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

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Abstract

Monomeric organic nitrogen (N) such as free amino acids (fAA) is an important resource for both plants and soil microorganisms and is, furthermore, 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 ^{15}N labelling. 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 warming and drought on these rates.

The turnover of fAA in the soil was ca. 3 h, 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 ($10 \pm 2.9 \mu\text{g N g}^{-1} \text{ day}^{-1}$) was eight-times smaller than the summed gross fAA production rate of five AAs (alanine, valine, leucine, isoleucine, proline: 127.4 to $25.0 \mu\text{g N g}^{-1} \text{ day}^{-1}$). Gross fAA mineralization ($3.4 \pm 0.2 \mu\text{g N g}^{-1} \text{ day}^{-1}$) contributed by 34 % to the gross N mineralization rate and is, thus, an important component of N mineralization. In the drought treatment, gross fAA production was reduced by 65 % and 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. Warming did not significantly affect N transformations, even though that gross fAA production was more than halved.

Overall our results suggest that heathland soil exposed to droughts has a shift in the composition of the SON being mineralized. Furthermore, compared to agricultural soils, fAA mineralization was relatively less important in the investigated heathland. This indicates a more complex mineralization dynamics in semi-natural ecosystems.

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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 plant available N and effects from global change factors threaten this ecosystem type.

Ammonium (NH_4^+) is produced during mineralization of organic N. Two alternative pathways for N mineralization have been discussed, “direct route” and mineralization-immobilization turnover: MIT (Barracough, 1997). In the first, organic N such as fAA is taken up by microorganism followed by excretion of NH_4^+ from excess N not needed for microbial assimilation. In contrast, MIT refers to the mineralization of the organic N by exo-enzymes, followed by microbial immobilization of released NH_4^+ (Barracough, 1997). Direct microbial uptake of intact fAAs was evident in a Danish heathland from dual labelled fAA tracing (^{13}C and ^{15}N ; Andresen et al., 2009, 2011), suggesting that the fAA mineralization took place inside bacterial cells (direct route). However, this does not rule out the possibility of a simultaneous NH_4^+ production via extracellular fAAs mineralization (MIT). For instance, a gradual change from direct route dominance to MIT was observed during wheat residue decomposition (Barracough, 1997; Giesseler et

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al., 2009). Gross mineralization is depending on the availability of fAAs and, hence, on the fAA production rate. 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). 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 -fAA pool dilution assay (Wanek et al., 2010; Wild et al., 2013) now enables us to study simultaneously gross fAA production rate (depolymerization rates), gross fAA mineralization and gross N mineralization rate. Thereby, investigating the relative importance of direct mineralization versus MIT is now possible by focussing on the fAA nitrogen fluxes.

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). 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 net 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 (Björnsne et al., 2014). Contrastingly, drought events decreased gross mineralization rates (Björnsne 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 mineralisation 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 shrub-lands 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 hypothesised 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 ca. 25 m a.s.l. 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 (Gimingham, 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 (Kopittke et al., 2012). Annual rainfall was 1072 mm and the annual average temperature 10.1 °C.

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

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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 is applied each year in early growing season (April to July). Precipitation is excluded for two to three months which reduced precipitation by a PVC curtain that is 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 15 April and ended the day before soil sampling (22 June). Rainfall is 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) is 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 23 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 depth. The 9 soil cores from each plot were mixed to a composite sample and stored until further processing within 48 h. Roots were discarded and the remaining soil homogenized by hand. Gravimetric soil moisture content was determined by drying 10 g soil for 24 h 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 h before the isotope labels were amended. This enabled homogenous mixing of the isotope label solution with the soil. Soil organic matter was determined by glowing 2 g of dried soil for 4 h at 500°C . Total N and C was determined with an elemental analyser (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’ (Barracough, 1997; Rütting et al., 2011; Fig. 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 ($^{15}\text{NH}_4$) $_2\text{SO}_4$ (98 % ^{15}N). Both isotope experiments received both N moieties, in which one was labelled, the other unlabelled. In total $9.1 \mu\text{g AA-N g}^{-1}$ dry soil and $60.4 \mu\text{g NH}_4\text{-N g}^{-1}$ 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 or 7 h 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 ultrasound during 30 s, 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 M Ω), 3 M NH_3 and 1 M 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 $^{15}\text{NH}_4^+$ labelled soil were extracted with 40 mL 1 M KCl, then shaken for 30 min at 100 rpm, centrifuged for 10 min at 3500 rpm and finally filtrated.

