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Effects of drought on nitrogen turnover and abundances of ammonia-oxidizers in mountain grassland

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Abstract

Future climate scenarios suggest an increased frequency of summer drought periods in the European Alpine Region. Drought can affect soil nitrogen (N) cycling, by altering N transformation rates, as well as the abundances of ammonia-oxidizing bacteria and archaea. However, the extent to which drought affects N cycling under in situ conditions is still controversial. The goal of this study was to analyse effects of drought on soil N turnover and ammonia-oxidizer abundances. To this end we conducted a rain-exclusion experiment at two differently managed mountain grassland sites, an annually mown and occasionally fertilized meadow and an abandoned grassland. Soils were sampled before, during and after drought and were analysed for gross rates of N mineralization, microbial uptake of inorganic N, nitrification, and the abundances of bacterial and archaeal ammonia oxidizers based on gene copy numbers of the *amoA* gene (AOB and AOA, respectively).

Our results showed that the response to drought differed between the two sites. Effects were stronger at the managed meadow, where NH_4^+ immobilization rates increased and AOA abundances decreased. At the abandoned site gross nitrification and NO_3^- immobilization rates decreased during drought, while neither AOB, nor AOA abundances were affected. The different responses of the two sites to drought were likely related to site specific differences, such as soil organic matter content, nitrogen pools and absolute soil water content, resulting from differences in land-management. At both sites rewetting after drought had only minor short-term effects on the parameters that had been affected by drought, and seven weeks after the drought no effects of drought were detectable anymore. Thus, our findings indicate that drought can have distinct transient effects on soil nitrogen cycling and ammonia-oxidizer abundances in mountain grasslands and that the effect strength could be modulated by grassland management.

1 Introduction

For the European Alpine Region future climate scenarios predict an increased irregularity of precipitation timing, which will enhance the frequency of drought periods and heavy rainfall events (IPCC, 2007, 2012; Schär et al., 2004; Seneviratne et al., 2006; Gobiet et al., 2013), causing temporal imbalances of the water budget in soils. Soil water is a key factor for physiological processes and determines plant productivity and the activity of soil microorganisms by influencing the osmotic potential, soil pore connectivity, substrate diffusion and nutrient availability (Knapp et al., 2002; Moyano et al., 2013; Stark and Firestone, 1995). Changes of precipitation patterns can therefore affect soil nutrient cycling, such as the nitrogen (N) turnover in the soil.

Nitrogen is the major growth limiting nutrient in most non-fertilized terrestrial ecosystems (LeBauer and Treseder, 2008), and is strongly influenced by the intensity of agricultural management (Cookson et al., 2007; Patra et al., 2006; Stempfhuber et al., 2014). Nonetheless, N turnover in soils is mainly controlled by microbial processing. Soil microbes fix atmospheric N, they decompose organic N sources and take up and release ammonium (NH_4^+) and nitrate (NO_3^-),during mineralization and nitrification processes (Booth et al., 2005; Schimel and Bennett, 2004). As plant and microbial N uptake, as well as microbial N mineralization and nitrification appear to differ in their environmental controls, and occur on different temporal scales (Chen et al., 2011; Schimel and Bennet, 2004), they may vary in the response to the predicted climatic changes.

Nitrogen mineralization, as well as microbial uptake of NH_4^+ and NO_3^- (immobilization), can be termed as “broad” processes that combine multiple distinct processes performed by a wide range of microorganisms covering a large spectrum of ecophysiological optima (Schimel and Schaeffer, 2012). Thus, “broad” processes could be more stable to altered environmental conditions, such as during drought, than “narrow” processes, carried out by a phylogenetically constrained group of microorganisms (Allison and Martiny, 2008; Schimel and Schaeffer, 2012). In field experiments, results are not as consistent: N mineralization was found to be only marginally affected (e.g. Auyeung et al., 2012), while other studies showed decreased rates during drought (Chen et al., 2011; Emmett et al., 2004).

In contrast to N mineralization, nitrification, i.e. the oxidation of ammonia (NH_3) via nitrite (NO_2^-) to nitrate (NO_3^-), is a comparatively "narrow" process (Schimel and Schaeffer, 2012). Nitrification has long been expected to be solely performed by a monophyletic group of bacteria (Kowalchuk and Stephen, 2001; Schimel et al., 1989), but also archaea of the phylum thaumarchaeota are able to conduct the first step of nitrification, the conversion of NH_3 to NH_2OH (Zhang et al., 2010). This step is catalysed by the enzyme ammonia-monooxigenase (AMO), of which the subunit A (*amoA*) is highly conserved encoded in ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Treusch et al., 2005). AOB and AOA seem to be functionally redundant and are both contributing to nitrification in soils (Leininger et al., 2006; Schauss et al., 2009), but they differ in physiology and ecology (Erguder et al., 2009). Although in many soils AOA are present at higher abundances than AOB (Alves et al., 2013; Leininger et al., 2006; Prosser and Nicol, 2008), AOB seem to outcompete AOA at high levels of NH_4^+ (Martens-Habbena et al., 2009; Offre et al., 2009; Schauss et al., 2009) and dominate nitrification in N-rich grasslands (Brankatschk et al., 2010; Di et al., 2009). In turn, archaeal AMO has a higher affinity for ammonia and they seem to have a clear advantage in environments with low ammonia concentrations (Gubry-Rangin et al., 2011; Höfferle et al., 2010; Offre et al., 2009; Tourna et al., 2008, 2011). Despite differences in NH_4^+ optima, both AOB and AOA were found to be more abundant in soils of grasslands with higher land-management intensity (Meyer et al., 2013; Stempfhuber et al., 2014). Moreover, AOB and AOA seem to differ in their sensitivities to changes in soil water availability (Gleeson et al., 2010; Stres et al., 2008; Szukics et al., 2012), with growth of AOB, but not of AOA, being favoured at higher levels of soil water content (Bates et al., 2010; Szukics et al., 2012). Effects of drought on nitrification seem to be inconsistent. During drought nitrification was shown to either increase (Auyeung et al., 2012; Gleeson et al., 2010), to decrease (Stark and Firestone, 1996) or to remain unaffected (Hartmann and Niklaus, 2012; Larsen et al., 2011). It is, however, still unclear, if there is a relation between the sensitivity of nitrification to drought and ammonia-oxidizer abundances in soils.

