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 in soil without drought history. To this end we conducted rain-exclusion experiments 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 potential 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).

Drought induced different responses at the two studied sites. At the managed meadow drought increased NH_4^+ immobilization rates and NH_4^+ concentrations in the soil water solution, but led to a reduction of AOA abundance compared to controls. At the abandoned site gross nitrification and NO_3^- immobilization rates decreased during drought, while AOB and AOA abundances remained stable. Rewetting had only minor, short-term effects on the parameters that had been affected by drought. Seven weeks after the end of drought no differences to control plots could be detected anymore. Thus, our findings demonstrated that in mountain grasslands drought had distinct transient effects on soil nitrogen cycling and ammonia-oxidizers, which could have been related to a niche differentiation of AOB and AOA with increasing NH_4^+ levels. However, the effect strength of drought was modulated by grassland management.

1 Introduction

Soil water availability is a key factor for physiological processes determining plant productivity and the activity of soil microorganisms (Knapp et al., 2002; Moyano et al., 2013; Stark and Firestone, 1995). Increasing frequencies of drought periods and heavy rainfall events, as predicted for the European Alpine Regions (Gobiet et al., 2013; IPCC, 2007; IPCC, 2012; Schär et al., 2004; Seneviratne et al., 2006), will lead to strong soil water imbalances and can therefore affect ecosystem 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 its turnover in soils is mainly controlled by microbial processing, such as fixation of atmospheric N, decomposition of organic N sources, as well as uptake and release of ammonium (NH_4^+) and nitrate (NO_3^-) during mineralization and nitrification processes (Booth et al., 2005; Schimel and Bennett, 2004). These individual steps in soil N cycling occur on different temporal and spatial scales (Schimel and Bennet, 2004) and have been shown to strongly differ in their response, both direction and magnitude, to drought (e.g., Auyeung et al., 2012; Chen et al., 2011; Emmett et al., 2004; Gleeson et al., 2010; Stark and Firestone, 1996).

Nitrogen mineralization and microbial immobilisation of NH_4^+ and NO_3^- can be termed as 'broad' processes combining multiple distinct pathways performed by a wide range of microorganisms covering a large spectrum of ecophysiological optima (Schimel and Schaeffer, 2012). 'Broad' processes could therefore be more stable to drought, than 'narrow' ones, such as nitrification (Allison and Martiny 2008; Schimel and Schaeffer 2012). Nitrification, the oxidation of ammonia (NH_3) via nitrite (NO_2^-) to nitrate (NO_3^-) has long been expected to be solely performed by a highly specialized group of autotrophic bacteria (Kowalchuk and Stephen, 2001; Schimel et al., 1989), but also archaea of the phylum thaumarchaeota conduct the first step of nitrification, the conversion of NH_3 to hydroxylamine (NH_2OH) (Könneke et al 2005). 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). As both AOB and AOA can contribute to ammonia-oxidation, however still unclear to which extent (Prosser and Nicol, 2012), they can be considered as potentially, functionally redundant (Leininger et al., 2006; Schauss et al., 2009). They have however been shown to differ in physiology and ecology (Erguder et al., 2009). In many soils archaeal *amoA* genes are more abundant than their bacterial counterparts (Alves et al., 2013; Leininger et al., 2006; Prosser and Nicol, 2008), but AOB seem to outcompete AOA and dominate nitrification in agricultural soils (Jia and Conrad, 2009), N-rich grasslands (Di et al., 2009), and at high levels of NH_4^+ (Brankatschk et al., 2010; Di et al., 2010; Verhamme et al., 2011). Archaeal AMO, in turn, appears to have a higher affinity, and lower inhibition constant for ammonia (Martens-Habbena et al., 2009; Prosser and Nicol, 2012), which could be advantageous at low ammonia concentrations.

Whereas dynamics of AOB and AOA to ammonia are well studied, much less is known about responses of ammonia-oxidizers, specifically of AOA, to drought and rewetting under *in situ* conditions. Generally, AOB and AOA feature different physiological prerequisites (Schauss et al., 2009), presumably also leading to different responses of AOB and AOA to soil drying and rewetting. During drought periods nutrient concentrations in the soil solution increase, forcing microbes to balance the increasing osmotic potential by accumulating or producing osmolytes (Roeßler and Müller, 2001), which could lead to large amounts of N tied up in the microbial biomass (Schimel et al., 2007). Rainfall events in turn, introduce a water pulse causing a sudden decrease of the osmotic soil potential; microbes release accumulated osmolytes to avoid lysis (Schimel et al., 2007, Roeßler and Müller, 2001) and are triggered back from low activity or dormant states (Barnard et al, 2013; Placella and Firestone, 2013) resulting in peaks of carbon and N mineralization (Birch, 1958; Fierer and Schimel, 2002; Evans and Wallenstein, 2012), and of nitrification (Fierer and Schimel, 2002).

Thus, both drying and rewetting could lead to high ammonium concentrations is the soil solution, which has been shown to adversely affect AOA, but not AOB abundances in non-drought adapted grassland soils, likely related to a niche differentiation of AOB and AOA (Thion and Prosser 2014).

