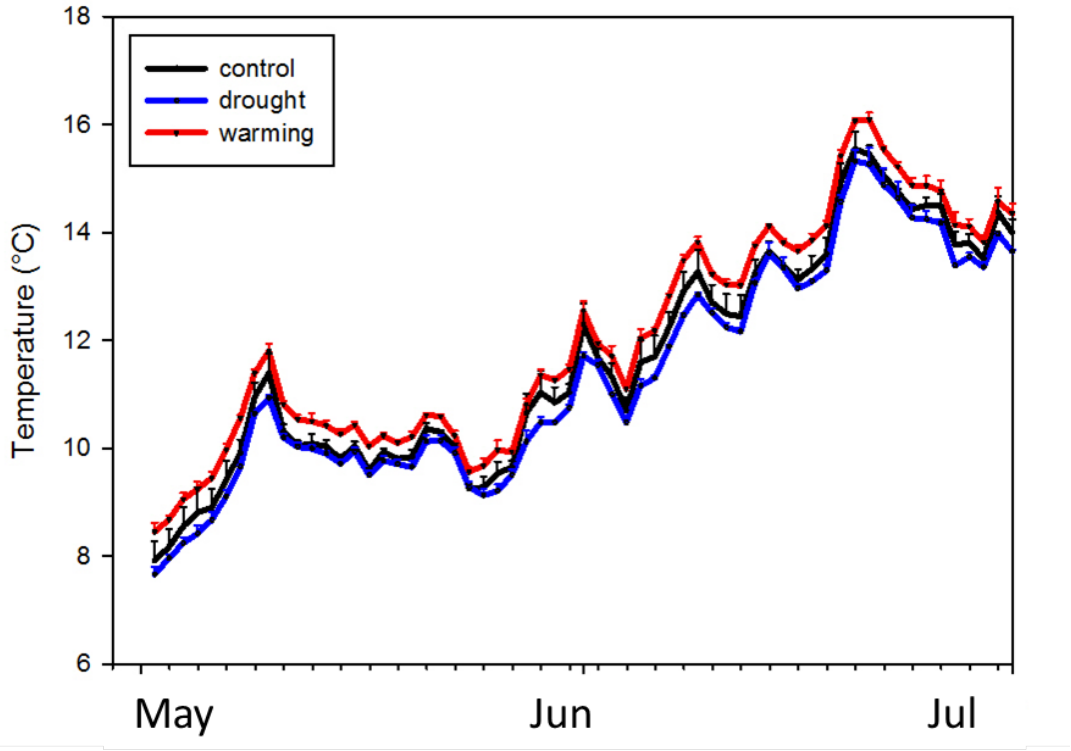
Figure 2


100
80
60
40
20
0
accumulated rainfall (mm)
control
drought
warming
Feb
March
April
May
June

A

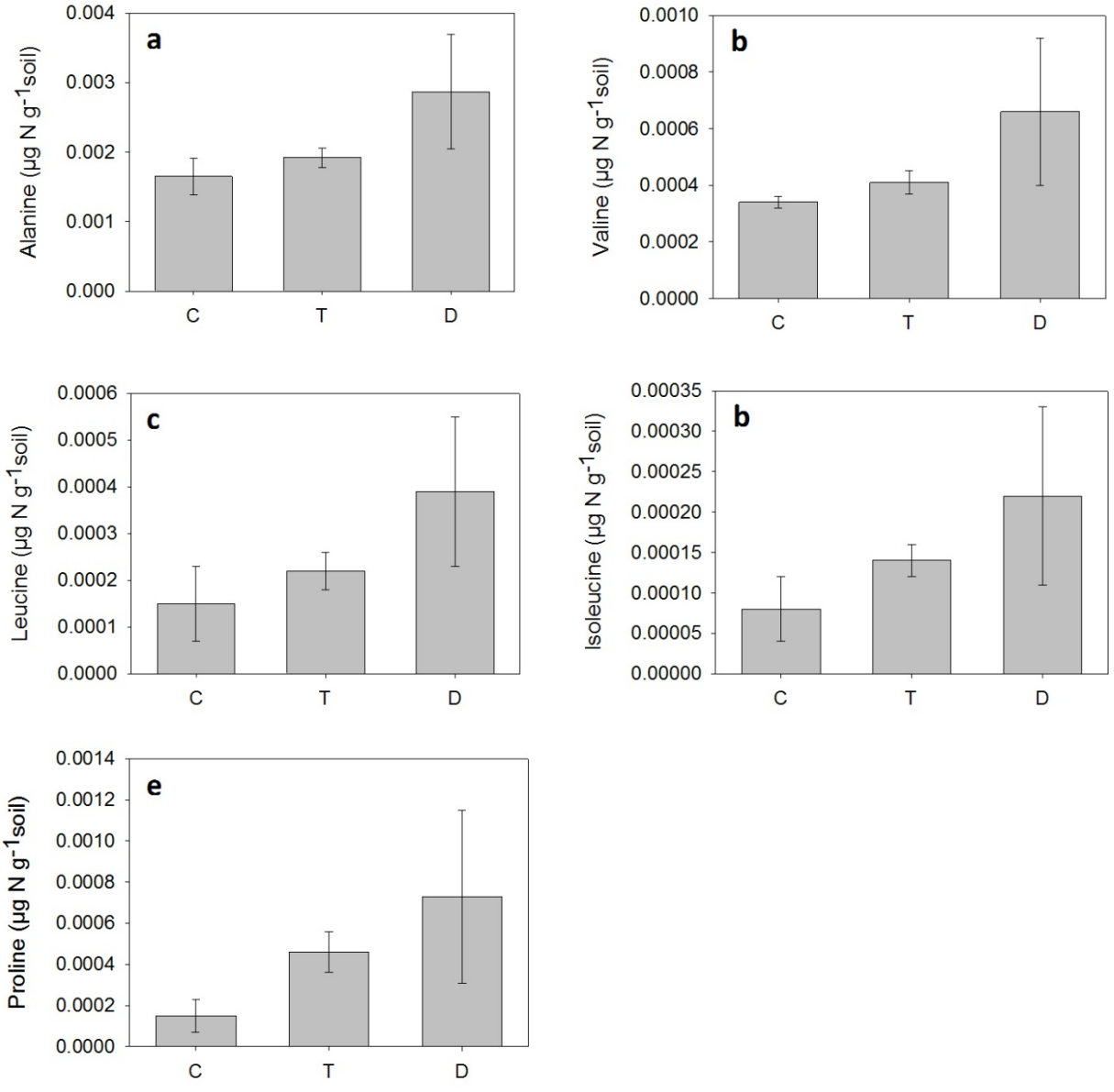

0.00
-0.02
-0.04
-0.06
-0.08
-0.10
-0.12
-0.14
moisture index (-)
Feb
March
April
May
June

B


control
drought
warming
Temperature (°C)
18
16
14
12
10
8
6
May
Jun
Jul

C

Figure 3


a
Alanine (µg N g-1soil)
0.004
0.003
0.002
0.001
0.000
C
T
D
b
Valine (µg N g-1soil)
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
C
T
D
c
Leucine (µg N g-1soil)
0.0006
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
C
T
D
b
Isoleucine (µg N g-1soil)
0.00035
0.00030
0.00025
0.00020
0.00015
0.00010
0.00005
0.00000
C
T
D
e
Proline (µg N g-1soil)
0.0014
0.0012
0.0010
0.0008
0.0006
0.0004
0.0002
0.0000
C
T
D