During drought periods microbes may balance the increasing osmotic soil water potential by accumulating osmolytic compounds, which could lead to large amounts of N tied up in the microbial biomass (Schimel et al., 2007). Rainfall events, specifically after longer dry periods, introduce a water pulse causing a sudden decrease of the osmotic soil potential, which forces microbes to release accumulated osmolytic compounds in order not to burst (Schimel et al., 2007, but see Boot et al., 2012). In addition, accumulated nutrients become available for microbes, but water pulses can also stimulate microbes that may have shifted to dormancy during drought (Placella and Firestone, 2013). Rewetting has therefore been shown to induce short-term increases of carbon and N mineralization (Birch, 1958; Fierer and Schimel, 2002; Evans and Wallenstein, 2012), and of nitrification (Fierer and Schimel, 2002).

However, most data on effects of drought and rewetting on grassland N-cycling originate either from seasonal dry (e.g. Fierer and Schimel, 2002) or from intensively managed grasslands (e.g. Hartmann and Niklaus, 2012). Data on effects of drought on typically well water-supplied sites with low or without management, such as mountain meadows, are still missing so far. The aim of this study was therefore to investigate effects of drought and subsequent rewetting, in situ, on the potential rates of gross N mineralization, gross nitrification, and abundances of ammonia-oxidizers of mountain meadows. We conducted a rain-exclusion experiment to simulate a drought period during the growing season at an extensively managed and an abandoned meadow located in the Austrian Central Alps. We hypothesized that (i) drought decreases nitrification rates, but has only minor effects on N mineralization and that (ii) drought has stronger effects on bacterial than on archaeal *amoA* gene copy numbers. As land-management has been shown to affect soil properties, such as soil organic matter content (Meyer et al., 2012), N pools, and N turnover, as well as the abundance of the related microbial communities (Cookson et al., 2007; Meyer et al., 2013; Patra et al., 2006; Stempfhuber et al., 2014), we further hypothesized (iii) that drought has a stronger impact on the managed meadow than on the abandoned site.

2 Material and methods

2.1 Study sites

The study sites are located in the Austrian Central Alps near Neustift, Stubai Valley (47°07′ N, 11°19′ E) and are characterized by a mean annual temperature of 3 °C and a mean annual precipitation of 1097 mm. Drought simulations were conducted on a typical extensively-managed mountain meadow (will be referred to as “meadow”, 1850 m a.s.l.) and on an abandoned meadow site (will be referred to as “abandoned site” 1900 m a.s.l.). Soils at both sites were characterized as dystric cambisols (FAO classification) with a pH in the uppermost 10 cm soil depth of 5.5 (determined in $CaCl_2$). At the meadow total aboveground plant biomass is cut and harvested once a year; parts of the meadow are slightly grazed by cattle in spring and autumn, and fertilized with manure every two to three years (Bahn et al., 2006). The meadow is characterized by high plant primary production (Schmitt et al., 2010), by higher soil respiration rates (Bahn et al., 2008), as well as by lower soil organic matter (SOM), total carbon (C_{tot}) and nitrogen (N_{tot}) contents compared to the abandoned site (Table 1, and Meyer et al., 2012). At the abandoned site all management activities were terminated in 1983. The dominant plant community was determined as Trisetetum-Flavescentis at the meadow, and as Seslerio-Cariecetum at the abandoned site (Schmitt et al., 2010).

2.2 Experimental set-up and soil sampling

At both sites drought and corresponding control plots ($n = 4$, respectively) were established in spring 2011 in a paired plot design. To simulate drought rain-out-shelters, covering an area of 3.0 m × 3.5 m, were installed on 31 May 2011 and left on the plots for 10 weeks. The shelters were equipped with light- and UV-B-permeable plastic foil (UV-B Window, Foiltec GmbH, Germany, light-permeability ca. 95 %, UV-B permeability > 70 %) to exclude any precipitation. To maintain the traditional land-management, the total area of the meadow was mown (i.e. aboveground biomass was cut and removed) during the drought period (2 August 2011), while there was no land-management activity at the abandoned site. After 10 weeks of drought (10 August 2011) all plots received previously collected rainwater over a time period of 3 min to simulate a short heavy rainfall event of 20 mm; subsequently rain-out shelters were removed and all plots were again exposed to natural precipitation.

Soils were sampled one week before the onset of drought simulation, then every two to three weeks during drought, one day and seven weeks after rewetting (Fig. 1). For each sample two soil collars (5 cm × 7 cm, 10 cm depth) were pooled after the uppermost litter layer had been removed. Then soil collars were homogenized and sieved to 2 mm. Aliquots of soil were immediately frozen at −80 °C for molecular analyses, the remaining samples were stored at 4 °C until further processing.