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2.4 ¹⁵N 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 ¹⁵N enrichment were determined using gas chromatography – mass spectrometry (GC – MS, Trace GC – DSQ, Thermo Fisher). Separation was done on a VF 5-MS 30 m × 0.25 mm ID × 0.25 μm film. We focussed on five detectable amino acids (alanine *m/z*: 116/117, valine *m/z*: 144/145, leucine *m/z*: 158/159, isoleucine *m/z*: 158/159, and proline *m/z*: 142/143).

2.5 ¹⁵N-NH₄ determination

The ¹⁵N enrichment of NH₄⁺ in the KCl soil extracts was determined, using an ANCAT-GII 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 (NH₃) 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 rate (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).

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

For the few cases with (nearly) constant NH₄⁺ concentration throughout the incubation time of 7 h, the gross N mineralization was calculated as follows (Kirkham and

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Bartholomew, 1954):

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

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.

Total fAA production rate was equal to the sum of the individual fAA production rate. Each treatment had 2 replicates 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 at the time steps 10 min and 7 h, and was calculated according to Watkins and Barraclough (1996):

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

Hereafter fAA mineralization was calculated as

$$m_{AA} = \alpha \times m [\mu\text{g N g}^{-1} \text{ day}^{-1}]. \quad (4)$$

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. (5)

m is the gross NH_4^+ production (gross mineralisation) calculated from Eqs. (1) or (2).

Statistical analysis of effect of climatic treatment was conducted using SigmaPlot 11; t test, by comparing drought (D) or warming (T) to control (C).

3 Results

3.1 Climate and soil properties

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

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

3.2 N transformations

In control soil, gross fAA production was $76.2 \mu\text{g AA-N g}^{-1} \text{ day}^{-1}$, which was ca. eight-fold larger than the gross N mineralization rate of $10.0 \mu\text{g N g}^{-1} \text{ day}^{-1}$ (Table 2). fAA mineralization ($\text{NH}_4\text{-N}$ production rate directly from fAAs) was $3.4 \mu\text{g N g}^{-1} \text{ day}^{-1}$ in control, representing 34 % of the total gross N mineralization (Table 2). In the drought treatments only fAA mineralization was significantly reduced ($P = 0.006$; Fig. 3), but also gross fAA production declined at drought and warming (Table 2). AA turnover time

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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). Turnover times were not significantly affected by climate manipulations.

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 $\mu\text{g N g}^{-1}$ (Abuarghub and Read, 1988a, b; Kielland, 1995; Finzi and Berthrong, 2005; Andresen et al., 2008, 2011). These studies furthermore showed that the standing 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 have to be seen as an indicator for total depolymerization, as the rates are based on five AAs only. Nevertheless, total fAA production rate was ca. 8 times larger than gross N mineralization (control plots), which was also observed by Wanek et al. (2010) and Wild et al. (2013). Immobilization of fAAs via a direct uptake pathway by microbes is evident from previous dual-labelling studies at similar *Calluna* heathlands (Andresen et al., 2009, 2011), and is from our study further supported by the high fAA production and mineralization rates, and short AA turnover times. This supports the paradigm that the “direct route” is the main pathway for N mineralization, whereby microorganisms take up fAAs and excrete excess N as NH_4^+ (Barraclough, 1997; Schimel and Bennett, 2004).

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

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by simultaneous warming (Björnsne et al., 2014). However, changes in the N availability for plants, as we have observed, potentially occurring at droughts, followed by sudden rain events during the main growing season, could be part of a negative feedback effect during climate change that threatens the heathland ecosystem functioning and diversity.

Overall, we conclude that N transformation processes during drought events will shift to dominance of inorganic N production, with consequences for the N availability for vegetation at future frequent drought events. We suggest further analysis of seasonal effects on these production rates, and a look into the combination of drought and warming treatments. A resource based N-niche differentiation of co-occurring species would result in a drought induced shift from species relying on free amino acid N uptake to species relying on inorganic N uptake (McKane et al., 2001; Nordin et al., 2004), which may threaten the heathland ecosystem.

