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) 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

42   mineralization was relatively less important in the investigated heathland. This indicates more

43   complex mineralization dynamics in semi-natural ecosystems.

44   **Keywords:** depolymerization, ammonification, N cycle, ^{15}N pool dilution, amino acid

45   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

71 was much slower than from amino acid to ammonium, which suggest that the

72 depolymerization rate is the main important constraining factor of N availability in forest

73 ecosystems (Jones and Kielland, 2002; 2012). Carbon (C) to N ratio of amino acids is not a

74 good predictor of FAA mineralization rates (Roberts et al., 2009; Rothstein, 2010), because

75 microbial assimilation of FAAs differs between small C-poor and large C-rich amino acids

76 (Knowles et al., 2010; Mooshammer et al., 2014). Recent developments of a ^{15}N-AA pool

77 dilution assay (Wanek et al., 2010; Wild et al., 2013) now enables us to study simultaneously

78 gross FAA production rates (depolymerization rates), gross FAA mineralization and gross N

79 mineralization rates.

80 For NW Europe (including the Netherlands) it is expected that the future climate will be

81 characterized by longer dry periods during summer and 1 to 2 °C warmer air temperatures

82 (IPCC 2013). Changes in soil N dynamics occurring in response to these conditions diverge

83 for the two factors warming and drought. At experiments using field scale future climate

84 change scenarios, net production of IN increased in response to warming (Emmett et al.,

85 2004; Andresen et al., 2010; Bai et al., 2013). Furthermore, results studying *Calluna* litter

86 mineralization rates suggested a positive correlation of IN production with temperature and

87 moisture (van Meeteren et al., 2007). Likewise, in response to warming, gross mineralization

88 rate increased in a *Calluna - Deschampsia* dominated heathland (Björsne et al., 2014).

89 Contrastingly, drought events decreased gross mineralization rates (Björsne et al., 2014) and

90 net mineralization rates (Emmett et al., 2004; Andresen et al., 2010). However, in the event of

91 re-wetting following drought stress, gross N mineralization may rapidly increase to

92 compensate the drought response (Pulleman and Tietema 1999; Chen et al., 2011). Overall,

93 effects from changes in microclimate may increase (warming treatment) or decrease (drought

94 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

119   intensity increased and also during night rain-events. During heavy winds (wind speeds over

120   10 m s^{-1}) or frost and snowfall the curtains were not active. Throughout the 14 years, the

121   warming treatments increased the top soil temperature by 0.5 to 1.5 °C. The drought treatment

122   was applied each year in early growing season (April to July). Precipitation was excluded for

123   two to three months which reduced precipitation by a PVC curtain that was automatically

124   drawn over the vegetation during rain events. Throughout the 14 years, the treatment reduced

125   precipitation by 6-29% annually (Kopittke et al., 2012). The recent drought period in 2013

126   started on April 15 and ended the day before soil sampling (June 22). Rainfall was recorded in

127   all plots by funnels (75 mm diameter at 1 m height). Soil moisture and soil temperature (at a

128   soil depth of 4-7 cm) was recorded in each plot by Decagon sensors. Soil moisture index was

129   calculated for each probe relative to an average obtained from the wettest month (December

130   2012), where no drought treatment was active and all soils were water saturated.

131   **2.2 Soil sampling and soil handling**

132   Soil sampling was conducted on the 23rd of June 2013. Vegetation and loose litter were

133   pushed gently aside. From three locations within each plot, three soil cores were sampled with

134   a corer of 4.5 cm diameter to a depth of 5 cm. The 9 soil cores from each plot were mixed to a

135   composite sample and stored until further processing within 48 hours. Roots were discarded

136   and the remaining soil homogenized by hand. Gravimetric soil moisture content was

137   determined by drying 10 g soil for 24 hours at 100 °C. Because of the extreme dryness of the

138   drought soil (partly hydrophobic) the drought soils were adjusted to the same water content

139   as the control plots, 12 hours before the isotope labels were amended. This enabled

140   homogenous mixing of the isotope label solution with the soil. Soil organic matter was

141   determined on 2 g of dried soil samples by loss of ignition (4 hours at 500° C). Total N and C

142   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$_4$)$_2$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 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

167 the $^{15}NH_4^+$ labelled soil were extracted with 40 mL 1M KCl, then shaken for 30 min at 100

168 rpm, centrifuged for 10 min at 3500 rpm and finally filtrated.