2.3 Soil parameters and N pools

For both sites maximum soil water holding capacity (WHC_{max}) was determined by adding excess amounts of deionized water to aliquots of soil samples and leaving them for 48 h over water to allow maximum saturation. Then 2 g of water saturated soils were dried for 48 h at 60 °C. Soil water content (SWC) was measured gravimetrically by drying of 5 g of fresh soil for two days at 60 °C in a drying oven and calculated relative to WHC_{max}. Total carbon (C_{tot}) and N (N_{tot}) content were determined from dried and ground soil samples by EA-IRMS (EA 1110, CE Instruments, Italy, coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, MA, USA). Total extractable N was determined from K_2SO_4 extracts (2 g of soil were extracted with 20 mL 0.5 M K_2SO_4) using a TOC/TN analyser (TOC-V CPH E200V/TNM-122V; Shimadzu, Austria). NH_4^+ was measured photometrically from K_2SO_4 extracts using a modified indophenol reaction method (Kandeler and Gerber, 1988). NO_3^- was determined from water extracts (2 g of fresh soil were extracted with 20 mL of MilliQ water) by chemically suppressed ion-chromatography (DX500, Dionex, Austria) on a Dionex AS11 column. Extractable organic nitrogen (EON) was calculated by subtracting inorganic (NH_4^+ and NO_3^-) from total extractable N.

2.4 Gross N transformation rates

Gross NH_4^+ and NO_3^- transformation rates were determined using ^{15}N pool dilution techniques described by Kaiser et al. (2011). For determination of microbial gross N mineralization and NH_4^+ immobilization fresh aliquots of soil (2 g in duplicates) received 0.5 mL $(NH_4)_2SO_4$ (0.125 mM; 10 atom% ^{15}N). For determination of gross nitrification and microbial NO_3^- uptake (NO_3^- immobilization) 2 g of fresh soil samples (in duplicates) received 0.5 mL KNO_3 (0.25 mM, 10 atom% ^{15}N). Labelled samples were incubated for 4 and 24 h at room temperature and finally extracted with 20 mL 2 M KCl. Both control and drought treated soils received solute ^{15}N label, which could have altered the conditions, specifically in drought treated soils (Chen et al., 2011). Therefore, the reported rates should be considered as the N-turnover potential of the soil microbial community.

Gross N mineralization and NH_4^+ immobilization were determined by microdiffusion of NH_3 from KCl-extracts into acid traps, which were analysed for nitrogen concentration and atom-percent excess of ^{15}N by EA-IRMS (EA 1110, CE Instruments, Italy, coupled to a Finnigan MAT Delta Plus IRMS, Thermo Fisher Scientific, MA, USA). For gross nitrification rates NH_3 was removed from the extracts, before converting NO_3^- to NH_3 by adding Devarda's Alloy. Again, NH_3 was trapped and analysed for N concentration and atom-percent excess of ^{15}N. Gross N mineralization, gross NH_4^+ immobilization, as well as gross nitrification and gross NO_3^- immobilization rates were calculated as described in detail by Wanek et al. (2010).

2.5 Nucleic acid extraction

DNA was extracted from 0.35 g of soil using the FastDNA® SPIN Kit for Soil (MP Biomedicals, CA, USA) and the Precellys24 Instrument (Bertin Technologies, France). After extraction, the DNA was tested in quantity and quality with a spectrophotometer (Nanodrop, PeqLab, Germany) and stored at −20 °C until further processing.

2.6 Quantitative Real-Time PCR

The abundances of the bacterial and archaeal ammonia-monooxygenase gene (*amoA*) served as proxy for ammonia-oxidizers and were detected by quantitative Real-Time PCR, which was carried out on a 7300 Real-Time PCR System (Applied Biosystems, Germany) using SYBR green as fluorescent dye. The PCR was performed in 96-well plates (Applied Biosystems, Germany) for all investigated genes. The reaction mixes were performed according to Töwe et al. (2010), the thermal profiles of the PCRs are given in Table 2. In a preliminary test dilution series of the DNA extracts were tested to avoid inhibition of PCR, resulting in an optimal dilution of 1 : 128 for all samples. Serial plasmid dilutions of the respective functional genes ranging from 10^6 to 10^1 gene copies μL^{-1} were used as standards for the determination of the gene abundances of each sample (Table 2). To confirm the specificity of the amplicons after each PCR run, a melting curve and a 2 % agarose gel stained with ethidium bromide were conducted. The efficiencies (Eff) of the amplification were calculated from the standard curve with the formula $\text{Eff} = [10^{(-1/\text{slope})} - 1] \times 100\,\%$ and resulted in the following values: *amoA* of ammonia-oxidizing archaea (AOA) 83.4 % to 91.8 % and *amoA* of ammonia-oxidizing bacteria (AOB) 94.8 % to 95.4 %.

2.7 Statistics

To determine site specific differences all available parameters were compared by repeated-measures ANOVA using only the controls of both sites. As we found significant differences between sites, we subsequently tested for effects of drought by comparing control samples from each study site with drought plots over the course of the experiment by repeated-measures ANOVA, using the plot number as within-factor. To test for differences between drought and control samples at single sampling time points we used paired *t* tests with Bonferroni-adjusted levels of significance. All data were tested for normality by Shapiro–Wilk test and for homoscedasticity by Levene's test. If data did not meet ANOVA assumptions they were log-transformed or rank-normalized. All data, including SWC, C_{tot}, N_{tot}, all N pools and turnover data, as well as ammonia-oxidizer abundances were subjected to principal component analyses (PCA), after they were transformed to meet PCA assumptions. To determine differences between sites and drought on the distribution of the samplings along the PC-axes, we conducted a two-way ANOVA. All statistical analyses were performed using R 2.15.2 (R Core Team, 2012).