Most studies on effects of drought and rewetting on grassland N-cycling and on ammonia-oxidizer abundances originated either from seasonal dry sites (e.g. Fierer and Schimel, 2002; Gleeson et al., 2010; Placella et al., 2013), or from soil mesocosm incubation studies (Thion and Prosser, 2014). However, well water-supplied ecosystems, such as many grasslands in mountainous areas, will be experiencing a higher frequency of drought and heavy rainfall events (Gobiet et al., 2013; IPCC 2012). These grasslands, which are often in transitions from land-management to abandonment, play a pivotal role in nutrient retention and erosion protection with repercussions on densely populated watersheds downstream. Thus, there is an urgent need study possible effects of such climate extremes on soil N cycling in situ.

The aim of this study therefore was to investigate effects of drought and subsequent rewetting on gross N mineralization, gross nitrification, and the abundances of ammonia-oxidizers of mountain meadows under different land-management levels, an extensively managed meadow and an abandoned grassland site in the Austrian Central Alps. At both sites we conducted rain-exclusion experiments to simulate drought and subsequent rewetting, which allowed us examining effects of drought in soils with different preconditions, such as soil organic matter content (Meyer et al., 2012), organic and inorganic N availability (specifically NH_4^+), and with potentially different abundances of bacterial and archaeal ammonia-oxidizers. We hypothesized that the phylogenetically 'broad' process of N mineralization is less affected by drought than the more 'narrow' process of nitrification. We expected AOB and AOA to respond differentially to drought; more specifically, that archaeal *amoA* abundances will decrease as a consequence of rising ammonium concentrations in the

soil solution. Additionally, we hypothesized that the impact of drought on N-turnover and ammonia-oxidizer abundances will be stronger on the managed meadow than on the abandoned site, as the higher soil organic matter content at the abandoned site could act as buffer against soil drying (Brady and Weil, 2002; Franzluebbers et al., 2002).

2 Material and Methods

2.1 Study sites

The two studied grasslands 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 about 1100 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). At both sites soils 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 the meadow and the abandoned site, four drought and corresponding control plots were established in spring 2011 in a paired plot design. Drought was simulated by installing rain-out-shelters, covering an area of 3.0 x 3.5 m, for 10 weeks starting on 31 May 2011. 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 (Figure1). For each sample two soil collars (5x7 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 Potential gross N transformation rates

Potential gross NH_4^+ and NO_3^- transformation rates were determined using a ^{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.

Potential gross N mineralization and NH_4^+ immobilization rates were determined by microdiffusion of NH_3 from KCl-extracts into acid traps, which were analysed for nitrogen concentrations 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 potential gross nitrification and NO_3^- immobilization 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. Potential gross N mineralization, gross NH_4^+ immobilization, as well as potential 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 (2-5 ng DNA μl^{-1} per sample). 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 $Eff = [10^{(-1/slope)} - 1] * 100\%$ and resulted in the following values: *amoA* of ammonia-oxidizing archaea (AOA)

83.4% to 91.8% (R^2 = 0.997) and *amoA* of ammonia-oxidizing bacteria (AOB) 94.8% to 95.4% (R^2 = 0.999).

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 potential N-turnover data, as well as ammonia-oxidizer gene 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) when concentrations were calculated per gram dry soil, 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). However, when N concentrations were calculated per g soil water after five weeks of drought, EON and NH_4^+ concentrations were significantly increased at both sites, and remained on a higher level until one day after rewetting (Table 1, Fig 3). In particular at the meadow, drought induced five-fold increases of NH_4^+ concentrations, while at the abandoned site NH_4^+concentrations at most tripled. In contrast, NO_3^- concentrations in the soil solution were not affected by drought, but strongly increased one day after rewetting at the meadow, while at the abandoned site no clear effect of drought was detected (Table 1, Fig 3). Whereas at both sites seven weeks after the end of drought EOC and NH_4^+ concentrations were similar in drought and control plots, only in soils of the meadow NO_3^- concentrations in drought treated soils were significantly increased (Table 1, Fig. 3).

3.2 Effects of drought on microbial N transformation rates

Potential gross rates of microbial N mineralization and NH_4^+ immobilization were similar at both sites (Table 1, Fig. 4). However, only at the meadow drought significantly increased potential gross NH_4^+ immobilization rates, which consequently decreased the mean residence time (MRT) of NH_4^+ (per g dw) from 27.4 ±6.8h to 14.4 ±1.3h. At the abandoned site, in contrast, potential 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. 4). At neither site potential N mineralization, nor NH_4^+ immobilization responded to rewetting (Fig. 4).

Potential 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 potential nitrification and microbial NO_3^- immobilization rates were influenced by sampling time indicating a strong seasonal variability (Table 1, Fig. 4). At the meadow drought affected neither potential nitrification, nor potential NO_3^- immobilization rates. At the abandoned site, in contrast, drought significantly altered both potential nitrification and NO_3^- immobilization rates, which showed less variance compared to controls (Table 3, Fig. 4). One day after rewetting at both sites potential nitrification and NO_3^- immobilization rates slightly increased in drought treated plots, but seven weeks after rewetting, the rates were again similar to the controls (Fig. 4).

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 $8.1x10^5$ to $3.2x10^6$ copies g^{-1} DW soil at the meadow and from $5.2x10^5$ to $3.7x10^6$ copies g^{-1} DW soil at the abandoned site. At the meadow bacterial *amoA* gene copies (as a proxy for AOB), ranging from $2.5x10^5$ to $1.7x10^6$ copies g^{-1} DW soil, were as abundant as archaeal *amoA* gene copies, thus AOA:AOB ratios ranged between 1 and 10. At the abandoned site, in contrast, AOB numbers ranged from $8.0x10^4$ to $7.8x10^5$ 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 5).

