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

L. C. Andresen^{1,*}, S. Bode², A. Tietema³, P. Boeckx², and T. Rütting¹

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

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

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

*current address: Department of Plant Ecology, Justus-Liebig-Universität-Gießen, Heinrich-Buff-Ring 26, 35392 Gießen, Germany

Correspondence to: L. C. Andresen (louise.andresen@bot2.bio.uni-giessen.de)

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Abstract. Monomeric organic nitrogen (N) compounds such as free amino acids (FAAs) 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 of warming and drought treatment on these rates.

The turnover of FAA in the soil was ca. 3 h, which is almost 2 orders of magnitude faster than that of NH_4^+ (i.e. ca. 4 days). This suggests that FAA 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 8 times smaller than the total 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 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 pool is 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.

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 char-

acteristic ecosystem type requires management of the vegetation (Webb, 1998; von Oheimb et al., 2009; García 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 develop 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, FAAs) 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 and Michelsen, 2005; Andresen et al., 2011; Clemmensen et al., 2008), and we need to understand how the interplay of available N and global change factors threatens this ecosystem type.

Ammonium (NH_4^+) is produced during mineralization of ON. Gross mineralization depends 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 has until now been poorly investigated and comprises 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 enable 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. In 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 (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 mineralization may rapidly increase to compensate for 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 in heathlands and shrublands across Europe, the field site “Oldebroek” took part in investigating the research question of whether heathlands are vulnerable or resilient to climate change. The effect of drought during growing season and passive night-time warming has been 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 they are 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 ca. 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 *C. vulgaris* plants were 28 years 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 year⁻¹ and N leaching is 29 kg N ha⁻¹ year⁻¹. Annual rainfall was 1072 mm and the annual average temperature 10.1 °C (Kopittke et al., 2012).

Climate change manipulations have been 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 m × 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 reflective, and they are pulled over the plots after sunset when light intensity is lower than 200 lx and removed at sunrise when the light intensity increases 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 topsoil 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 2–3 months, which reduced precipitation by a PVC curtain that was automatically drawn over the vegetation during rain events. Throughout the

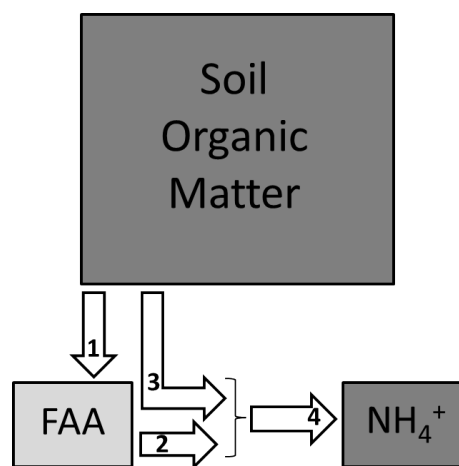


Figure 1. Concept model of investigated N transformations in heathland soil by ^{15}N tracer techniques: (1) free amino acid (FAA) production (^{15}N -AA pool dilution), (2) FAA mineralization (^{15}N - NH_4^+ production from ^{15}N -AA, ^{15}N -tracing), (3) mineralization from other soil organic matter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

14 years, the treatment reduced precipitation by 6–29 % annually (Kopittke et al., 2012). The recent drought period in 2013 started on 15 April and ended the day before soil sampling (22 June). Rainfall was recorded in all plots with funnels (75 mm diameter at 1 m height). Soil moisture and soil temperature (at a soil depth of 4–7 cm) were recorded in each plot with 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 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. The nine 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 homogeneous 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 h at 500 °C). Total N and C were 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 μg AA- N g^{-1} dry soil and 60.4 μg $\text{NH}_4\text{-N g}^{-1}$ 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 and 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 (20 mW cm^{-3} by Elma S 100 H) for 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 $\text{M}\Omega$), 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 and 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.

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 (30 m \times 0.25 mm ID \times 0.25 μm film). We focused 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).

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 an asterisk (*), whereas ns is non-significant.

	Control	Drought	Warming	Statistics
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

2.5 $^{15}\text{N-NH}_4$ 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).