169 ## 2.4 ^{15}N Amino acid analysis

170 The internal standard added to samples during purification was a mixture of two non-

171 biological amino acids: nor-valine and nor-leucine. The method described by Wanek et al.,

172 2010 was developed further for our instrumentation at ISOFYS, Ghent University.

173 Concentration and ^{15}N enrichment were determined using gas chromatography - mass

174 spectrometry (GC - MS, Trace GC - DSQ, Thermo Fisher). Separation was done on a VF 5-

175 MS column (30m x 0.25mm ID x 0.25µm film). We focused on five detectable amino acids

176 (alanine mz: 116/117, valine mz: 144/145, leucine mz: 158/159, isoleucine mz: 158/159, and

177 proline mz: 142/143).

178 ## 2.5 ^{15}N-NH$_4$ determination

179 The ^{15}N enrichment of NH_4^+ in the KCl soil extracts was determined, using an ANCATGII

180 Automated Nitrogen Carbon (Trace Gas) Analyzer (PDZ Europa, UK) coupled to a 20-20

181 Isotope Ratio Mass Spectrometer (IRMS; SerCon, UK), after conversion to nitrous oxide

182 (Hauck, 1982; Saghir et al., 1993). For this ammonia (NH_3) was liberated from the sample

183 extracts by adding magnesium oxide (MgO), and absorbed by an acid solution. Nitrous oxide

184 is produced by reaction with sodium hypobromite (NaOBr).

185 ## 2.6 Data analysis and calculations

186 Gross mineralization and gross FAA production rates (for each individual AA) were

187 estimated by using time steps 10 min and 7 h or 10 and 30 min, respectively, using analytical

188 equations (Kirkham and Bartholomew, 1954).

189 $$m = \frac{N_t - N_0}{t} * \frac{\ln(a'_0/a'_t)}{\ln(N_t/N_0)} \quad [\mu g \ N \ g^{-1} \ day^{-1}] \qquad (1)$$

190   For the few cases with (nearly) constant NH_4^+ concentration throughout the incubation time

191   of 7 h, the gross N mineralization was calculated as follows (Kirkham and Bartholomew,

192   1954):

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

194   N_0 and N_t are the concentrations of the respective N pool (i.e. NH_4^+ or AA) at time 0 and

195   t, respectively; N_{av} is the average of N_t and N_0.

196   a'_0 and a'_t are the excess ^{15}N abundances at time 0 and t, respectively. Gross N

197   mineralization had three replicates per treatment analyzed at each time step.

198   Total FAA production rate was equal to the sum of the individual FAA production rate. Some

199   treatments had only two replicates successfully analyzed at each time step, both numbers are

200   reported in addition to the average. The turnover time (mean residence time) was calculated as

201   N_0 / m.

202   The fraction of mineralization derived from FAA mineralization (α) from the ^{15}N-AA mixture

203   was obtained by measuring ^{15}N-NH_4^+ production in three replicates per treatment at the time

204   steps 10 min and 7 hours, and was calculated according to Watkins and Barraclough, 1996:

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

206   Hereafter FAA mineralization was calculated as

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

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

209   two time steps;

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

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

212   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).

3. Results

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 =

226   0.007; Table 1). Soil temperature in the top layer (0-7 cm) was enhanced during the 14 years

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 $0.0024 \pm$

232   0.0006 µg N g^{-1} and total AA and individual AA concentrations were not significantly

233   affected by climate manipulation (Figure 3).

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

237   mineralization (NH_4-N production rate directly from FAAs) was 3.4 µg N g^{-1} day^{-1} in control,

238   representing 34% of the total gross N mineralization (Table 2). In drought treatment FAA

239   mineralization was reduced (P = 0.006; Table 2), and gross FAA production declined with

240   drought and warming (Table 2) though, due to limited amount of replicates this could not be

241   tested statistically. AA turnover time ranged between 1 h (valine, C) and 32 h (leucine, T),

242   while turnover time for NH_4^+ was 4.3 days in control (Table 2).