Author contributions. A. Tietema set up and maintained the long term field manipulation; L. C. Andresen carried out the fieldwork together with T. Rütting; L. C. Andresen made the ¹⁵N labelling laboratory experiment; S. Bode and L. C. Andresen set up the GC-MS method under supervision of P. Boeckx; calculations by T. Rütting, P. Boeckx and L. C. Andresen; L. C. Andresen wrote the first draft of the paper, while all authors contributed to writing and interpretation of results.

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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	$D: *; T: ns$

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Table 2. Nitrogen transformation rates and turnover times. Gross mineralization rate (Eqs. 1 and 2), free amino acid (fAA) mineralization (Eq. 4), represented by average \pm 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 ($\mu\text{g N g}^{-1} \text{ day}^{-1}$)	76.2 [127.4; 25.0]	27.0 [17.3; 36.6]	43.4 [34.6; 52.1]	nd.
Gross mineralization rate ($\mu\text{g N g}^{-1} \text{ day}^{-1}$)	10.0 ± 2.9	11.2 ± 1.6	9.3 ± 4.1	T : ns; D : ns
fAA mineralization ($\mu\text{g N g}^{-1} \text{ day}^{-1}$)	3.4 ± 0.2	2.0 ± 0.3	3.8 ± 0.2	D :**, T : ns
Turnover time NH_4 (days)	4.3 ± 2.2	3.2 ± 0.5	5.2 ± 3.1	T : ns; D : ns
Turnover time fAA (hours)	2.9	11.5	7.2	nd.

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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 (bold itl.), 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 ($\mu\text{g N g}^{-1} \text{h}^{-1}$)	gross min. rate ($\mu\text{g N g}^{-1} \text{h}^{-1}$)	α (%)
Barracough (1997)	Agri. (wheat)	Sandy loam (6.0)	1.05	0.07 Leucine	6	1.61	2.28	71
Barracough (1997)	Agri. (wheat)	Sandy loam (6.0)	1.05	0.07 Glycine	6	6.24	8.94	70
Hadas et al. (1992)	Agri.	Chromoxert (7.8)	0.82	0.08 Alanine	7	5.59	88	
Hadas et al. (1992)	Agri.	Camborthid (8.1)	1.31	0.12 Alanine	7	7.59	10.08	75
Stange and Döhling (2005)	Agri.	Haplic Phaeozem	2.1	1.7 Glycine	6	3.50	5.10	69
Geisseler et al. (2012)	Pasture, straw	Anthrosol (7.2)	1.44	0.15 Gly&Leu	1 (week)	0.62	1.60	39
Geisseler et al. (2012)	Pasture, straw	Cambisol (8.0)	1.13	0.11 Gly&Leu	1 (week)	0.42	0.90	47
Current study	Heathland	Haplic Podzol (3.9)	6.0	0.31 AA mix	7	0.140	0.420	34

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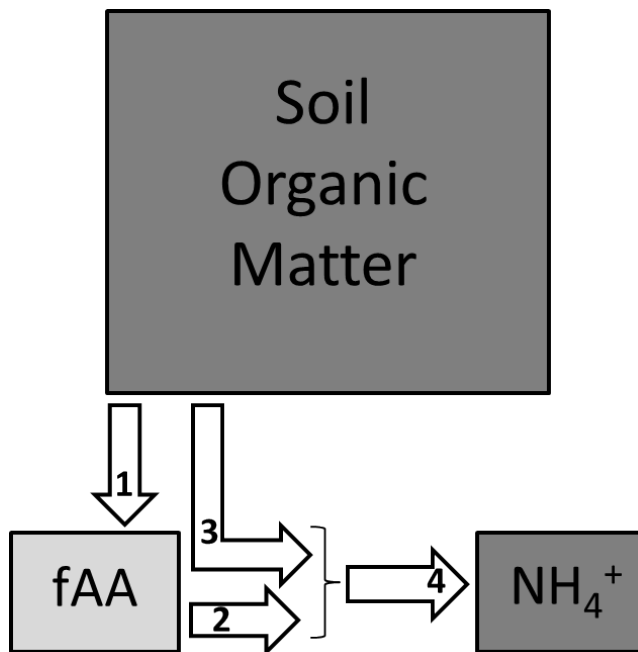


Figure 1. Concept model of investigated N transformations in heathland soil by ^{15}N tracer techniques; (1) free amino acid (fAA) production (^{15}N -AA pool dilution), (2) fAA mineralization (^{15}N - NH_4^+ production from ^{15}N -AA, ^{15}N -tracing), (3) mineralization from other soil organic matter (not measured directly), (4) gross N mineralization (^{15}N - NH_4^+ pool dilution).

