



# Short term effects of climate change and intensification of management on the abundance of microbes driving nitrogen turnover in montane grassland soils



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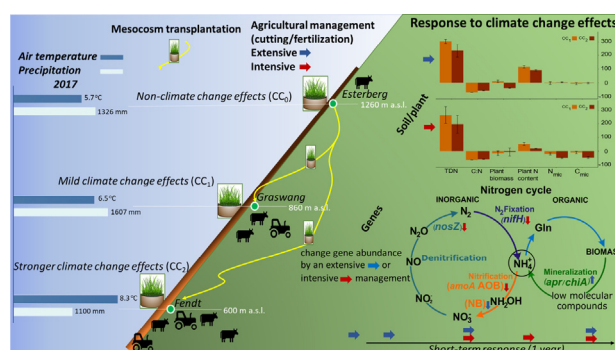
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## HIGHLIGHTS

- Climate change alone mostly influenced soil and plant properties.
- Climate change and management interactions caused shifts in microbial N turnover.
- Mineralizers and diazotrophs react differently.
- Ammonia oxidizing archaea and denitrifiers are resilient to short-term exposure.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Montane grasslands in Europe are exposed to increasing temperatures twice as fast as the global average. Changes in climatic conditions are possibly accompanied by an increase in land use intensity, caused by a prolongation of the vegetation period and the need to improve productivity. Therefore, the investigation of combined effects of climate change and land use intensity is needed to further implement agricultural management strategies. Here we present results from a study performed in the pre-alpine region of southern Germany, where intact plant-soil mesocosms from grasslands, were translocated along an altitudinal gradient, resulting in an increase in soil temperature (moderate treatment: +0.5 K; strong treatment: +1.9 K warming) during the experimental period. Additionally, we applied an extensive or intensive agricultural management (two vs. five times of mowing and slurry application) on the transplanted mesocosms. After an exposure of one year, we measured plant growth and soil properties and quantified abundances of soil microorganisms catalyzing key steps in the nitrogen (N) cycle. Our data indicate, significant interactions between climate change and management. For example, microbial biomass was significantly reduced (−47.7% and −49.8% for C<sub>mic</sub> and N<sub>mic</sub> respectively), which was further accompanied by lower abundances of N<sub>2</sub>-fixing bacteria (up to −89.3%), as well as ammonia oxidizing bacteria (−81.4%) under intensive management, whereas N-mineralizing bacteria increased in abundance (up to +139.8%) under extensive management. Surprisingly, the abundances of denitrifying bacteria as well as mean N<sub>2</sub>O emissions were not affected by the treatments. Overall, our data suggest pronounced shifts in the abundance of microbes driving the N cycle in soil as a result of combined climate change and land use intensification already after a short simulation period of one year.

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## 1. Introduction

Montane grassland soils, which are often rich in organic matter, support a large number of important ecosystem functions including carbon sequestration and nutrient retention, preservation of biodiversity and the protection of endangered plant- and animal species (Bardgett and Caruso, 2020; Wagg et al., 2019; Xiong et al., 2018). Furthermore, these grasslands are important local recreational areas and play a significant role for the tourism in the particular regions (Jobbágy and Jackson, 2000; Leifeld et al., 2009; Schirpke et al., 2013). However, montane grasslands are affected by climate change more than grasslands of other regions. Current regional climate models for the European Alpine region predict warming to progress as twice as fast as the global average (Auer et al., 2007; Gobiet et al., 2014). Until the end of this century, this may result in a loss of permanent snow cover in winter in altitudes of up to 1000 m and a decrease in precipitation mostly during summer (Etter et al., 2017; Gobiet et al., 2014; IPCC, 2018). Consequently, the frequency of freezing thawing cycles in winter may increase due to the loss of the snow cover, followed by prolonged periods of drought during summer.

It is obvious that these scenarios will have a strong impact on the ecosystem functions described above. In particular, soil quality will be strongly affected and shifts in soil respiration and nutrient cycling are well described (Carey et al., 2016; Dawes et al., 2017; Rogora et al., 2018; Schindlbacher et al., 2012). Those changes might induce negative feedback loops for above- (Berauer et al., 2019; Liu et al., 2018) and belowground biota and their activity, even in the short term (Castro et al., 2010; Maestre et al., 2015; Rogora et al., 2018; Schindlbacher et al., 2012). Consequences of shifts in climatic conditions on nitrogen (N) turnover and microorganisms, which catalyze the different transformation reactions, have been well described. Wang et al. (2016) demonstrated that soil N turnover is sensitive to warming (+2 K mean annual temperature), reduced precipitation (−500 mm mean annual precipitation) and increased numbers of freeze-thaw cycles. Under these climate change conditions, high gross N turnover together with an increase in nitrifying microorganisms has been observed in frozen soil (Wang et al., 2016), which might be a result of accumulation of dissolved carbon (C) and N substrates together with the microorganisms in the water film (Watanabe et al., 2019). N mineralization, nitrification rates and N<sub>2</sub>O formation increased due to warming (Hart, 2006; Hu et al., 2017; Larsen et al., 2011; Rustad et al., 2001) and are in addition affected by interactions of moisture and temperature (Auyeung et al., 2015; Auyeung et al., 2013; Dannenmann et al., 2016; Larsen et al., 2011). Therefore, climate change effects may induce N losses as a result of increased NO<sub>3</sub><sup>−</sup> leaching as well as N<sub>2</sub>O and N<sub>2</sub> gas emissions (Kuypers et al., 2018; Stein and Klotz, 2016; Zistl-Schlingmann et al., 2019) or by uptake into the plant biomass on the short run. On the long run this might induce imbalanced nitrogen contents in soils which negatively affect plant growth and crop productivity (LeBauer and Treseder, 2008).