At the meadow drought significantly decreased archaeal *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. 5). 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 the total 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=<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 potential 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. 6). 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 potential gross N mineralization and gross NH_4^+ immobilization (Fig. 6). Specifically in soil samples from the meadow subjected to drought the potential gross NH_4^+ immobilization rates correlated negatively with SWC (R=-0.62, p<0.001).

4 Discussion

4.1 Effects of drought on soil N dynamics and ammonia-oxidizer abundances

In our study the potential of the microbial community to mineralize organic nitrogen to ammonium was not affected by drought irrespectively of site-specific differences related to land-management. Thus, this comparably 'broad' soil process remained stable and could show that N mineralization may have been dominated by drought tolerant microbial generalists like e.g. fungi (Allison and Martiny 2008, Schimel and Schaeffer, 2012). Only in soils of the managed meadow potential gross rates of microbial NH_4^+ immobilization and therefore NH_4^+ turnover increased during drought. This points to a potentially increased microbial N demand to facilitate the synthesis of nitrogenous osmolytes to balance the osmotic potential between the soil solution and microbial cells (Schimel et al., 2007). At the abandoned site, however, the microbial NH_4^+ uptake potential remained unaffected by drought, which suggests a high stability of this process, or a microbial community that might use other than N-containing substrates for osmolyte production (Schimel et al. 2007).

As microbial nitrification appears to be a more 'narrow' process than microbial N mineralization (Schimel and Schaeffer, 2012), we expected nitrification rates to respond sensitive to dry conditions. In the presented study, however, responses of potential nitrification to drought were diverging between the studied sites. In accordance with other studies, where grassland and heathland sites had been subjected to drought (Hartmann et al., 2013; Larsen et al., 2011), the microbial nitrification potential was not affected by drought at the managed meadow. At the abandoned site, in turn, drought reduced the dynamics of potential 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, responses of potential nitrification rates to drought seem to be strongly context dependent and may not be generalized.

Drought distinctly affected bacterial and archaeal *amoA* gene copy numbers, which were used as proxy for the abundance of AOB and AOA. As expected, AOB were stable and not affected by drought at both sites. The observed decrease of AOA in response to drought at

the managed meadow in our study was similar to results reported by Thion and Prosser (2014), where the observed decrease of archaeal *amoA* abundance during drought was likely related to strong increases of NH_4^+ during drought. In our study soil NH_4^+ concentrations calculated per gram dry soil were not affected during drought at either site, similar to the study of Hartmann et al. (2013) in subalpine grassland soils. When NH_4^+ concentrations were calculated per g soil water however, five-fold increases at the meadow and up to three-fold increases at the abandoned site were observed. Thus, it is likely that the stronger increase of NH_4^+ in the soil solution of the meadow could have induced the decrease of archaeal *amoA* genes. Thus, our findings corroborate that responses of archaeal *amoA* abundances to drought could be rather driven by changes in soil NH_4^+ concentrations as a consequence of decreasing water content than by decreasing soil water content alone (Thion and Prosser, 2014; Delgado-Bazquerizo et al., 2013).

Overall, these findings partly support the hypothesis that the impact of drought on the abandoned site could be stronger at the meadow than at the abandoned site. In contrast to the managed meadow, where aboveground plant biomass is annually removed and harvested, at the abandoned site a thicker litter layer (Meyer et al., 2012), and higher SOM content were accumulated, which might have protected the soil from drying out (Brady and Weil, 2002; Franzluebbers, 2002; Knapp et al., 2008).

4.2 Effects of rewetting and recovery dynamics

Rewetting after drought has been shown to induce short term increases of N mineralization in soil, which are likely caused by microbial release 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 at both sites one day after rewetting neither gross N mineralization, nor NH_4^+

immobilization rates responded to water addition, 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.

The potential rates of nitrification, as well as of NO_3^- immobilisation were increased at both sites one day after rewetting, while neither AOB, nor AOA gene copy numbers were affected. Placella and Firestone (2013) detected strong increases in the transcript abundances of both bacterial and archaeal *amoA* within two hours after rewetting at constant gene copy numbers, accompanied by increases in nitrification rates, which suggests an activity pulse of ammonia-oxidizing organisms (Fierer and Schimel 2002). In non drought-adapted grassland soils, however, AOB were found to have benefited from the flush of NH_4^+ after rewetting and to be more tolerant and resilient after drought than AOA (Thion and Prosser, 2014). Nonetheless, rewetting after long dry periods may initially rather stimulate microbial activity, and DNA-based methods could underestimate potential dynamics (Barnard et al., 2013).

In spite of the fact that effects of drought were very diverse and divergent between sites, seven weeks after termination of drought all determined parameters, (except NO_3^- concentrations at the meadow), were similar to undisturbed controls, which indicates a high resilience of the studied grasslands after drought. Moreover, we detected a strong temporal variability of organic and inorganic N concentrations in soils at both sites that might be related to overall seasonal microclimatic fluctuations, and indirectly to altered levels of plant N demand and plant phenology (see eg. Kaiser et al., 2011).