3 Results

3.1 Effects of drought on soil parameters and soil N pools

During drought simulation, in total 358 mm of precipitation were excluded, equalling one third of mean annual precipitation. This led to a significant decrease of SWC to 16.9 % (±1.4 %) at the meadow, and to 21.2 % (±3.5 %) at the abandoned site (Table 1, Fig. 1). However, the initial maximum soil water holding capacity (WHC_{max}) was significantly lower at the meadow than at the abandoned site (Table 1), thus the amount of water remaining in meadow soils was lower during drought as compared to the abandoned site. At both sites, rewetting had no immediate effects on the SWC in drought-treated plots, but seven weeks after the end of drought the SWC-levels had approximated those of controls (Fig. 1). At both sites drought had no significant effect on the N pools (Table 2, Fig. 2), although the meadow was characterized by significantly lower EON and NH_4^+, but higher NO_3^- concentrations compared to the abandoned site (Table 1, Fig. 2).

3.2 Effects of drought on microbial N transformation rates

Gross rates of microbial N mineralization and NH_4^+ immobilization were similar at both sites (Table 1, Fig. 3). However, only at the meadow drought significantly increased gross NH_4^+ immobilization, which consequently decreased the mean residence time (MRT) of NH_4^+ from 27.4 ± 6.8 h to 14.4 ± 1.3 h. At the abandoned site, in contrast, gross N mineralization and immobilization, as well as the MRT of NH_4^+ (50.8 ± 10.5 h) were not affected by drought (Table 3, Fig. 3). At neither site N mineralization, or NH_4^+ immobilization were affected by rewetting (Fig. 3).

Gross rates of nitrification and microbial NO_3^- immobilization were significantly lower and the NO_3^- pool turned over much slower (30.3 ± 9.9 h) at the meadow than at the abandoned site (2.2 ± 0.7 h Table 1, Fig. A1). At both sites nitrification and microbial NO_3^- immobilization rates were influenced by sampling time indicating a strong seasonal variability (Table 1, Fig. 3). At the meadow drought affected neither nitrification nor NO_3^- immobilization rates. At the abandoned site, however, drought significantly altered both nitrification and NO_3^- immobilization rates, which showed less variance compared to controls (Table 3, Fig. 3). One day after rewetting nitrification and NO_3^- immobilization rates slightly increased in drought treated plots at both sites, but seven weeks after rewetting, the rates were similar to the controls again (Fig. 3).

3.3 Effects of drought on the abundance of ammonia-oxidizers

The abundance of archaeal *amoA* genes (as a proxy for AOA) was similar at both sites, ranging from 8.1×10^5 to 3.2×10^6 copies g^{-1} DW soil at the meadow and from 5.2×10^5 to 3.7×10^6 copies g^{-1} DW soil at the abandoned site. At the meadow bacterial *amoA* gene copies (as a proxy for AOB), ranging from 2.5×10^5 to 1.7×10^6 copies g^{-1} DW soil, were as abundant as archaeal *amoA* gene copies, thus AOA : AOB ratios ranged

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between 1 and 10. At the abandoned site, in contrast, AOB numbers ranged from 8.0×10^4 to 7.8×10^5 copies g^{-1} DW soil, which was significantly lower, consequently AOA : AOB ratios were significantly higher (ranging between 10 and 100) than at the meadow (Table 1, Fig. 4).

At the meadow drought significantly decreased archeal *amoA* gene copy numbers, while the abundance of bacterial *amoA* remained unaffected, thus the ratio of AOA : AOB significantly decreased compared to the controls (Table 3, Fig. 4). At the abandoned site, in contrast, drought affected neither AOA, nor AOB abundances.

3.4 Drought effects in relation to site-specific differences

To summarize and illustrate drought effects in relation to site-specific differences, a principal component analysis (PCA) was conducted, including all studied parameters. In total, three factors with eigenvalues > 1 were identified and accounted for 68.9 % of variance. In a biplot showing the first two factors (PC1 and PC2, accounting in total for 55.1 % of the variance) on PC1 samples were separated (34.9 %) according to sampling site (two-way ANOVA with factor loadings: ($F(1) = 102.4$; $p < 0.001$) and drought treatment ($F(1) = 5.9$; $p < 0.05$, interaction: ns). PC2 explained 20.1 % and primarily displayed drought effects ($F(1) = 9.1$; $p \leq 0.01$), but was not affected by site ($F(1) = 0.9$; $p =$ ns; interaction: ns). The main parameters responsible for the separation along PC1 were higher EON and NH_4^+ concentrations, as well as higher gross nitrification and gross NO_3^- immobilization rates at the abandoned site, which were in contrast to higher abundances of AOB and NO_3^- concentrations that were dominating at the meadow (Fig. 5). This pattern was supported by positive correlations between EON and NH_4^+ concentrations in controls of both, the meadow ($R = 0.59$, $p < 0.001$) and the abandoned site ($R = 0.57$, $p < 0.01$). Moreover, in controls of the meadow NO_3^- correlated negatively with EON concentrations ($R = -0.67$, $p < 0.001$), but positively with AOB abundances ($R = 0.42$, $p < 0.05$). Only in controls of the abandoned site EON correlated with gross N mineralization ($R = 0.60$, $p < 0.001$) and with SWC ($R = 0.60$, $p < 0.001$). PC2 separated the drought treatment from the controls, although this effect was stronger for the meadow than for the abandoned site. SWC was distributed along PC2 opposing the vectors for gross N mineralization and gross NH_4^+ immobilization (Fig. 5). Specifically in soil samples from the meadow subjected to drought the gross NH_4^+ immobilization rates correlated negatively with SWC ($R = -0.62$, $p < 0.001$).