In addition to climate change, montane grasslands are strongly affected by an increase in land use intensity as mainly sites which are easily accessible by farmers are used for husbandry or hay production more intensely. Land use intensity is negatively correlated to the richness of microbial communities not only in bulk soil, but also in the rhizosphere and root interior of typical plants grown in these grasslands (Estendorfer et al., 2017; Schöps et al., 2018), which can negatively impact plant performance and stress response. In addition, effects on N turnover in soil have been well documented. It is well accepted that N<sub>2</sub>O emissions, which contribute to global warming and ozone depletion can significantly increase with intensified N fertilization (Ravishankara et al., 2009; Shcherbak et al., 2014). Further, it has been shown that nitrification rates and abundance of nitrifiers increased with increasing land use intensity in grassland soils in Germany (Stempfhuber et al., 2014). Similar, the abundance of nitrifiers was driven by the management practice trajectories and soil environmental conditions along an intensity management gradient in European

montane grasslands (Szukics et al., 2019). Thus, increased N inputs through land use intensification result in increased NO<sub>3</sub><sup>−</sup> leaching and higher N<sub>2</sub>O emission (Bowles et al., 2018; Klaus et al., 2018).

Surprisingly, despite being the rule, not the exception, it is still unclear how combinations of climate change and land use intensification affect montane grassland ecosystems. In the frame of this study, we analyzed combined effects of climate change and management intensification on the abundance of microbiota involved in N turnover. The abundance of microbial functional groups characterizes the potential of a microbial community for a given transformation processes, which is further induced in situ depending on the actual environmental conditions. Thus, the genotype of microbiota, which is in the focus of this study can be considered as a more stable parameter compared to the microbial phenotype, which is highly fluctuating even during a single day. To simulate different intensities of climate change (i.e. mean annual temperature increase) and land use intensities, we transplanted intact plant-soil mesocosms from high altitude to two lower altitudes resulting in a temperature increase of 0.5 K and 1.9 K respectively. To account for management effects, the mesocosms underwent either an extensive or intensive agricultural management (two- versus five times of mowing and fertilization). We measured different N pools (plant, microbial, soil) and the abundance of microbiota, which catalyze major steps in the organic and inorganic N cycle including N-mineralization, N<sub>2</sub>-fixation, nitrification and denitrification to disentangle short-term effects of climate change and agricultural management after one year of transplantation on N turnover from a typical montane grassland.

Wiesmeier et al. (2013) demonstrated that SOC and N stocks in montane grasslands are protected, because of the lower temperature and the higher humidity of those soils. Thus, we hypothesized, that (1) already the short-term exposure to simulated climate change increases the abundance of N-mineralizing bacteria, which are able to directly decompose the organic N sources in montane grassland soils. Plants will benefit from this increased availability of N resulting in higher plant biomass mostly under extensive management. (2) Under intensive management the surplus of ammonia as a result of increased N-mineralization and fertilization will result in higher abundances of nitrifiers and denitrifiers together with a reduced abundance of N-fixing bacteria, triggering high N losses via leaching and gas emissions. (3) Effect sizes are correlated with the intensity of the simulated change in climate conditions.

## 2. Materials and methods

### 2.1. Study sites and experimental design

This study was conducted in the montane region in southern Germany close to Garmisch-Partenkirchen. We used a space-for-time approach along an altitudinal gradient from Esterberg (47°31' N, 11°09' E, 1260 m a.s.l.) via Graswang (47.57°N; 11.03°E, 860 m a.s.l.) to Fendt (47.83°N; 11.07°E, 600 m a.s.l.) to simulate climate change. The high elevation site Esterberg is a typical extensively used montane grassland. The management included one fertilization in spring, one biomass harvest in July followed by cattle grazing until the end of the growing season (20 animal per ha<sup>−1</sup> during summer). The soil has been classified as a Phaeozem (I.W.G. WRB, 2015), which is rich in soil organic carbon (129.8–188.9 mg g<sup>−1</sup>). In the top 0–15 cm the soil has a TN content of 13.8–18.8 mg g<sup>−1</sup>, resulting in a C:N ratio of 9.4–10.0 (Garcia-Franco et al., 2020). Further the bulk density of the soil is low (0.5–0.6 g cm<sup>−3</sup>) and a pH of 6.1 has been measured (Garcia-Franco et al., 2020; Schlingmann et al., 2020). The soil is characterized by a 30 cm thick Ah horizon, followed by the calcareous parent material. Mean annual N deposition rates were in the range of about 33 ± 7 kg N ha<sup>−1</sup> year<sup>−1</sup> (LFU, 2011).

In July 2016, a total number of 54 intact plant-soil mesocosms (stainless steel cylinders of 17 cm diameter and 25 cm height) were transplanted. Therefore, stainless steel cylinders were inserted into the

soil by hammering at the Esterberg site and excavated. A custom-made stacking frame protected the vegetation from damage. Details are described by Wang et al. (2016). 18 of the mesocosms were transplanted from Esterberg to Graswang (in the following referred as to CC<sub>1</sub>: mild climate change effects), 18 from Esterberg to Fendt (referred as to CC<sub>2</sub>: stronger climate change effects) and 18 served as transplantation control, which were re-transplanted in Esterberg again (hereafter referred to as CC<sub>0</sub>). After translocation, the intact plant-soil mesocosms remained undisturbed for eight months, only one cutting/fertilization was conducted in October 2016 for all mesocosms. Meteorological data during the experimental period in 2017 were described by Schlingmann et al. (2020). During the experimental period, from the first manure application to the second sampling date (see below), mean air temperature and accumulative precipitation along the altitudinal gradient was 11.2 °C and 890 mm, 11.7 °C and 669 mm, and 13.1 °C and 546 mm, respectively for CC<sub>0</sub>, CC<sub>1</sub> and CC<sub>2</sub>.

From mid of March 2017, the mesocosms from all three altitudes were divided into two groups and treated either intensively or extensively to simulate two agricultural management scenarios. The extensive (Ext) management included two manure applications (each ca. 35 kg N ha<sup>-1</sup>) and two mowing events per year. The intensive (Int) management consisted of four to five manure applications and three to four mowing events per year. Management was adapted to environmental conditions and phenology of the plants. No mowing and fertilization was conducted in CC<sub>0</sub>-mesocosms in August, due to insufficient biomass production. The manure fertilization- and mowing regime is summarized in Fig. S1.