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

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):

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

N_0 and N_t are the concentrations of the respective N pool (i.e. NH_4^+ or FAA) 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 analysed 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 analysed 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 $^{15}\text{N-AA}$ mixture was obtained by measuring $^{15}\text{N-NH}_4^+$ production in three replicates per treatment

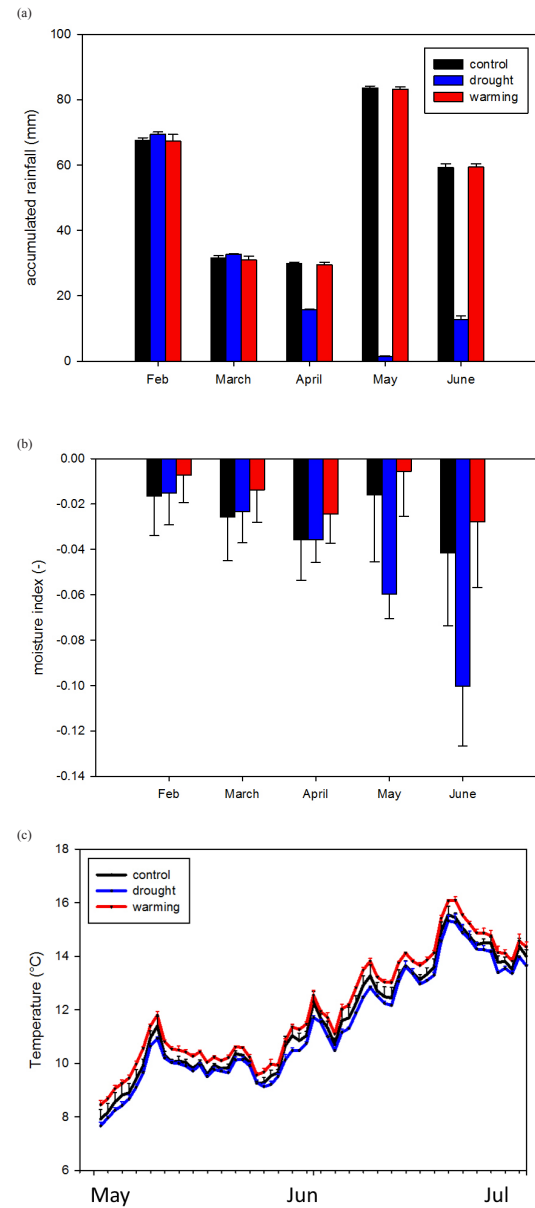


Figure 2. Climatic data for the treated plots control (black), drought (blue) and warming (red): (a) precipitation (accumulated mm rainfall for the given month), (b) soil moisture (index) for 0 to 5 cm depth and (c) temperature in the soil for 0 to 5 cm depth. This year drought treatment started 15 April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

at the time steps 10 min and 7 h, and was calculated according to Watkins and Barraclough (1996):