4 Discussion

In line with our hypothesis, drought induced distinct responses at the two studied mountain grasslands. During drought, NH_4^+ immobilization increased at the meadow (but not at the abandoned grassland), thereby reducing the mean residence time of NH_4^+. At the meadow the additional disturbance by mowing could have strengthened the effects of drought by altering the input of plant derived exudates (Hamilton et al., 2008), and could have enforced the competition for N between microorganisms and plants (Busso et al., 2001). However, the observed increased potential of the soil microbes to take up NH_4^+ could have facilitated the synthesis of nitrogenous osmolytes to balance the osmotic potential between soil solution and microbial cells (Schimel et al., 2007). Microbial N mineralization, however, was not affected by drought at either site. This could indicate that a critical threshold to affect this comparably “broad” soil process might not have been reached yet, or that N mineralization was dominated by drought tolerant microbial generalists like fungi (Allison and Martiny, 2008; Schimel and Schaeffer, 2012). In soils of the managed meadow, gross nitrification rates were not affected by drought, which is in accordance to other studies, where grassland and heathland sites had been subjected to drought (Hartmann and Niklaus, 2012; Larsen et al., 2011). At the abandoned site, drought reduced the dynamics of nitrification and NO_3^- immobilization, which was also shown e.g. for forest soils (Chen et al., 2011) or grassland under oak forest (Stark and Firestone, 1996). Thus, the sensitivity of nitrification to drought seems to be strongly context dependent.

Drought distinctly affected AOB and AOA abundances in the studied grasslands. Throughout the experiment bacterial *amoA* gene copy numbers were not affected by drought at either site, although they have been found to respond more sensitive and faster to altered environmental conditions, in particular to altered soil water availability, than archaeal *amoA* (Zhang et al., 2014; Gleeson et al., 2010). Archaeal *amoA* copy numbers, in contrast, significantly decreased during drought, however only in soils of the managed meadow, while they remained stable at the abandoned site. This, on the one hand, was in contrast to other studies, where AOA growth was even enhanced at low levels of soil water content (Bates et al., 2010; Szukics et al., 2012), and on the other hand suggests that at the meadow drought might have had stronger impacts on the archaeal ammonia-oxidizer (community) than at the abandoned site.

Abundances of bacterial and archaeal ammonia-oxidizers have been shown to strongly differ in soil NH_4^+ concentration optima (Gubry-Rangin et al., 2010; Offre et al., 2009; Schauss et al., 2009). Moreover, Meyer et al. (2013) reported a close link between nitrification rates and abundances of bacterial and archaeal ammonia-oxidizers. In our study, however, we found significant differences, specifically for archaeal *amoA* gene copy numbers, between the two sites, but neither AOB nor AOA were related to NH_4^+ concentrations or to gross nitrification rates. This lack of correlations could indicate that the ammonia oxidizers in the studied soils may not have fully exploited their nitrification potential (Prosser and Nicol, 2012). It might also hint to functional differences between the AOA populations (Alves et al., 2013), as well as the AOB community diversity, which were extracted to determine nitrification activity in soils (Gleeson et al., 2010). Overall our results did not confirm the hypothesis of a general pattern of drought sensitivities of AOB and AOA. However, responses of AOB and AOA to drought differed between sites with differing AOA : AOB ratios.

In contrast to the managed meadow, where aboveground plant biomass is annually removed and harvested, a thicker litter layer had accumulated at the abandoned site (Meyer et al., 2012), which might have protected the soil from drying out (Brady and Weil, 2002; Knapp et al., 2008). While at both sites the relative decrease of the soil water content was similar, the absolute amount of water remaining in the soils was higher at the abandoned site. This was caused by an initial higher soil water content at

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the abandoned site and could have been attributed to the higher SOM content, which has been shown to increase the water holding capacity in soils (Franzluebbers, 2002). However, as similarly observed by Hartmann and Niklas (2012) for subalpine grassland ecosystems, drought only marginally affected the studied N pools at both sites.

Rewetting after drought has been shown to induce short term increases of N mineralization in soil, which could be due to microbial excretion of accumulated osmolytes to avoid lysis, but also by re-connecting soil pores and increasing the nutrient availability for microbes (Evans and Wallenstein, 2012; Fierer and Schimel, 2002; Saetre and Stark, 2005). We therefore expected a strong increase of N mineralization rates in the soils after rewetting, but one day after rewetting neither gross N mineralization, nor NH_4^+ immobilization rates at both sites were affected, which is similar to findings by Chen et al. (2011). Due to the described short-term character of N mineralization peaks after rewetting we might have missed a possible increase. Our results showed that rewetting-effects on AOA and AOB gene copy numbers one day after rewetting were negligible, while nitrification, as well as NO_3^- immobilization rates was increased at both sites. This suggests an activity pulse of the ammonia-oxidizing organisms (Fierer and Schimel, 2002). Moreover, Placella et al. (2013) detected strong increases in the transcript abundances of both bacterial and archaeal *amoA* after rewetting at constant gene copy numbers, accompanied by increases in nitrification rates. In our experiment, seven weeks after termination of drought all determined parameters were similar to the controls (despite NO_3^- concentrations at the meadow).

5 Summary and conclusions

Our study showed that experimental drought under in situ conditions distinctly affected N cycling and ammonia-oxidizing organisms in soils of differently managed mountain grassland sites. N mineralization was less affected by drought than nitrification. Effects of drought on nitrification rates, however, were not related to the abundances of ammonia-oxidizers. Generally, impacts of drought were more pronounced at the man-

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aged as compared to the abandoned grassland, which could have been partly caused by beneficial effects of a thicker litter layer and higher soil organic matter content on soil water content at the abandoned grassland. In addition, differences found in the prevalent N-pools, nitrification rates, as well as abundances of ammonia-oxidizing organisms between the two grasslands suggest that effects of drought on soil N dynamics could have been modulated by the level of land-use.