## 2.2. Soil and plant sampling

During the management period 2017, two samplings were performed resulting in a total number of 36 sampled mesocosms. The first sampling (six mesocosms per site) took place in May at the beginning of the growing season when only one mowing and one fertilization was done for all mesocosms. The second sampling (six mesocosms per site, three per Int and three per Ext agricultural management) took place when peak plant biomass was reached. Due to the climatic differences of the sites this was performed in August at CC<sub>1</sub> and CC<sub>2</sub>, and in September at CC<sub>0</sub> (Fig. S1). Soil was sampled from 0 to 5 cm depth and stored at 4 °C and -80 °C for biochemical and molecular analyses, respectively. In addition, aboveground biomass was collected from each mesocosm for measurement of dry weight and nitrogen content. The most dominant plant species in the sampled mesocosms from all treatments and sites were the perennial grasses: *Festuca pratensis* (Huds.), *Agrostis capillaris* (L.), *Elymus repens* (L.), and *Anthoxanthum odoratum* (L.); the perennial herbs: *Carum carvi* (L.), *Taraxacum officinale* (L.), *Ranunculus acris* (L.), and *Myosotis alpestris* (F.W. Schmid) and the perennial legumes: *Trifolium pratense* (L.), and *T. repens* (L.), which accounted for approximately 8% of the plant community (Schlingmann et al., 2020).

## 2.3. Soil and plant analyses

Triplicate soil samples per mesocosm were used to quantify total dissolved nitrogen (TDN) comprising dissolved organic and inorganic N, total dissolved organic carbon (DOC), microbial biomass C (C<sub>mic</sub>) and N (N<sub>mic</sub>) using 0.5 M K<sub>2</sub>SO<sub>4</sub> for extraction. C<sub>mic</sub> and N<sub>mic</sub> were assessed after chloroform-fumigation extraction of soil extracts (Brookes et al., 1985) using the efficiency coefficient K<sub>E</sub> (K<sub>EC</sub> = 0.45 for C and K<sub>EN</sub> = 0.54 for N; according to Joergensen (1996) and Joergensen and Mueller (1996), respectively). Furthermore, 5 g soil were diluted in 20 ml deionized water for pH measurements with a pH meter after shaking for 30 min. Soil moisture and temperature were obtained by means of TDR probes (5TM, METER Group, Germany) attached to a data logger (EM50, METER Group, Munich, Germany). Bulk density (BD) was calculated based on total fresh weight

of the soil layers (0–5 and 6–15 cm) of the entire mesocosm and the respective gravimetric water content. Stone content was negligible in the first 15 cm of the soil and therefore not included in the calculation. Plant biomass was dried at 55 °C until constant weight and grinded. Plant N content (PNC) was assessed using an elemental analyzer (Flash EA, Thermo Scientific, USA). The exchange of N<sub>2</sub>O between soil and atmosphere was measured for 21 sampling dates between April and October 2017. For that purpose, a manual static chamber technique with stainless steel dark chambers directly fitted to the mesocosms was used. Chamber and gas sampling design, analytics using a gas chromatograph with an electron capture detector, and flux calculation as well as quality control procedures are described in detail in Unteregelsbacher et al. (2013). For N<sub>2</sub>O flux measurements, we used three replicated mesocosms for extensive and intensive treatments each at all sites (overall 18 mesocosms).

## 2.4. DNA extraction

DNA was extracted from ca. 0.5 g fresh weight of soil (Lueders et al., 2004; Töwe et al., 2011) with the following modifications: For microbial cell lysis, 750 µl of NaPO<sub>4</sub> buffer (120 mM, pH 8) and 250 µl of TNS (500 mM Tris-HCl, 100 mM NaCl, 10% SDS (wt/vol), pH 8.0) were added for bead beating a Precellys24 homogenizer was used (Bertin Technologies, France) once at 5500 ×g for 30 s. The supernatant was transferred into a 2 ml DNase/RNase free tube on ice. Purification was performed with phenol:chloroform:isoamylalcohol (25:24:1) pH 8 (AppliChem GmbH, Germany) and a solution of chloroform:isoamylalcohol (24:1) according to Lueders et al. (2004). The extracted DNA was dissolved in 50 µl of sterile diethyl pyrocarbonate (DEPC) water. DNA concentrations were quantified by means of a spectrophotometer (Nanodrop, PeqLab, Germany). DNA quality was estimated by the ratios of absorbance at 260 nm to 280 nm, and 260 nm to 230 nm. Soil DNA concentrations were in the range of 114 to 679 µg g<sup>-1</sup> soil dry weight (sdw). DNA extracts were stored at -20 °C until further analysis.

## 2.5. Quantitative real-time PCR assay

Total bacterial abundance and the abundance of microbes catalyzing major steps of the N cycle were quantified by quantitative Real-Time PCR (qPCR). The approach used includes the assessment of the different microbial functional groups using specific marker genes for key transformation steps. For N-mineralizing bacteria the genes *apr* (coding for an alkaline protease) and *chiA* (coding for a chitinase subunit) were used. For ammonia oxidizers the bacterial and archaeal *amoA* gene (coding for a subunit of the ammonium monooxygenase gene) was selected. For *Nitrospira* like nitrite oxidizers (NS) a specific primer targeting the 16S rRNA gene was used, whereas for *Nitrobacter*-like nitrite oxidizers (NB) a subunit of the oxidoreductase gene (*nxrA*) was used. For denitrifiers, nitrite reducers were quantified using the two nitrite reductases *nirK* and *nirS* as proxy, N<sub>2</sub>O reducers were analyzed based on the N<sub>2</sub>O reductase gene *nosZ*. Finally, N<sub>2</sub>-fixing bacteria were quantified based on a subunit of the dinitrogenase reductase gene (*nifH*). For the assessment of total bacterial abundance, universal primers for the 16S rRNA gene were used. Details on the marker genes and qPCR conditions are described in Table S1. qPCRs were carried out using 96-well plates (Applied Biosystems, Germany) with SYBR green as fluorescent dye and performed on a 7300 Real-Time PCR System (Applied Biosystems, Germany). A pre-experiment was conducted to determine the optimal DNA dilution to avoid qPCR inhibition due to co-extracted humic substances, which resulted in an optimal sample dilution of 1:256 (data not shown). Serial plasmid dilutions (10<sup>1</sup>–10<sup>6</sup> gene copies ml<sup>-1</sup>) specific for each gene were used for the determination of standard curves (Table S1). A negative control for the extraction and negative controls (only DEPC water) for qPCR were included in each 96-well plate. The PCR efficiencies of the amplifications were

calculated as  $E = -1 + 10(-1/\text{slope})$  (Töwe et al., 2010) and resulted in the following values: *apr* 87% ( $\pm 2.0$ ), *chiA* 88% ( $\pm 0.4$ ), archaeal *amoA* 84% ( $\pm 2.5$ ), bacterial *amoA* 83% ( $\pm 6.2$ ), NS 80% ( $\pm 1.2$ ), NB 86% ( $\pm 6.2$ ), *nirK* 89% ( $\pm 4.3$ ), *nirS* 86% ( $\pm 0.8$ ), *nosZ* 80% ( $\pm 0.9$ ), *nifH* 82% ( $\pm 1.7$ ) and 16S-rRNA 87% ( $\pm 5.2$ ). The coefficient of determination ( $R^2$ ) of the standard curves was determined to be above 0.99 for each qPCR. The specificity of the amplified products was checked by melting curves of the amplicons and on 2% agarose gels of randomly selected samples.