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

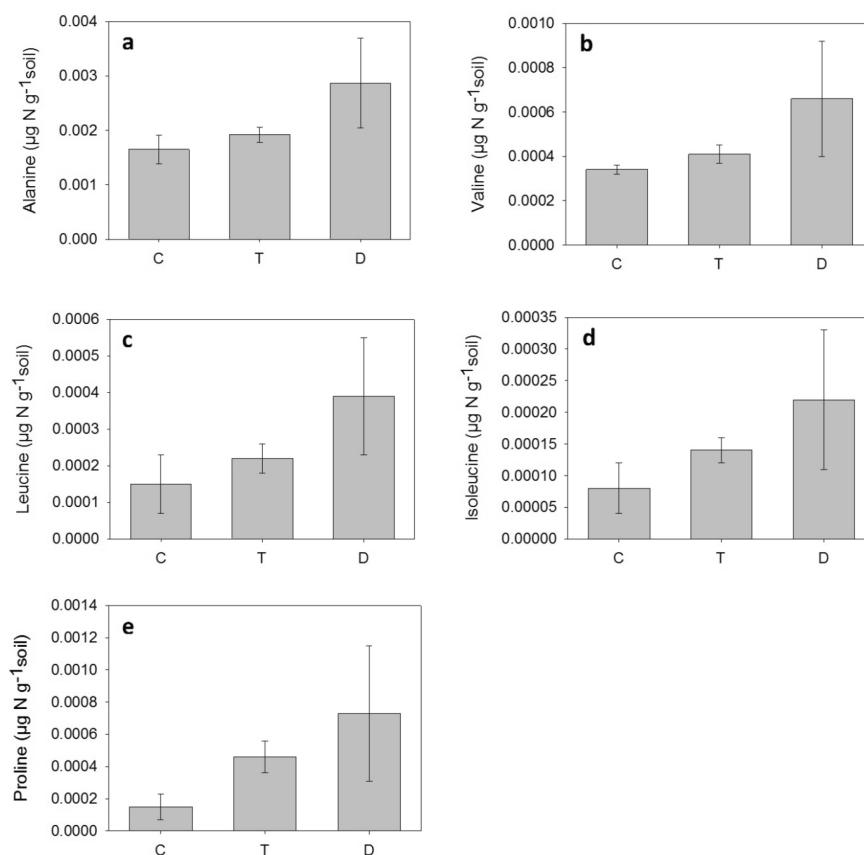


Figure 3. Initial amino acid concentrations for (a) alanine, (b) valine, (c) leucine, (d) isoleucine and (e) proline; in a heathland soil exposed to the climatic manipulations. T – warming treatment, D – drought treatment and C – control.

Hereafter FAA mineralization was calculated as

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

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

Statistical analysis was conducted using SigmaPlot 11 and a 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 among years since 1999 when the manipulations started. The drought treatment imposed via 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

to 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 most during early summer in drought treatment (Fig. 2b). The gravimetric 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 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 were 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).

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 AAs, 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 asterisks (**). ns is non-significant, and nd is non-determined.

	Control	Drought	Warming	Statistics
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 \pm 2.9	11.2 \pm 1.6	9.3 \pm 4.1	T: ns; D: ns
FAA mineralization ($\mu\text{g N g}^{-1} \text{ day}^{-1}$)	3.4 \pm 0.2	2.0 \pm 0.3	3.8 \pm 0.2	D:**; T: ns
Turnover time NH_4^+ (days)	4.3 \pm 2.2	3.2 \pm 0.5	5.2 \pm 3.1	T: ns; D: ns
Turnover time FAA (hours)	2.9	11.5	7.2	nd

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 ($\mu\text{g N g}^{-1} \text{ h}^{-1}$)	Gross min. rate ($\mu\text{g N g}^{-1} \text{ h}^{-1}$)	α (%)	
Barraclough (1997)	Agriculture (wheat)	Sandy loam (6.0)	1.05	0.07	Leucine	6	1.61	2.28	71
Barraclough (1997)	Agriculture (wheat)	Sandy loam (6.0)	1.05	0.07	Glycine	6	6.24	8.94	70
Hadas et al. (1992)	Agriculture	Chromoexert (7.8)	0.82	0.08	Alanine	7	4.93	5.59	88
Hadas et al. (1992)	Agriculture	Camborthid (8.1)	1.31	0.12	Alanine	7	7.59	10.08	75
Stange and Döhling (2005)	Agriculture	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

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. 8-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 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 number 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 $36 \mu\text{g N g}^{-1}$ (Abuarghub and Read, 1988a, b; 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 indicates a longer residence time than 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 %; Table 3) in agricultural systems. This indicates more complex mineralization dynamics in semi-natural ecosystems.

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

The relative contribution from FAA mineralization to gross N mineralization rate was differently affected by drought and warming treatments. While under drought FAA mineralization became relatively less important for total N mineralization, its importance was unchanged by warming. This implies that the various proteolytic enzymes involved in N mineralization are inhibited in the drought-affected soil, and as the observed gross mineralization was not affected, other sources for ammonium became relatively more important than the amino acids. This – together with the fact that drought-treated soils had a markedly smaller amount of total soil N percentage – reflects the many years of soil disturbance by severe droughts (Sowerby et al., 2008), combined with smaller organic matter input to the soils from the drought-inhibited vegetation. Down-regulation of N dynamics in drought-treated ecosystems can be a temporary phenomenon, which is alleviated by peak rain events (Pulleman and Tietema, 1999; Chen et al., 2011) or by simultaneous warming (Björnsne 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. Tietema set up and maintained the long-term field experiment; L. C. Andresen carried out the field-work together with T. Rütting; L. C. Andresen carried out the ¹⁵N labelling laboratory experiment; S. Bode and L. C. Andresen set up the GC-MS method under supervision of P. Boeckx; calculations were done 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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