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Table 1. Soil parameters, nitrogen pools (µg N g^{-1} DW soil), nitrogen turnover rates (µg N g^{-1} DW soil d^{-1}), as well as microbial abundances (gene copies g^{-1} DW soil) in soils of the meadow and the abandoned site ($n = 28$; means ± standard error). Effects of site and sampling time, as well as their interaction were assessed by repeated-measures ANOVA for non-drought treated controls. Asterisks mark levels of significance: $^{\circ} \leq 0.1$; $^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$.

	"Meadow"	"Abandoned site"	Site		Time		Site × Time	
	Mean (± SE)	Mean (± SE)	$F(1)$	p	$F(6)$	p	$F(1,6)$	p
SOM content (%)	13.3 (±0.8)	22.5 (±1.5)		^{***1}				
WHC_{max} (g H_2O g^{-1} DW)	1.5 (±0.1)	2.0 (±0.3)		^{***1}				
SWC (% of WHC_{max})	44.2 (±1.7)	45.4 (±1.8)	0.8		5.2	***	2.6	*
C_{tot} (%)	7.0 (±0.2)	11.2 (±0.7)	33.0	***	1.5		0.9	
N_{tot} (%)	0.7 (±0.1)	0.9 (±0.1)	15.2	***	1.3		0.8	
$C_{tot} : N_{tot}$	10.1 (±0.1)	12.0 (±0.2)						
EON	31.4 (±1.5)	55.6 (±5.5)	33.5	***	2.1		2.9	*
NH_4^+	5.7 (±0.6)	11.1 (±0.9)	42.7	***	2.1		2.8	*
NO_3^-	2.2 (±0.6)	0.4 (±0.1)	14.2	***	1.1		3.6	**
$MRT_{NH_4^+}$	27.4 (±6.8)	33.3 (±6.1)	1.7		2.2	$^{\circ}$	1.3	
$MRT_{NO_3^-}$	30.3 (±10.0)	2.2 (±0.7)	29.4	***	1.8		2.5	*
Gross N Min	8.5 (±1.0)	11.1 (±2.0)	0.1		0.9		1.4	
Gross NH_4^+ Immo	8.1 (±0.8)	13.4 (±1.7)	1.1		0.5		1.4	
GrossNit	5.9 (±0.7)	11.1 (±1.4)	12.4	**	2.9	*	1.7	
Gross NO_3^- Immo	4.6 (±0.9)	12.4 (±1.1)	47.4	***	4.7	***	2.9	
AOA	1.79×10^6	1.82×10^6	0.0		0.5		0.5	
AOB	8.68×10^5	2.25×10^5	37.2	***	1.3		0.4	
AOA/AOB ratio	2.8 (±0.6)	26.3 (±13.2)	27.0	***	0.4		0.7	

[1] Differences between sites for WHC_{max} $n = 16$, and SOM content, $n = 12$ were analysed by t tests.

Table 2. Thermal profiles and primers used for real-time PCR quantification of functional genes of ammonia-oxidation.

Target gene	Thermal profile	No. of cycles	Primer	Source of standard
amoA AOA	95 °C –10 min 94 °C – 45 s/55 °C – 45 s/ 72 °C–45 s	40	amo19F, CrenamoA16r48x (Leininger et al., 2006; Schauss et al., 2009)	Fosmid clone 54d9
amoA AOB	95 °C – 10 min 94 °C – 60 s/58 °C – 60 s/ 72 °C – 60 s	40	amoA1F, amoA2R (Rotthauwe et al., 1997)	*Nitrosomona* ssp.

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Table 3. Effects of the drought simulation and sampling time on all measured soil parameters and microbial abundances for the meadow and the abandoned site assessed by repeated-measures ANOVA ($n = 28$), within-factor was plot identity ($n = 4$); asterisks mark levels of significance: $^{\circ} = p < 0.1$; $^{*} = p < 0.05$; $^{**} = p < 0.01$; $^{***} = p < 0.001$.

	"Meadow"						"Abandoned site"					
	Drought		Time		Drought × Time		Drought		Time		Drought × Time	
	$F(1)$	p	$F(6)$	p	$F(1,6)$	p	$F(1)$	p	$F(6)$	p	$F(1,6)$	p
SWC	175.2	***	5.5	***	7.7	***	69.2	***	4.4	**	8.5	***
C_{tot}	3.3		1.4		1.0		2.2		3.8	**	2.1	°
N_{tot}	2.7		1.3		1.0		1.8		3.5	**	1.9	°
EON	2.9	°	4.4	**	3.4	**	1.1		11.3	***	1.1	
NH_4^+	0.4		2.0	°	2.3	°	< 0.1		4.0	**	1.3	
NO_3^-	0.2		2.8	*	3.6	**	0.1		0.6		1.2	
$MRT_{NH_4^+}$	3.7	°	1.4		1.6		1.8		2.0		2.8	
$MRT_{NO_3^-}$	< 0.1		1.0		2.3		0.6		1.3		0.4	
Gross N Min	1.9		3.6	**	1.2		< 0.1		2.8	*	0.9	
Gross NH_4^+ Immo	4.5	*	2.8	*	1.5		< 0.1		0.3		0.5	
GrossNit	0.1		1.2		0.6		4.9	***	3.6	°	4.1	**
Gross NO_3^- Immo	1.1		1.5		3.3	**	7.5	**	8.6	***	2.2	°
AOA	10.8	**	0.8		0.7		0.1		1.1		0.4	
AOB	2.4		1.6		0.8		0.1		0.5		1.2	
AOA/AOB	19.9	***	1.1		1.0		2.5		1.1		0.4	