## 2.6. Statistical analyses

All data was checked for normal distribution by applying the Shapiro-Wilk normality test and linear models “lm”. Homoscedasticity was tested by Levene’s test (Fox, 2016). If data were not normally distributed or with no homogeneous variance, the values were log- or Box-Cox-transformed (Yeo and Johnson, 2000). The effect of only climate change on gene abundance and soil/plant properties was tested in spring when management did not yet differ across all mesocosms ( $n = 6$ ), while for the summer sampling ( $n = 3$ ), the effects of climate change, agricultural management and their interaction on soil/plant properties and gene abundances were tested, using the R package “nlme” for mixed effect models (Pinheiro et al., 2019). For the mixed effect models, climate change (CC<sub>0</sub>, CC<sub>1</sub> and CC<sub>2</sub>) and management (Ext and Int treatments) were selected as fixed factors, while mesocosms were considered as random factor. Differences were considered statistically significant at  $p < 0.05$ . If one of the fixed factors was significantly different, least-squares means for factor combinations were estimated using the R package “lsmeans” pairwise comparison with emmeans test (Lenth, 2016). Correlations between soil/plant properties and gene abundances were calculated by Spearman correlations using the R package “Hmisc” and “corrplot” separately for Ext and Int agricultural management. Multiple stepwise regression analysis was performed to identify the potential drivers of gene abundance for each season, choosing the best model by Akaike’s Information Criterion (AIC, both forward selection and backward elimination) implemented in the R package “MASS”. In addition, principal component analyses were done using

the packages “FactoMineR” and “factoextra”; for missing values ( $n = 3$ ) the “imputePCA” function of the “missMDA” package was applied. In addition, the seasonal effect of climate change CC<sub>1</sub> and CC<sub>2</sub> was calculated as the relative change (RC) for all response parameters (soil/plant properties or gene abundances) compared to the respective control CC<sub>0</sub> according to Berauer et al. (2019). This RC was calculated as:

$$RC = ((\text{Sample} - \bar{x}CC_0) / \bar{x}CC_0) \quad (1)$$

In Eq. (1) *Sample* denotes a single translocated mesocosm and  $\bar{x}CC_0$  the mean of all reinserted mesocosms in CC<sub>0</sub>.

After checking for normality and homoscedasticity, differences in RC between elevations and agricultural management were analyzed by mixed effect models as mentioned before. To test if a RC at a single elevation and management type was significantly different from zero, we used one sample *t*-test under the null hypothesis  $\mu = 0$ . All statistical analyses were conducted using R software version 4.0.2 (R Core Team, 2020).

## 3. Results

### 3.1. Abundance of microbial key players driving N turnover in montane grassland soils

At the first sampling time point in spring, all mesocosms only differed in climate change effects but were managed similarly (Fig. S1). While already significant effects of climate change on most of the measured soil/plant properties were found (Table 1), except for BD and DOC:TDN ratio in soil, the abundance of microbes catalyzing major steps in N mineralization, nitrification, denitrification were not affected by the simulated climate change (Table S2; Fig. S2). Only for N<sub>2</sub>-fixing bacteria a slight decrease towards CC<sub>2</sub> was observed which did not reach statistical significance.

At the second sampling time point in summer, climate change was a significant factor for explaining the variances of N-mineralizing bacteria ( $P = 0.02$  for *apr* and  $P = 0.009$  for *chiA*; Table S2). N-mineralizing

**Table 1**

Soil physiochemical and plant properties during the short-term exposure to climate change effects (CC) and agricultural management (T) in pre-alpine grasslands. Values are means  $\pm$  standard error ( $n = 3$ ). Significant differences ( $P < 0.05$ ) between CC in the same T (lowercase for extensive and capital letters for intensive) are indicated by different letters. Significant differences ( $P < 0.05$ ) between T in the same CC are indicated by bold characters.