5. Summary and conclusions

Our study showed that experimental drought under in situ conditions distinctly affected N cycling and gene abundances of ammonia-oxidizers in soils of differently managed mountain grassland sites. Potential N mineralization was less affected by drought than the

nitrification potential. However, we could not detect a link between the potential nitrification rates and abundances of bacterial and archaeal ammonia-oxidizer, as was reported by Meyer et al. (2013) for intensively managed agricultural soils. This lack of correlation could indicate that the ammonia oxidizers may not have fully exploited their nitrification potential (Prosser and Nicol, 2012). Alternatively, they may have down-regulated their transcriptional activity (Barnard et al., 2013 Placella et al., 2013) or that heterotrophic nitrification by bacteria or fungi could have contributed to NO_3^- production (e.g, Pedersen et al., 1990). It might also hint to functional and structural differences of AOA (Alves et al., 2013) and AOB populations between the studied sites (Gleeson et al., 2010).

The distinct responses of ammonia-oxidizer in the studied grasslands, specifically of AOA, could likely be related to increases of NH_4^+ in the soil solution rather than to decreases of soil water content *per se*, but our results only partially confirmed this idea. Generally, impacts of drought were more pronounced at the managed as compared to the abandoned grassland, which could have been caused by beneficial effects of a thicker litter layer and higher soil organic matter content on soil moisture 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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Tables

Table 1. Soil parameters, nitrogen pools (µg N g^{-1}DW soil and in µg N g^{-1} soil water: *water sol.*), nitrogen turnover rates (µg N g^{-1} DW soil d^{-1}), mean residence times (MRT) of ammonium and nitrate (h), 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: °=<0.1; *=p<0.05; **=p<0.01;***=p<0.001 ([1]differences between sites for WHC_{max}; *n=16*, and SOM content *n=12* were analysed by t-tests).

	Meadow	**Abandoned site**	**Site**		**Time**		**Site x 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)	73.7	***	1.3		0.2	
$\delta^{13}C$ (‰) *bulk soil*	-26.5 (± 0.2)	-25.6 (± 0.1)	44.1	***	0.9		1.4	
$\delta^{15}N$ (‰) *bulk soil*	4.9 (± 0.2)	4.1 (±0.2)	11.2	**	1.4		0.9	
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_{NH4+}	27.4 (± 6.8)	33.3 (± 6.1)	1.7		2.2	°	1.3	
MRT_{NO3-}	30.3 (±10.0)	2.2 (± 0.7)	29.4	***	1.8		2.5	*
EON *water sol.*	51.3 (± 3.4)	60.6 (± 4.6)	3.8	°	3.9	*	2.5	*
NH_4^+ *water sol*	9.2 (± 0.8)	12.0 (± 0.7)	12.0	**	2.3	°	2.0	°
NO_3^- *water sol*	3.3 (± 0.9)	0.5 (± 0.1)	20.1	***	0.7		4.0	**
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	$1.79x10^6$	$1.82x10^6$	0.0		0.5		0.5	
AOB	$8.68x10^5$	$2.25x10^5$	37.2	***	1.3		0.4	
AOA:AOB ratio	2.8 (± 0.6)	26.3 (±13.2)	27.0	***	0.4		0.7	

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	*amoA*1F, *amoA*2R (Rotthauwe et al., 1997)	*Nitrosomonas*sp.

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: °=p<0.1; *=p<0.05; **=p<0.01; ***=p<0.001.

	Meadow						**Abandoned site**					
	Drought		Time		Drought x Time		Drought		Time		Drought x 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	°
C_{tot}:N_{tot}	2.2		1.5		0.6		6.4	*	6.2	***	1.9	
$\delta^{13}C$ (‰) *bulk soil*	3.6	°	1.1		0.7		3.9	°	1.9		0.3	
$\delta^{15}N$ (‰) *bulk soil*	14.9	***	3.6	**	1.8		<0.1		2.8	*	1.0	
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_{NH4+}	3.7	°	1.4		1.6		1.8		2.0		2.8	
MRT_{NO3-}	<0.1		1.0		2.3		0.6		1.3		0.4	
EON *water sol.*	82.2	***	5.1	***	6.0	***	66.1	***	15.0	***	4.0	**
NH_4^+ *water sol*	47.6	***	4.3	**	4.7	**	42.2	***	5.7	***	3.2	*
NO_3^- *water sol*	4.2	*	2.2	**	1.8		4.0		0.8	°	2.7	*
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	

Figures

Figure 1. Precipitation (a) and soil water content (b, SWC) calculated as percentage of the respective WHC_{max}. Grey bars indicate precipitation (in 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). The period of drought treatment is marked as grey background.

Figure 2. Soil N concentrations per g^{-1} dry soil 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). Effects of drought and sampling time were assessed by two-way repeated measures ANOVA, for further details see Table 3.

Figure 3. Soil N concentrations per g^{-1} soil water 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; °p<0.1; *p<0.05; **p<0.01, ***p<0.001, (*n=4,* respectively, bars show means; error bars indicate standard error). Effects of drought and sampling time were assessed by two-way repeated measures ANOVA, for further details see Table 3.

Figure 4. 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-testswith Bonferroni corrected levels of significance(*n=4,* respectively,error bars indicate standard error). Effects of drought and sampling time were assessed by two-way repeated measures ANOVA, for further details see Table 3.

Figure 5. 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. Effects of drought and sampling time were assessed by two-way repeated measures ANOVA, for further details see Table 3.

Figure 6. 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).