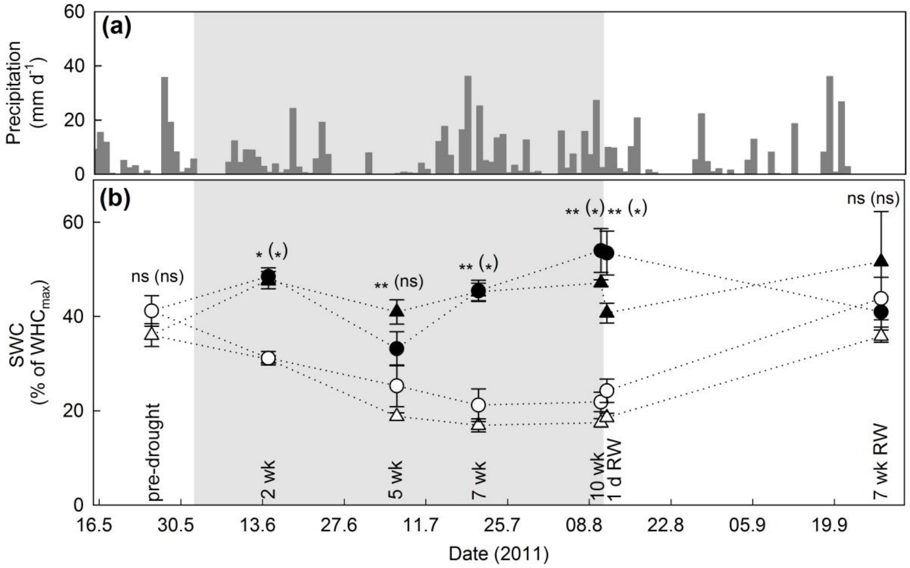

Figure 1. Precipitation **(a)** and soil water content (**b**, SWC) calculated as percentage of the respective $\mathrm{WHC}_{\mathrm{max}}$. Grey bars indicate precipitation (in $\mathrm{mm\,d^{-1}}$). Filled symbols represent controls of the meadow (triangles) and abandoned site (circles), open symbols show the respective drought treated plots (error bars indicate standard error, $n = 4$). Asterisks indicate differences between controls and drought treatments at single sampling points (levels of significance Bonferroni-corrected; $^{*}p < 0.05$; $^{**}p < 0.01$, symbols without parentheses refer to the meadow; symbols in parentheses to the abandoned site). Drought treatment is marked as grey background.

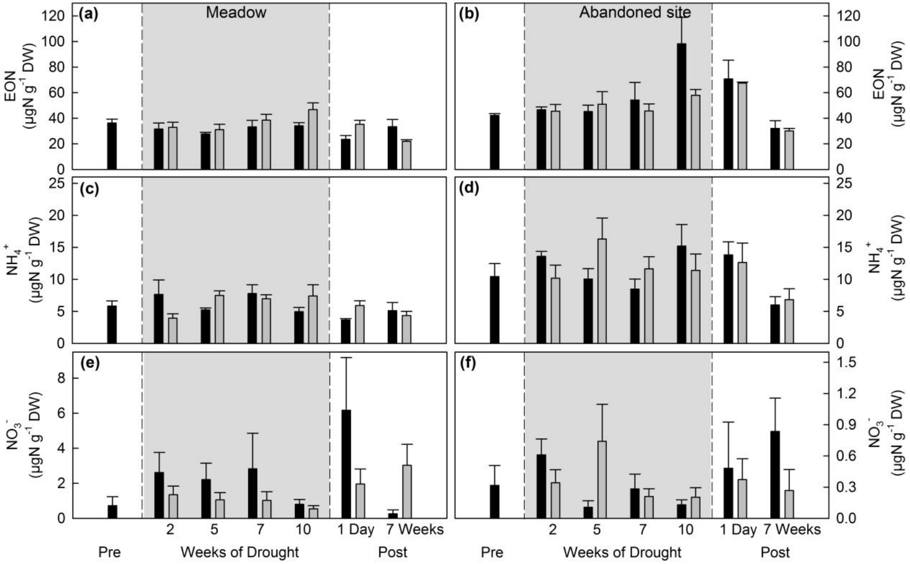

Figure 2. Soil N concentrations over the course of the experiment at the meadow (left panels) and the abandoned site (right panels) in controls (black bars) and drought treated plots (grey bars). **(a, b)** Extractable organic N (EON), **(c, d)** ammonium (NH_4^+) and **(e, f)** nitrate (NO_3^-, note the different scaling!). The grey background indicates the period of drought treatment. Differences between control and drought at single sampling points were assessed by *t* tests with Bonferroni corrected levels of significance ($n = 4$, respectively, bars show means; error bars indicate standard error).