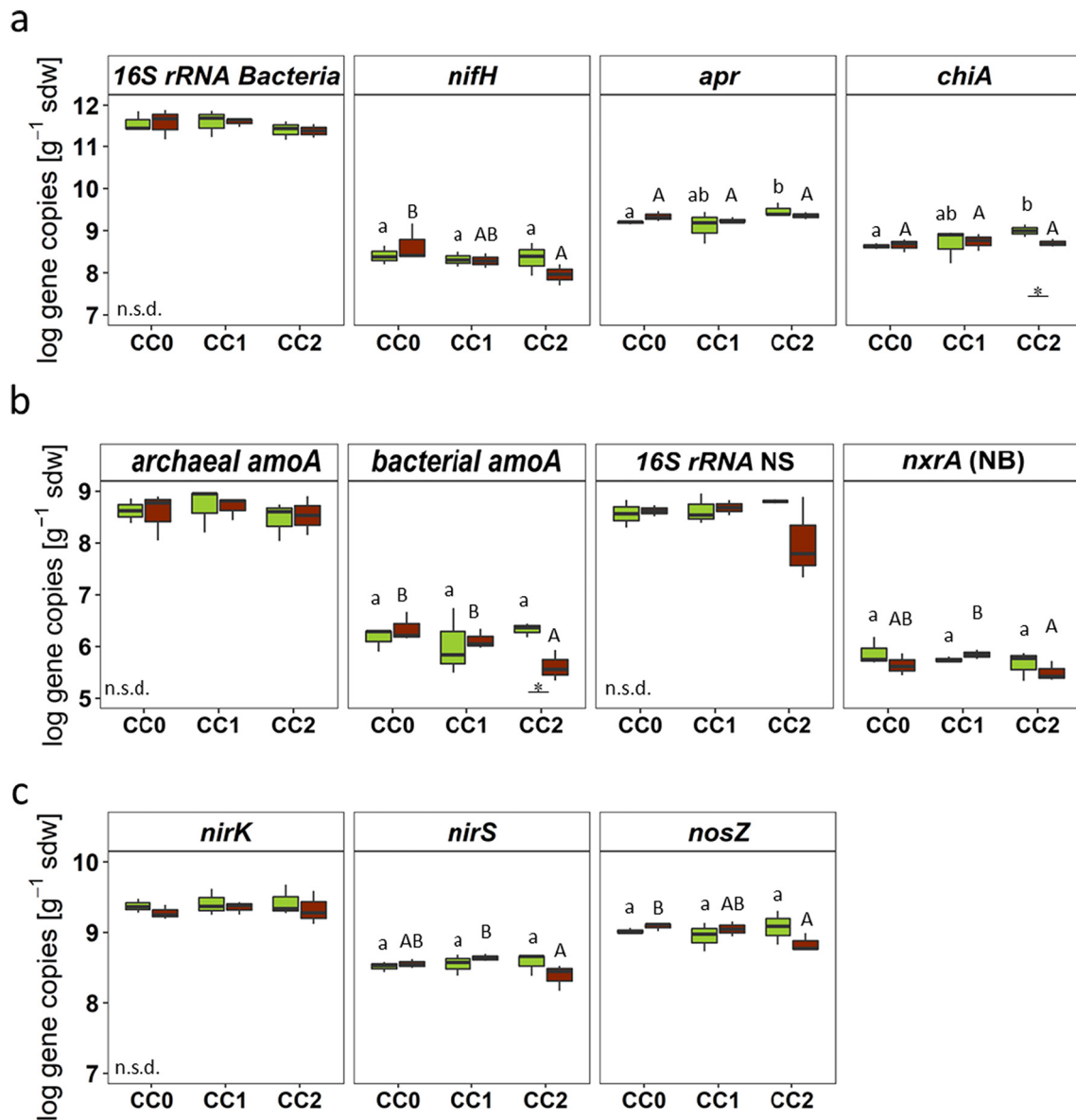
Season	Spring				Summer						* Factors/ interaction $P < 0.05$
	Extensive			CC	Extensive			Intensive			
Management	CC <sub>0</sub>	CC <sub>1</sub>	CC <sub>2</sub>			CC <sub>0</sub>	CC <sub>1</sub>	CC <sub>2</sub>	CC <sub>0</sub>	CC <sub>1</sub>	CC <sub>2</sub>
Climate change	Mean values from 17 <sup>th</sup> March to sampling date				Mean values from 1 <sup>st</sup> June to sampling date						
Air temperature (°C)	4.74 $\pm$ 0.41 a	5.87 $\pm$ 0.45 a	7.67 $\pm$ 0.43 b	CC	14.50 $\pm$ 0.40 a	16.30 $\pm$ 0.43 b	18.15 $\pm$ 0.41 c	14.50 $\pm$ 0.40 A	16.30 $\pm$ 0.43 B	18.15 $\pm$ 0.41 C	CC
Soil temperature (°C)	4.07 $\pm$ 0.08 a	7.35 $\pm$ 0.09 b	8.96 $\pm$ 0.17c	CC	16.2 $\pm$ 0.16 a	18.5 $\pm$ 0.26 b	19.7 $\pm$ 0.08 c	15.9 $\pm$ 0.17 A	18.9 $\pm$ 0.28 B	20.0 $\pm$ 0.31 C	CC
Soil moisture (cm <sup>3</sup> cm <sup>-3</sup> )	0.32 $\pm$ 0.01 a	0.30 $\pm$ 0.01 a	0.41 $\pm$ 0.02 b	CC	0.36 $\pm$ 0.02 b	0.31 $\pm$ 0.01 ab	0.27 $\pm$ 0.02 a	0.33 $\pm$ 0.03 A	0.29 $\pm$ 0.03 A	0.32 $\pm$ 0.03 A	CC
	Measurements from sampling date				Measurements from sampling date						
TDN (mg N kg <sup>-1</sup> sdw)	42.8 $\pm$ 3.3 a	44.7 $\pm$ 3.1 a	144.0 $\pm$ 23.1 b	CC	41.0 $\pm$ 3.0 a	165.6 $\pm$ 6.4 b	137.3 $\pm$ 19.4 b	38.4 $\pm$ 1.4 A	140.0 $\pm$ 23.7 B	113.5 $\pm$ 25.2 B	CC
DOC (mg C kg <sup>-1</sup> sdw)	193.5 $\pm$ 13.9 a	232.8 $\pm$ 27.5 a	633.1 $\pm$ 98.0 b	CC	270.5 $\pm$ 6.3 a	328.8 $\pm$ 13.6 b	392.0 $\pm$ 71.7 ab	259.7 $\pm$ 20.0 A	324.2 $\pm$ 48.6 A	307.2 $\pm$ 58.7 A	CC
DOC:TDN	4.54 $\pm$ 0.14 a	5.14 $\pm$ 0.27 a	4.46 $\pm$ 0.22 a	NSD	6.68 $\pm$ 0.60 c	1.98 $\pm$ 0.01 a	2.82 $\pm$ 0.17 b	6.75 $\pm$ 0.29 C	2.34 $\pm$ 0.07 A	2.74 $\pm$ 0.08 B	CC, CC*T
Bulk density (g cm <sup>-3</sup> )	0.32 $\pm$ 0.02 a	0.30 $\pm$ 0.01 a	0.33 $\pm$ 0.02 a	NSD	0.31 $\pm$ 0.03 a	0.29 $\pm$ 0.01 a	0.32 $\pm$ 0.02 a	0.32 $\pm$ 0.02 A	0.31 $\pm$ 0.03 A	0.38 $\pm$ 0.04 A	NSD
Plant biomass (g)	4.69 $\pm$ 0.71 a	9.51 $\pm$ 0.73 b	10.95 $\pm$ 0.93 b	CC	14.05 $\pm$ 0.50 b	15.38 $\pm$ 1.28 b	8.60 $\pm$ 0.40 a	12.00 $\pm$ 0.40 A	10.15 $\pm$ 1.95 A	11.23 $\pm$ 3.58 A	CC, T
Biomass N content (%)	2.52 $\pm$ 0.15 b	2.55 $\pm$ 0.10 b	1.89 $\pm$ 0.09 a	CC	0.93 $\pm$ 0.09 a	2.01 $\pm$ 0.09 c	1.77 $\pm$ 0.05 b	0.92 $\pm$ 0.02 A	1.40 $\pm$ 0.10 C	1.10 $\pm$ 0.02 B	CC, CC*T
Microbial C (mg C kg <sup>-1</sup> sdw)	1548 $\pm$ 68.1 b	1465 $\pm$ 52.5 b	1166 $\pm$ 52.8 a	CC	3444 $\pm$ 39.4 a	3209 $\pm$ 286.9 a	3363 $\pm$ 179.0 a	4013 $\pm$ 367.3 B	3620 $\pm$ 341.5 B	2100 $\pm$ 245.5 A	CC*T
Microbial N (mg N kg <sup>-1</sup> sdw)	262.4 $\pm$ 13.1 b	225.1 $\pm$ 19.3 b	145.8 $\pm$ 13.3 a	CC	463.7 $\pm$ 23.2 a	451.2 $\pm$ 43.9 a	475.1 $\pm$ 13.6 a	637.7 $\pm$ 137.1 B	508.9 $\pm$ 49.2 B	320.2 $\pm$ 26.7 A	CC*T
C <sub>mic</sub> :N <sub>mic</sub>	5.91 $\pm$ 0.09 a	6.67 $\pm$ 0.39 a	8.19 $\pm$ 0.58 b	CC	7.47 $\pm$ 0.40 a	7.13 $\pm$ 0.27 a	7.08 $\pm$ 0.31 a	6.60 $\pm$ 0.74 A	7.14 $\pm$ 0.36 A	6.53 $\pm$ 0.24 A	NSD
pH	6.86 $\pm$ 0.06 b	7.06 $\pm$ 0.05 c	6.39 $\pm$ 0.12 a	CC	6.72 $\pm$ 0.11 a	6.55 $\pm$ 0.16 a	6.68 $\pm$ 0.15 a	6.87 $\pm$ 0.10 A	6.52 $\pm$ 0.39 A	6.70 $\pm$ 0.16 A	NSD