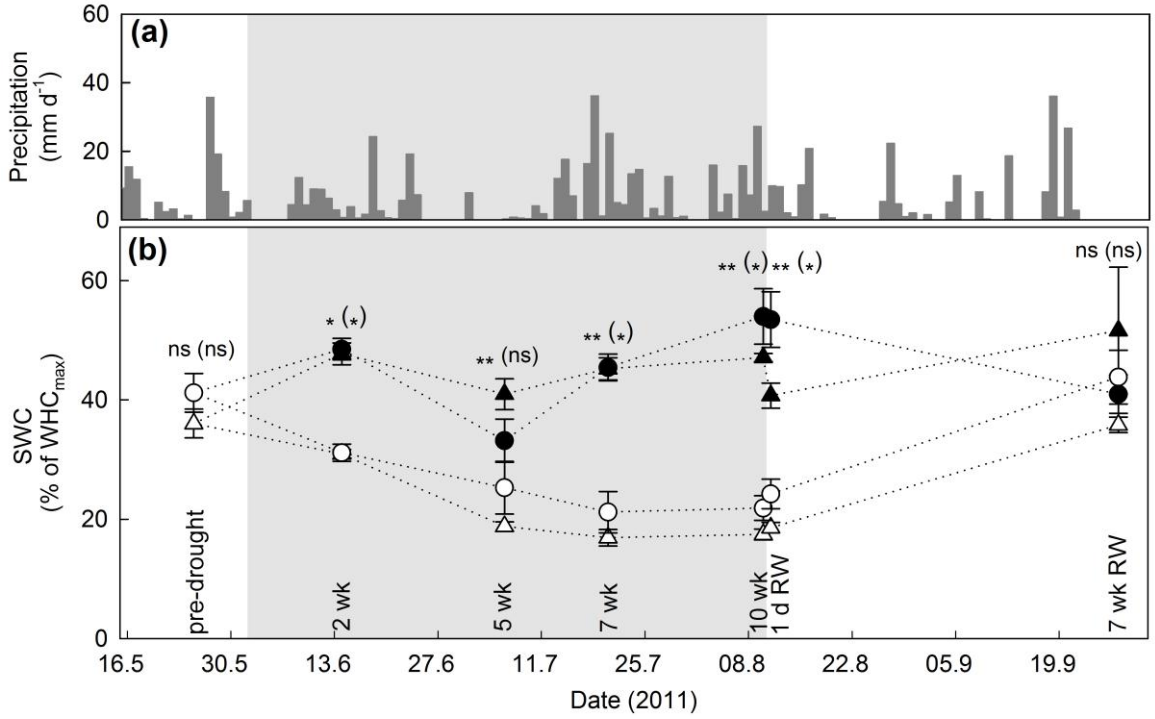
(a)
Precipitation (mm d-1)
60
40
20
0
(b)
SWC (% of WHCmax)
60
40
20
0
ns (ns)
* (*)
** (ns)
** (*)
** (*) ** (*)
ns (ns)
pre-drought
2 wk
5 wk
7 wk
10 wk 1 d RW
7 wk RW
16.5
30.5
13.6
27.6
11.7
25.7
08.8
22.8
05.9
19.9
Date (2011)

Figure 1

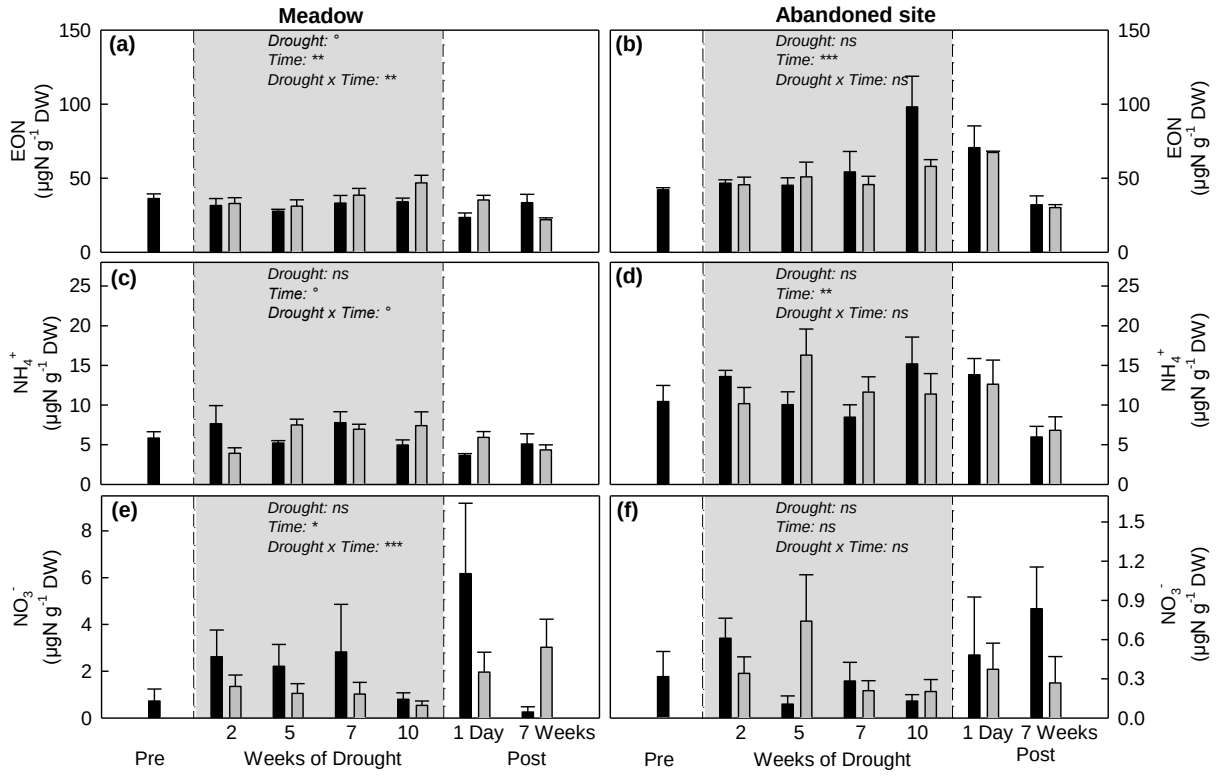
Meadow
Abandoned site
(a)
Drought: °
Time: **
Drought x Time: **
(b)
Drought: ns
Time: ***
Drought x Time: ns
(c)
Drought: ns
Time: °
Drought x Time: °
(d)
Drought: ns
Time: **
Drought x Time: ns
(e)
Drought: ns
Time: *
Drought x Time: ***
(f)
Drought: ns
Time: ns
Drought x Time: ns
EON (µgN g-1 DW)
NH4+ (µgN g-1 DW)
NO3- (µgN g-1 DW)
Pre
2
5
7
10
1 Day
7 Weeks
Weeks of Drought
Post