4. Discussion

244   In heathland ecosystems, FAAs are only sparsely studied, but previous research suggested a

245   wide concentration range in soil of 0.02 to 36 µg N g^{-1} (Abuarghub and Read, 1988a; 1988b;

246   Kielland, 1995; Finzi and Berthrong, 2005; Andresen et al., 2008; 2011). These studies

247   showed that the FAA-N pool was in general smaller than the NH_4^+-N pool. The turnover time

248   of FAAs in our study indicate a longer residence time compared with results from ^{15}N

249   labelling studies in forest litter and agricultural soils (0.5 to 1.5 h in Wanek et al., 2010; 3.5 h

250   in Geisseler et al., 2012). Moreover, studies using ^{14}C methodologies suggest a maximum

251   turnover of 12 h (Jones et al., 2009; Farrell et al., 2014; Wilkinson et al., 2014).

252   The gross FAA production rates quantified in the current study can be considered as an

253   indicator for total depolymerization, as the rates are only based on five AAs. Nevertheless,

254   total FAA production rate was ca. 8 times larger than gross N mineralization (control plots),

255   which is in line with observations by Wanek et al., (2010) and Wild et al., (2013).

256   FAA mineralization, which was calculated based on the addition of 20 ^{15}N-labelled

257   AAs, was an important component of gross N mineralization. However, due to the addition of

258   a large amount of AA-N, the FAA mineralization rates are potentially overestimated.

259   Nevertheless, the contribution of FAA mineralization to total gross N mineralization of 18-

260   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

285 et al., 2014). However, changes in the N availability, as observed in this study, is a potential

286 realistic effect during droughts, followed by sudden rain events in the main growing season.

287   Overall, we conclude that N transformation processes in response to drought will shift

288 towards a dominance of inorganic N rather than organic N (*e.g.* FAA) production. We suggest

289 further analysis of seasonal effects on these production rates, and a look into the combination

290 of drought and warming treatments.

291

Author contributions

293 A. T. set up and maintained the long term field experiment; L. C. A. carried out the fieldwork

294 together with T. R.; L. C. A. carried out the ^{15}N labelling laboratory experiment; S. B. and L.

295 C. A. set up the GC-MS method under supervision of P. B.; calculations by T. R., P. B. and L.

296 C. A.; L. C. A. wrote the first draft of the paper, while all authors contributed to writing and

297 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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463

464 **Table 2**: Nitrogen transformation rates and turnover times. Gross mineralization rate (*Eq. 1 &*
465 *2*), free amino acid (FAA) mineralization (*Eq. 4*), represented by average ± standard error.
466 FAA production rate is the sum of the five AA, the two measurement points in square
467 brackets, NH_4^+ turnover time (days) and turnover time of amino acids (hours). Significant
468 effect ($P < 0.01$) of treatment is indicated by asterisk (**), ns. is non-significant, and nd. is
469 non-determined.