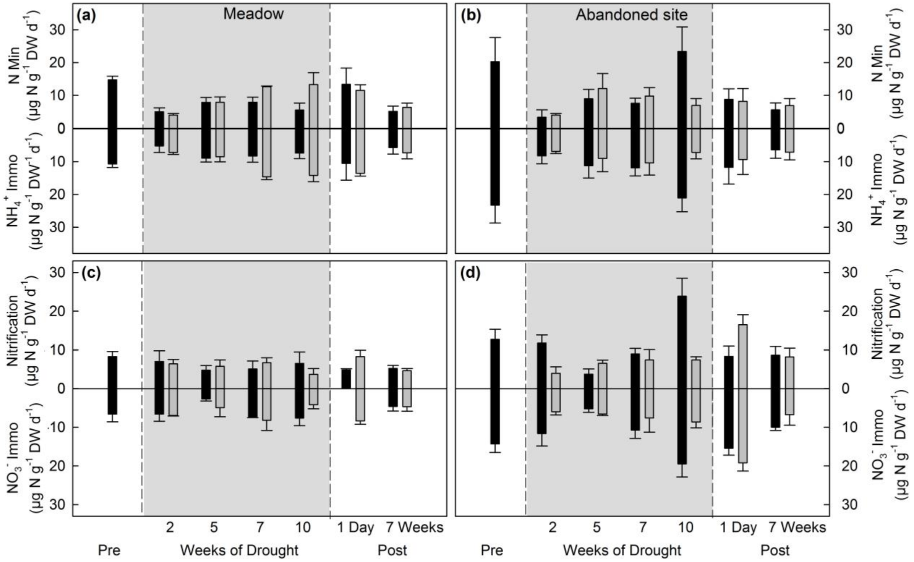

Figure 3. N transformation rates over the course of the experiment at the meadow (left panel) and the abandoned site (right panel) in control (black bars) and drought treated plots (grey bars). **(a, b)** Gross N mineralization (upper part) and gross microbial NH_4^+ immobilization rates (lower part); **(c, d)** gross nitrification (upper part) and gross microbial NO_3^- immobilization rates (lower part). The grey background indicates the period of drought treatment. Differences between control and drought treated plots at single sampling points assessed by *t* tests with Bonferroni corrected levels of significance ($n = 4$, respectively, error bars indicate standard error).

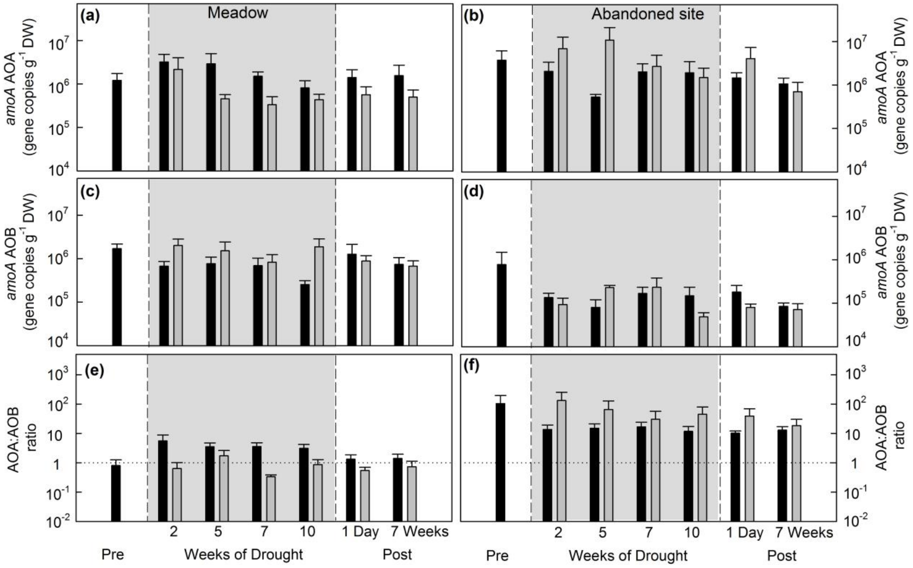

Figure 4. Gene copy numbers of ammonia oxidizers at the meadow (left panel) and abandoned site (right panel) over the course of the experiment. **(a, b)** Display archaeal (*amoA* AOA) and **(c, d)** bacterial (*amoA* AOB) *amoA* gene copy numbers, and **(e, f)** the ratio of AOA : AOB gene copy numbers. Black bars show controls, grey bars drought treatments ($n = 4$, error bars indicate standard error). The grey background indicates the period of drought treatment. Differences between control and drought treated plots at single sampling points assessed by t tests with Bonferroni corrected levels of significance ($n = 4$, respectively, error bars indicate standard error).

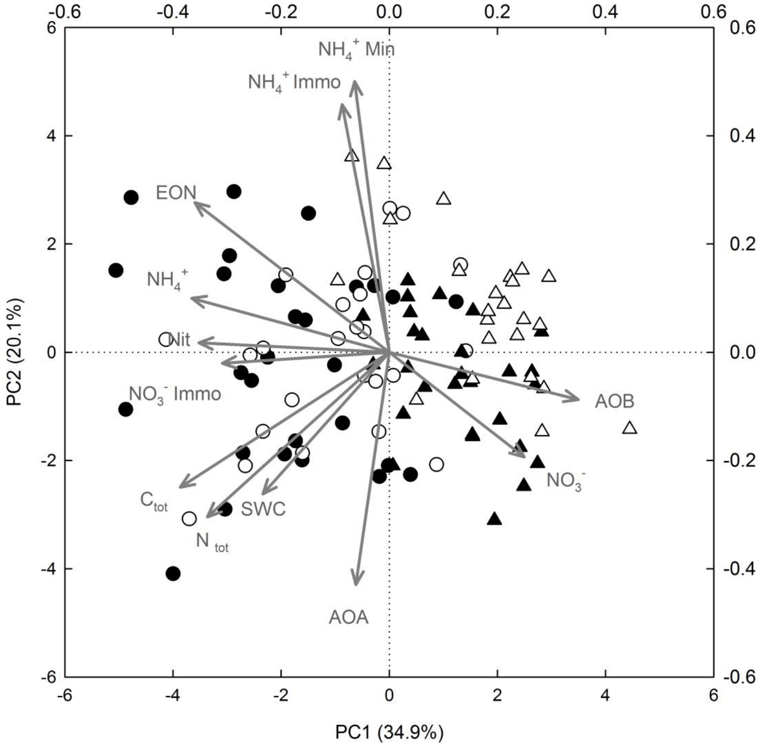

Figure 5. Biplot, displaying PC1 and PC2 derived from principal component analysis. Filled symbols represent control plots at the meadow (triangles) and the abandoned site (circles), open symbols show the drought treatment of the respective sites. Vectors display the variables contributing to PCA (C_{tot} = total carbon, N_{tot} = total nitrogen, for further abbreviations see results).