Figure 2

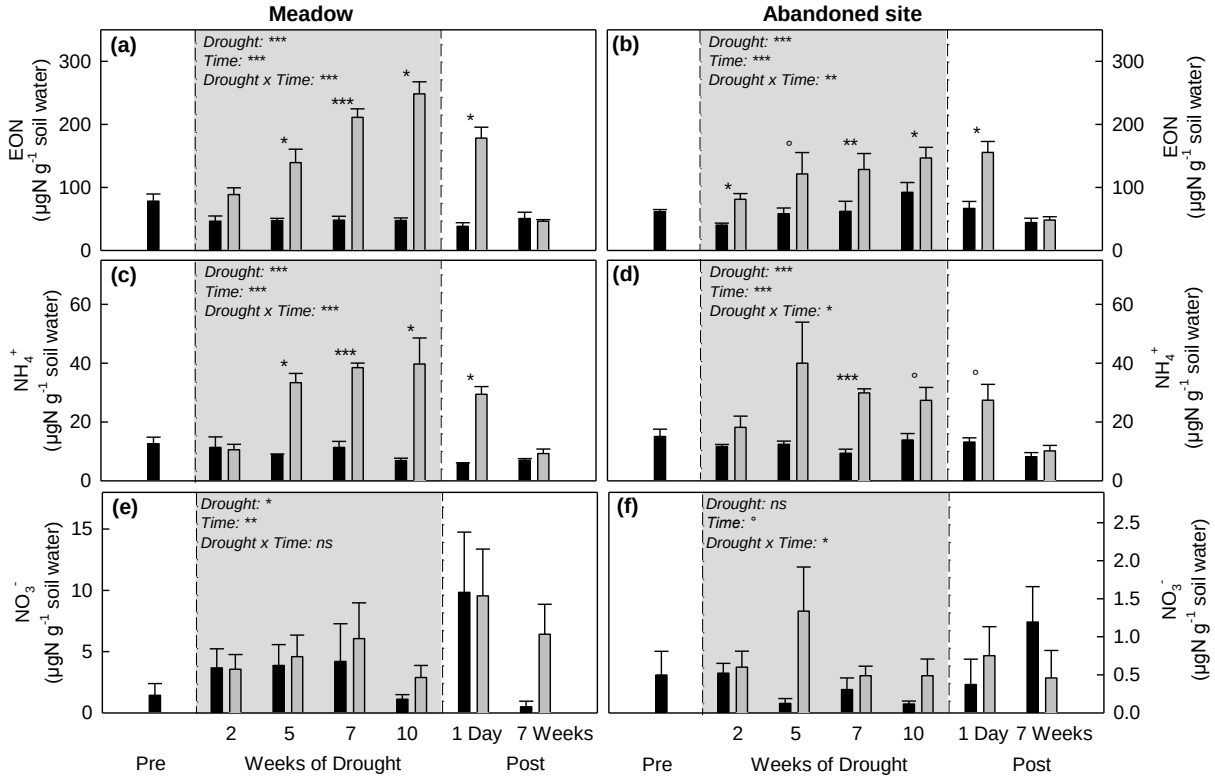
Meadow
Abandoned site
(a)
(b)
(c)
(d)
(e)
(f)
Drought: ***
Time: ***
Drought x Time: ***
Drought: ***
Time: ***
Drought x Time: **
Drought: ***
Time: ***
Drought x Time: ***
Drought: ***
Time: ***
Drought x Time: *
Drought: *
Time: **
Drought x Time: ns
Drought: ns
Time: °
Drought x Time: *
EON (µgN g-1 soil water)
NH4+ (µgN g-1 soil water)
NO3- (µgN g-1 soil water)
Pre
2
5
7
10
1 Day
7 Weeks
Weeks of Drought
Post