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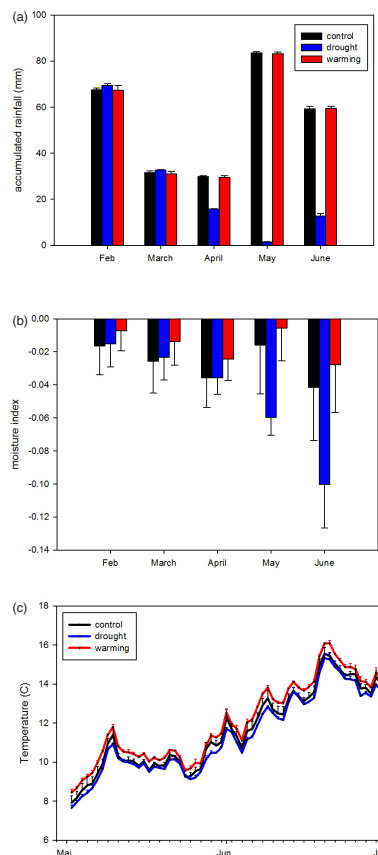


Figure 2. Climatic data for the treated plots control (black), drought (blue) and warming (red) **(a)**. precipitation (accumulated mm rainfall for the given month) **(b)**: soil moisture (index) for 0 to 5 cm depth; and **(c)**: temperature in the soil for 0 to 5 cm depth. This year drought treatment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

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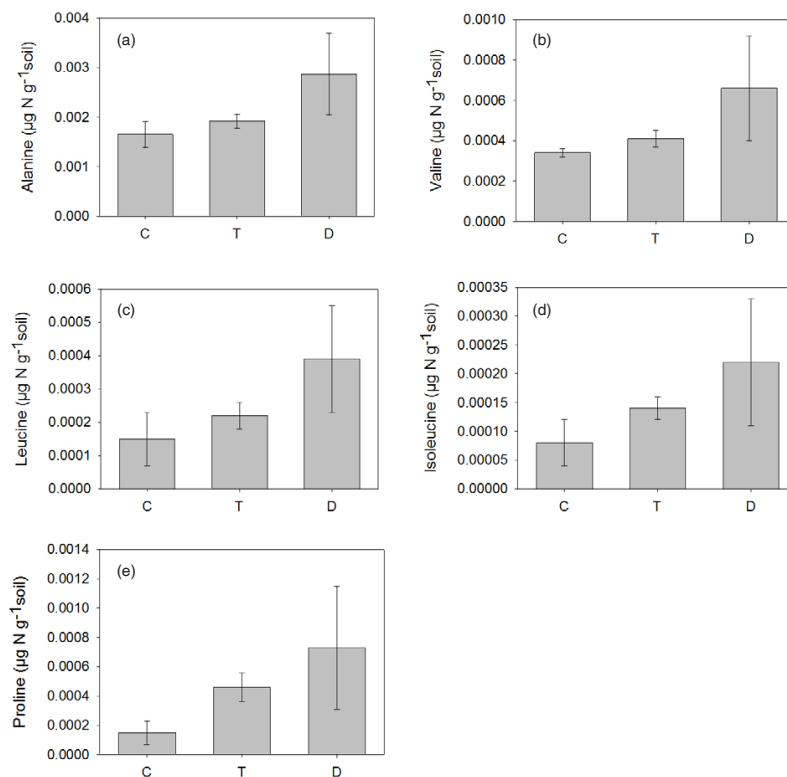


Figure 3. Initial amino acid concentrations for: (a) alanine, (b) valine, (c) leucine, (d) isoleucine and (e) proline; in a heathland soil exposed to the climatic manipulations T = warming treatment, D = drought treatment and C = control.

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