470

	Control	Drought	Warming	stat.
FAA production rate ($\mu g \ N \ g^{-1} \ day^{-1}$)	76.2 [127.4; 25.0]	27.0 [17.3; 36.6]	43.4 [34.6; 52.1]	nd.
Gross mineralization rate ($\mu g \ N \ g^{-1} \ day^{-1}$)	10.0 ± 2.9	11.2 ± 1.6	9.3 ± 4.1	T: ns; D: ns
FAA mineralization ($\mu g \ N \ g^{-1} \ day^{-1}$)	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*, 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 ($\mu g\ N\ g^{-1}\ h^{-1}$)	gross min. rate ($\mu g\ N\ g^{-1}\ h^{-1}$)	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.
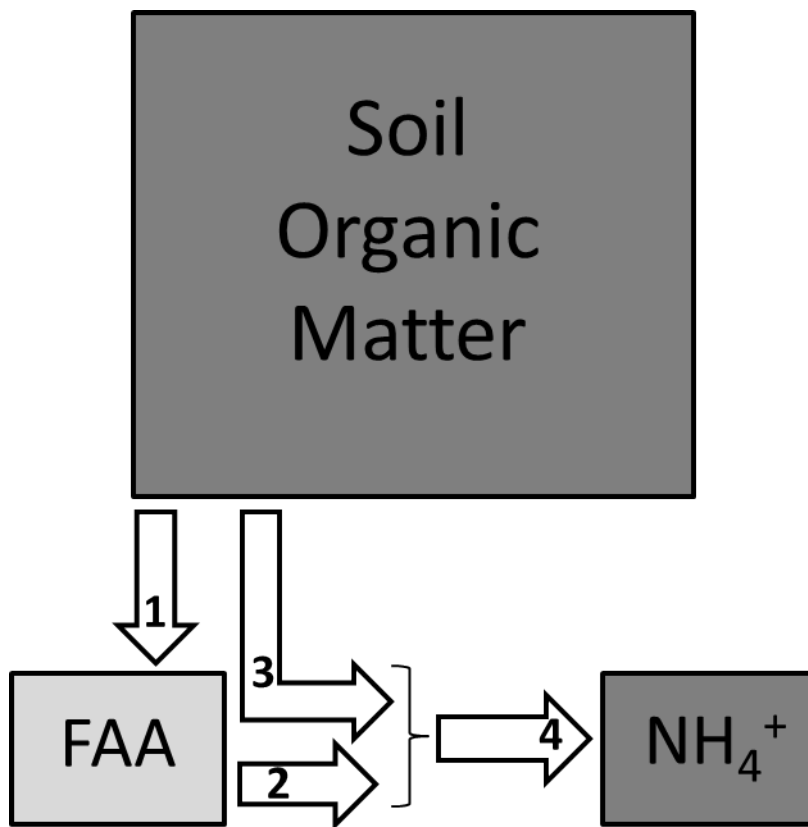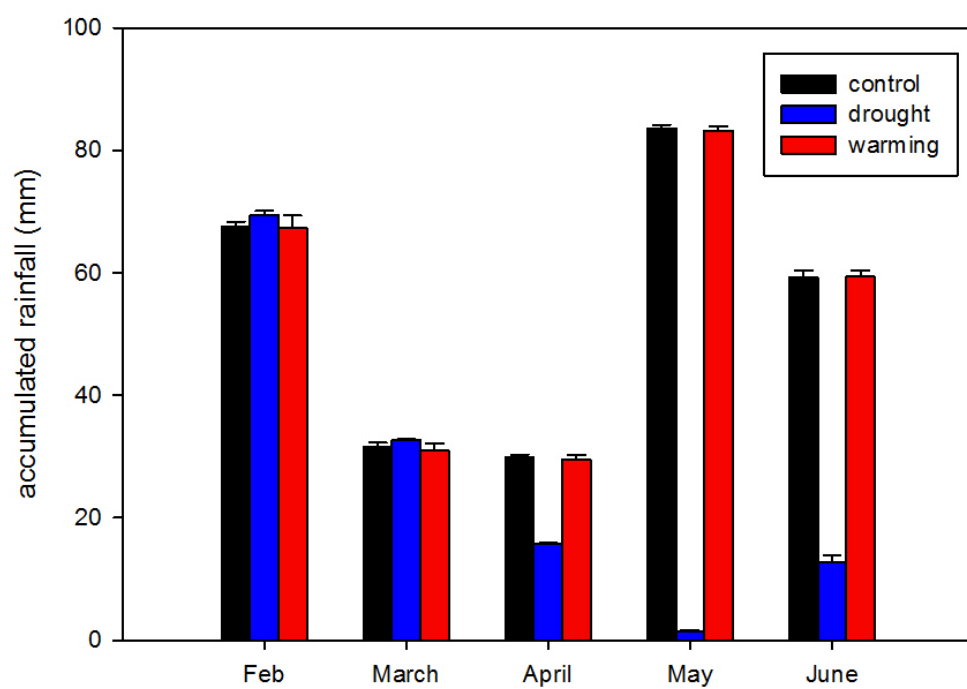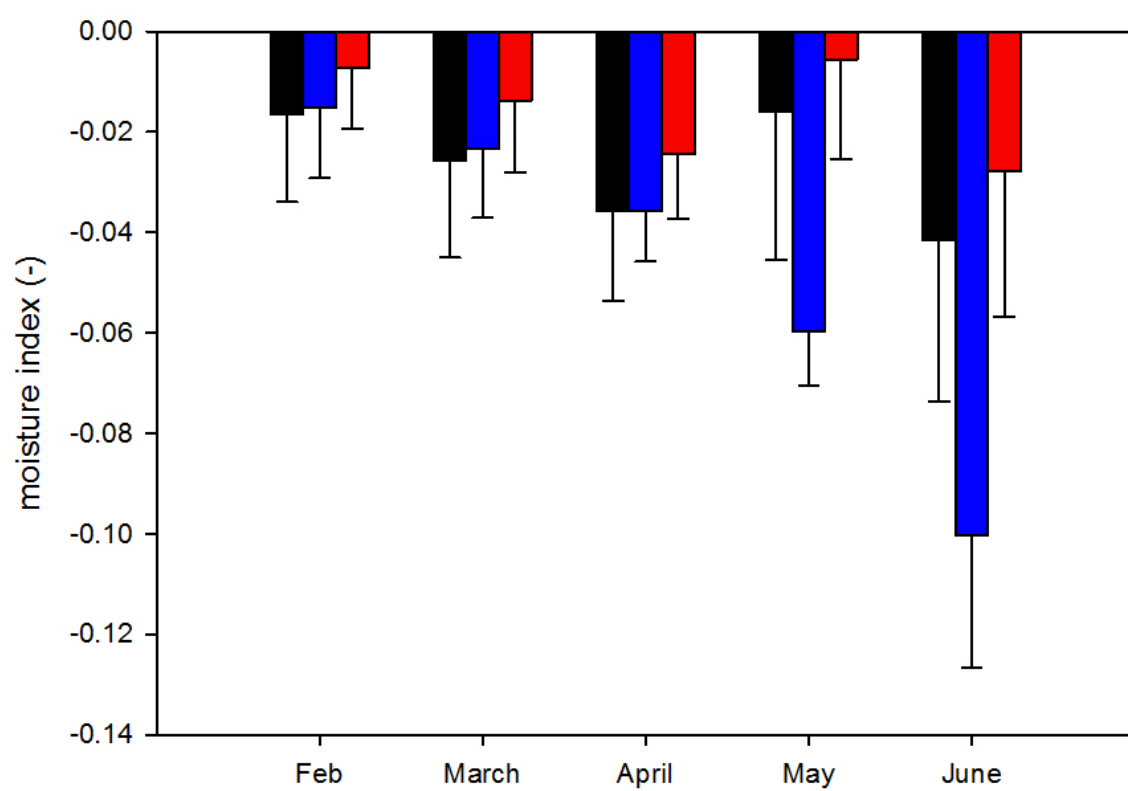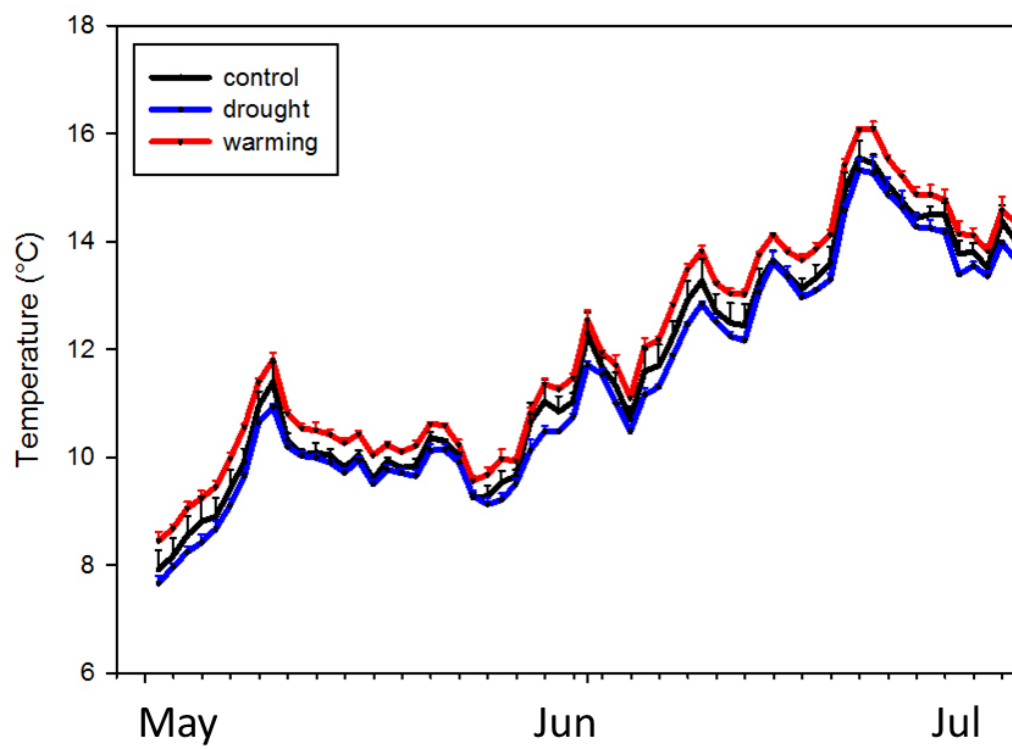
Figure 1

Soil Organic Matter

FAA

1

3

2

4

NH_4^+

Figure 2

A

B

C

Figure 3