Figure 3

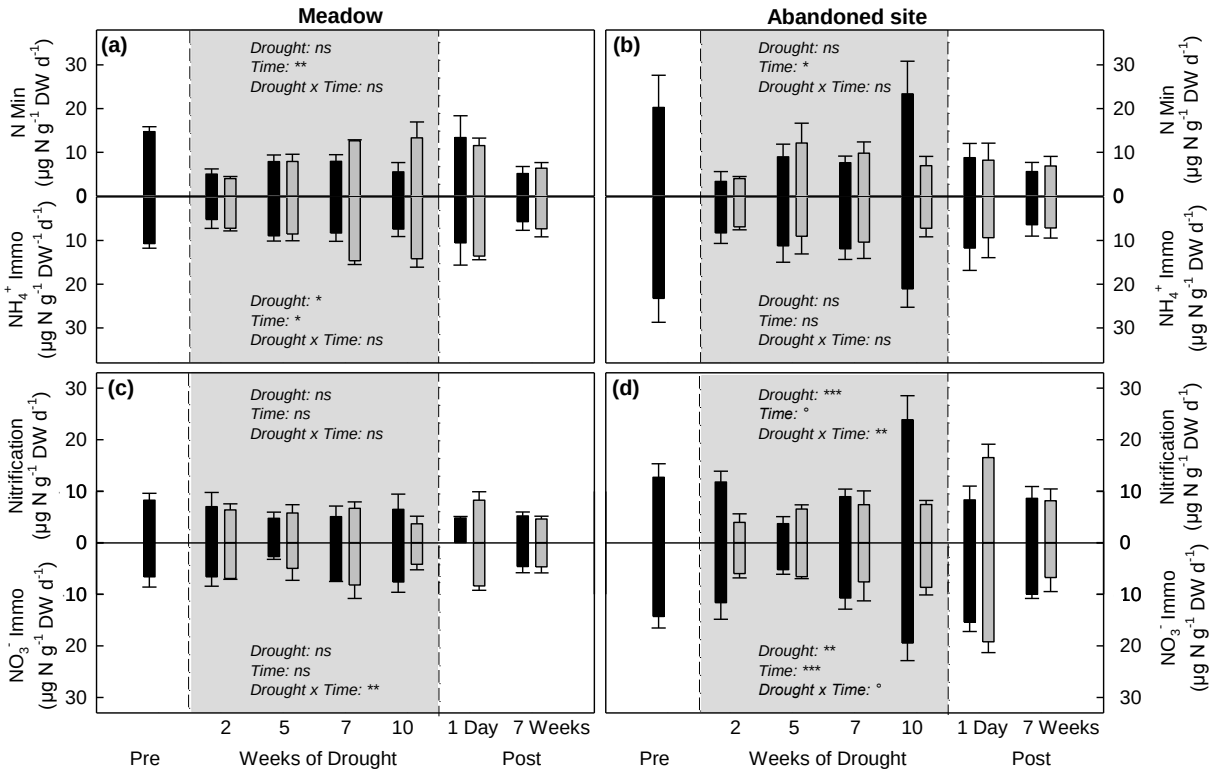
Meadow
Abandoned site
(a)
(b)
(c)
(d)
Drought: ns
Time: **
Drought x Time: ns
Drought: *
Time: *
Drought x Time: ns
Drought: ns
Time: *
Drought x Time: ns
Drought: ns
Time: ns
Drought x Time: ns
Drought: ns
Time: ns
Drought x Time: **
Drought: ***
Time: °
Drought x Time: **
Drought: **
Time: ***
Drought x Time: °
N Min (µg N g-1 DW d-1)
NH4+ Immo (µg N g-1 DW d-1)
Nitrification (µg N g-1 DW d-1)
NO3- Immo (µg N g-1 DW d-1)
30 20 10 0 10 20 30
Pre
2 5 7 10
Weeks of Drought
1 Day 7 Weeks
Post

Figure 4

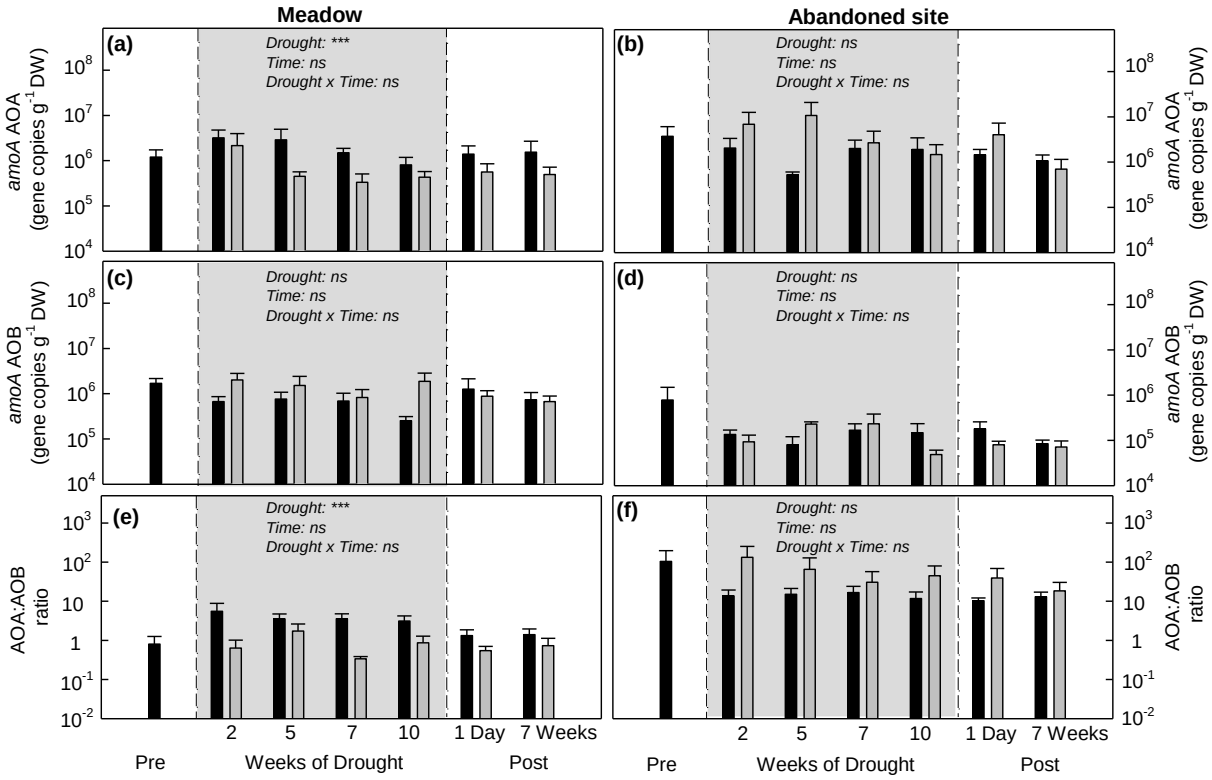
Meadow
Abandoned site
(a)
Drought: ***
Time: ns
Drought x Time: ns
(b)
Drought: ns
Time: ns
Drought x Time: ns
(c)
Drought: ns
Time: ns
Drought x Time: ns
(d)
Drought: ns
Time: ns
Drought x Time: ns
(e)
Drought: ***
Time: ns
Drought x Time: ns
(f)
Drought: ns
Time: ns
Drought x Time: ns
amoA AOA (gene copies g-1 DW)
amoA AOB (gene copies g-1 DW)
AOA:AOB ratio
Pre
2
5
7
10
1 Day
7 Weeks
Weeks of Drought
Post