TDN: Total dissolved nitrogen. DOC: dissolved organic carbon. NSD: not significant difference. \* Significant fixed factors (CC, T) and their interaction (CC\*T) are given after ANOVA of nlme models. Temperatures and soil moisture are mean values of data recorded from the beginning of the management application in March to the sampling date in Spring, and from June to the sampling date in summer.

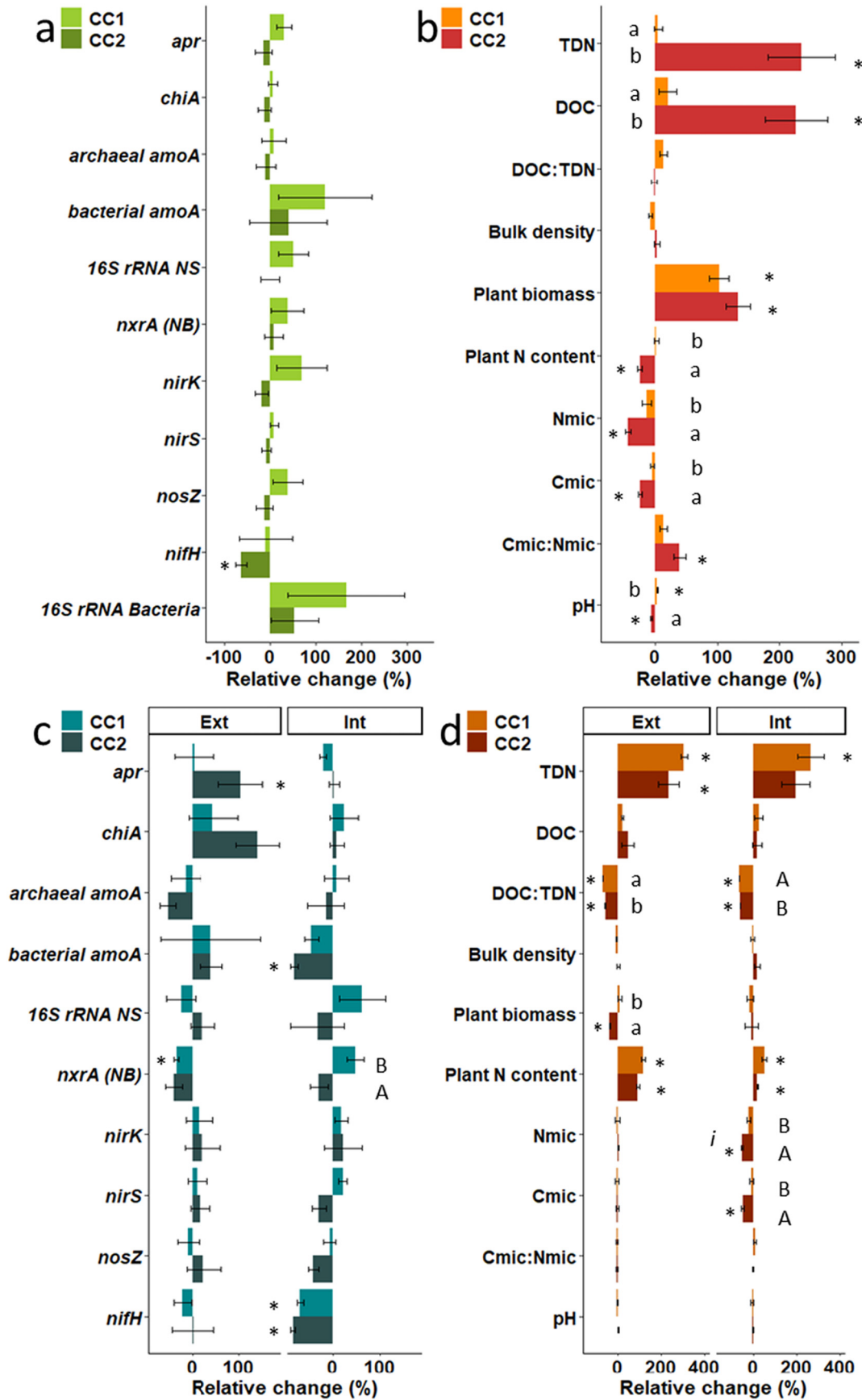
bacteria increased at CC<sub>2</sub> when an Ext management was applied as compared with the same management at CC<sub>0</sub> ( $P = 0.05$  for *apr* and  $P = 0.01$  for *chiA*; Fig. 1). Agricultural management alone had no significant effect on the abundance of N mineralizing bacteria, but the interaction of agricultural management and climate change (CC\*T) altered the abundance of N-mineralizing bacteria, particularly those with chitinolytic activity (*chiA*;  $P = 0.03$ ). Specifically, a significantly higher abundance of *chiA* was observed under extensive management compared to intensive management at CC<sub>2</sub> (Fig. 1; Table S2). In addition, the abundance of bacterial ammonia oxidizers, but not of their archaeal counterpart, was affected by the interaction of climate change and management ( $P = 0.003$ ), as only in the Int treatment the gene abundance significantly dropped from CC<sub>0</sub> to CC<sub>2</sub> (Fig. 1). Additionally, this resulted in significantly lower abundance of bacterial ammonia oxidizers in the Int compared the Ext treatment at CC<sub>2</sub> ( $P = 0.03$ ). Also, gene abundance of nitrite oxidizers (NS and NB) trended to decrease at CC<sub>2</sub> in the Int