Figure 5

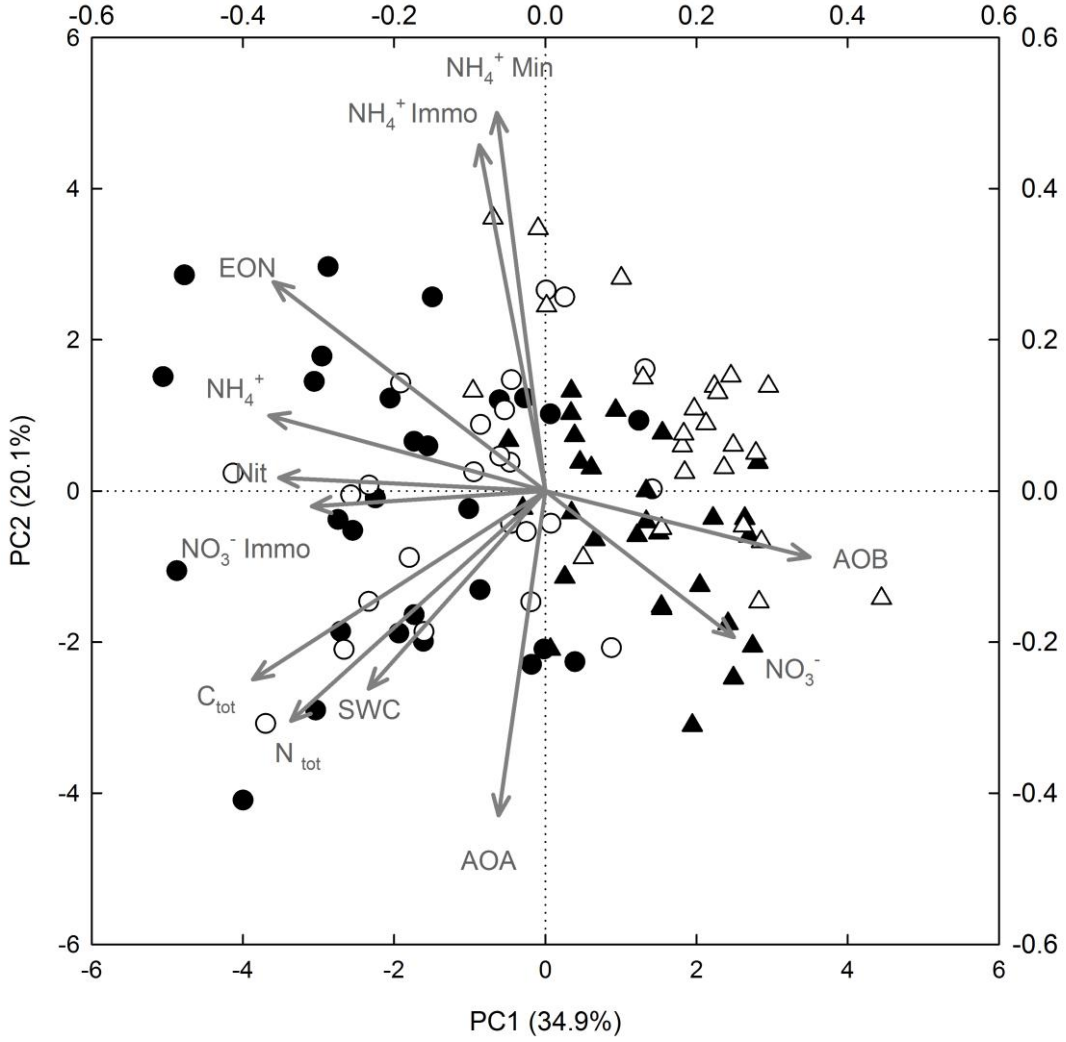
NH4+ Min
NH4+ Immo
EON
NH4+
Nit
NO3- Immo
Ctot
SWC
N tot
AOA
AOB
NO3-
PC1 (34.9%)
PC2 (20.1%)

Figure 6