treatment. Similar observations were made for nitrite reducing bacteria harboring the *nirS* gene, where a significant difference between CC<sub>1</sub> and CC<sub>2</sub> was visible in Int treatment ( $P = 0.02$  for NB and  $P = 0.01$  for *nirS*). Comparing CC<sub>0</sub> with CC<sub>2</sub>, significant differences were found only under Int management for nitrous oxide reducing bacteria (*nosZ* gene;  $P = 0.008$ ), and N<sub>2</sub>-fixing bacteria (*nifH* gene;  $P = 0.04$ ).

In order to measure the magnitude of the impact of the climate change effects and agricultural management on the gene abundances and soil/plant parameters, relative changes (RC) compared to the control (CC<sub>0</sub>) were calculated for CC<sub>1</sub> and CC<sub>2</sub> (Berauer et al., 2019) in spring, and summer for each agricultural management (Fig. 2). Again, climate change effects were already visible for abiotic soil and plant properties. TDN was strongly increased when soil mesocosms were translocated to CC<sub>2</sub> confirming the above-described data. The same was observed for plant biomass, already at CC<sub>1</sub>, and C<sub>mic</sub>:N<sub>mic</sub> ratio. The latter was caused by a stronger drop of N<sub>mic</sub> concentrations



**Fig. 1.** Gene abundances per gram of soil dry weight (sdw) in summer for: total (16s rRNA), N<sub>2</sub>-fixing (*nifH*) and N mineralizing (*apr* and *chiA*) bacteria (a); Nitrifiers (b): ammonia oxidizing archaea (archaeal *amoA*) and bacteria (bacterial *amoA*), nitrite oxidizing (NS and NB) bacteria; Denitrifiers (c): nitrite (*nirK* and *nirS*) and nitrous oxide reducing (*nosZ*) bacteria. The mesocosms at the high-elevation (CC<sub>0</sub>) were translocated to CC<sub>0</sub> (Esterberg, 1260 m a.s.l.), CC<sub>1</sub> (Graswang, 860 m a.s.l.) and CC<sub>2</sub> (Fend, 600 m a.s.l.) and treated similarly by an extensive (green) and intensive (brown) agricultural management. Boxplots represent soil gene copy numbers (on a log<sub>10</sub> scale), medians (black lines inside the box) and maximal and minimal values (n = 3). Significant differences ( $P < 0.05$ ) after pairwise comparisons are represented by different letters for each management. Capital letters show significant differences for intensively and lowercase letters for extensively managed mesocosms. \* indicates significant difference between agricultural management in the same climate change condition. n.s.d.: not significant difference.



**Fig. 2.** Relative change (represented in percentage) of gene abundances (a, c) or soil properties (b, d) compared to the respective control at CC<sub>0</sub> for spring (a, b; n = 6), and for extensive (Ext) and intensive (Int) agricultural management in summer (c, d; n = 3). Significant differences ( $P < 0.05$ ) are indicated by different lower case (for Ext) and capital (for Int) letters. \*: Mean value ( $\pm$  standard error) significantly different from zero (CC<sub>0</sub>); i: significant interaction between climate change effects and agricultural management ( $P < 0.05$ ). Gene name description on Fig. 1. TDN: total dissolve nitrogen; Nmic and Cmic: microbial N and C biomass; DOC: dissolved organic carbon, DOC:TDN: ratio dissolved organic C to total dissolved N.

compared to  $C_{mic}$  concentrations after the translocation to  $CC_2$ . The  $N_2O$  emissions were characterized by high temporal and spatial variability (Fig. S3). Mean  $N_2O$  fluxes until the first sampling ranged from  $0.02 \pm 0.006$  to  $10.86 \pm 3.34 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_0$ ,  $2.70 \pm 0.87$  to  $10.33 \pm 3.06 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_1$  and  $-1.31 \pm 0.44$  to  $3.18 \pm 0.83 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_2$ . Also, the strong resilience of the investigated microbial key functional groups towards climate change was again visible in spring (Fig. 2a). Only the abundance of  $N_2$ -fixing bacteria decreased significantly at  $CC_2$  ( $-65 \pm 12\%$ ). In summer, mainly the significantly negative RCs for AOB ( $-45 \pm 15\%$  at  $CC_1$  and  $-81 \pm 7.5\%$  at  $CC_2$ ) and diazotrophs ( $-69 \pm 7.1\%$  at  $CC_1$  and  $-85 \pm 4.8\%$  at  $CC_2$ ) indicated that the application of Int management reduced their abundance at  $CC_1$  and even stronger at  $CC_2$  (Fig. 2c). Moreover, at  $CC_2$  we observed an increase of N-mineralizing bacteria (*apr*) under Ext management ( $103 \pm 47\%$ ), but a negative RC for NB ( $-35 \pm 4.8\%$ ). These changes in the abundance of the microbial key players in N turnover were also observed when abiotic soil properties and analyses of plant biomass were done. A significant reduction of the aboveground biomass (by  $-40\%$ ) was shown at  $CC_2$  under extensive but not under intensive management (Fig. 2d). Negative RCs were observed for the microbial biomass ( $-26 \pm 0.4\%$  and  $-42 \pm 2.5\%$  respectively for  $C_{mic}$  and  $N_{mic}$ ) in the Int managed mesocosms at  $CC_2$  (Fig. 2d). The  $N_2O$  emissions in summer until the second sampling ranged from  $-0.70 \pm 3.35$  to  $5.64 \pm 1.27 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_0$ ,  $-0.03 \pm 0.21$  to  $2.52 \pm 2.12 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_1$  and  $-0.06 \pm 0.14$  to  $6.92 \pm 5.02 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_2$  under Ext management (Fig. S3). For Int managed mesocosm single fluxes ranged from  $0.32 \pm 0.36$  to  $5.31 \pm 3.42 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_0$ ,  $-2.76 \pm 3.0$  to  $2.0 \pm 0.72 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_1$  and  $0.39 \pm 0.37$  to  $21.15 \pm 10.21 \mu\text{g N m}^{-2} \text{h}^{-1}$  for  $CC_2$ .

### 3.2. Correlations between microbes catalyzing N turnover and soil/plant properties

Correlation analyses were done in order to determine whether the changes in the gene abundances were associated with the above observed shifts of abiotic soil and plant properties (Fig. 3). Spearman correlations showed that the abundances of N-mineralizing bacteria (*chiA* gene) were positively correlated to  $C_{mic}$  ( $r = 0.81$ ,  $P = 0.01$ ) at  $CC_2$  when Ext management was performed, while negative correlations with DOC:TDN ratio ( $r = 0.87$ ,  $P = 0.0025$ ) and plant biomass ( $r = 0.67$ ,  $P = 0.05$ ) were visible in the same treatment (Fig. 3e; Table S3). No correlations were detected for the abundance of the alkaline protease gene (*apr*) under the same scenarios. The abundance of AOB was positively correlated to TDN ( $r = 0.83$ ,  $P = 0.005$ ), while negative correlations were observed to BD ( $r = -0.87$ ,  $P = 0.002$ ) and microbial biomass ( $r = -0.70$ ,  $P = 0.036$  for  $N_{mic}$  and  $r = -0.72$  and  $P = 0.030$  for  $C_{mic}$ ) when climate change effects were not present (at  $CC_0$ ) and Int management was applied (Fig. 3b; Table S3). The abundance of ammonia oxidizing archaea could not be linked to any of the measured parameters independent of the treatment. The abundance of  $N_2$ -fixing bacteria was correlated with plant N content ( $r = -0.67$ ,  $P = 0.05$  for Ext management and  $r = -0.75$ ,  $P = 0.02$  for Int management) and microbial biomass ( $r = 0.70$ ,  $P = 0.036$  for  $N_{mic}$  and  $r = 0.78$ ,  $P = 0.013$  for  $C_{mic}$  for Ext management and  $r = 0.78$ ,  $P = 0.012$  for  $N_{mic}$  and  $r = 0.9$ ,  $P = 0.0009$  for  $C_{mic}$  for Int management) at  $CC_0$  only (Fig. 3a and b; Table S3). Regarding denitrification, only denitrifiers harboring the *nosZ* gene showed clear correlations to soil/plant properties. These correlations were only observed at  $CC_0$  and included plant biomass ( $r = 0.73$ ,  $P = 0.02$ ) and DOC:TDN ratio ( $r = 0.67$ ,  $P = 0.05$ ) under Ext management. Under Int management these correlations were stronger ( $r = 0.85$ ,  $P = 0.004$  for plant biomass and  $r = 0.78$ ,  $P = 0.01$  for C:N ratio) and additional correlations to microbial biomass ( $r = 0.67$ ,  $P = 0.05$  for  $N_{mic}$  and  $r = 0.72$ ,  $P = 0.030$  for  $C_{mic}$ ) and PNC were detected ( $r = -0.73$ ,  $P = 0.024$ ; Fig. 3).

A principal component analysis (PCA) integrating all the investigated variables across the climate change gradient and all management conditions was used to investigate the relationships separately for

spring and summer (Fig. 4). In spring, PC1 explained 35.2% of the variance that separated  $CC_2$  from  $CC_0$  and  $CC_1$ .  $CC_2$  mostly differed in moisture, soil temperature,  $C_{mic}:N_{mic}$  ratio, TDN concentrations, pH and plant N content. PC2 explained 22.5% of the variance and separated  $CC_0$  and  $CC_1$ . The bacterial biomass, as well as the abundance of nitrifiers (ammonia oxidizing archaea, and NB) and denitrifiers (*nirK*, *nirS* and *nosZ*) contributed to the separation of the cluster  $CC_1$  and  $CC_0$  (Fig. 4a). In summer,  $CC_1$  and  $CC_2$  were clearly separated from  $CC_0$  due to differences mainly in soil temperature, TDN concentration, plant N content, precipitation, moisture, and soil DOC:TDN ratio. The abundances of N-mineralizing bacteria (*chiA*), denitrifiers (*nirS* and *nosZ*),  $N_2$ -fixing bacteria and ammonia oxidizing bacteria as well as microbial biomass contributed to the separation not only of  $CC_0$  from  $CC_2$ , but also of Ext and Int agricultural management at  $CC_2$  (Fig. 4b).

To identify dominant environmental controls for N-mineralizing bacteria, nitrifiers, denitrifiers and  $N_2$  fixing bacteria, stepwise multiple regression analyses were conducted at the seasonal scale (Table S4). In spring, the levels of TDN, plant N content and  $C_{mic}$  were related to the climate change effects (soil moisture and temperature) and explained almost 90% of the dynamics of  $N_2$ -fixing bacteria. In summer, TDN and  $C_{mic}$  were closer correlated to moisture, BD, pH, and the ratio of DOC:TDN, which together were driving the abundance of  $N_2$ -fixing bacteria but to a lesser extent than in spring (50%). Nevertheless, these factors had even a stronger explanatory power (77–82%) for the climate change effects on N-mineralizing bacteria (*apr*, *chiA*) and denitrifiers (*nosZ*) in summer (Table S4).

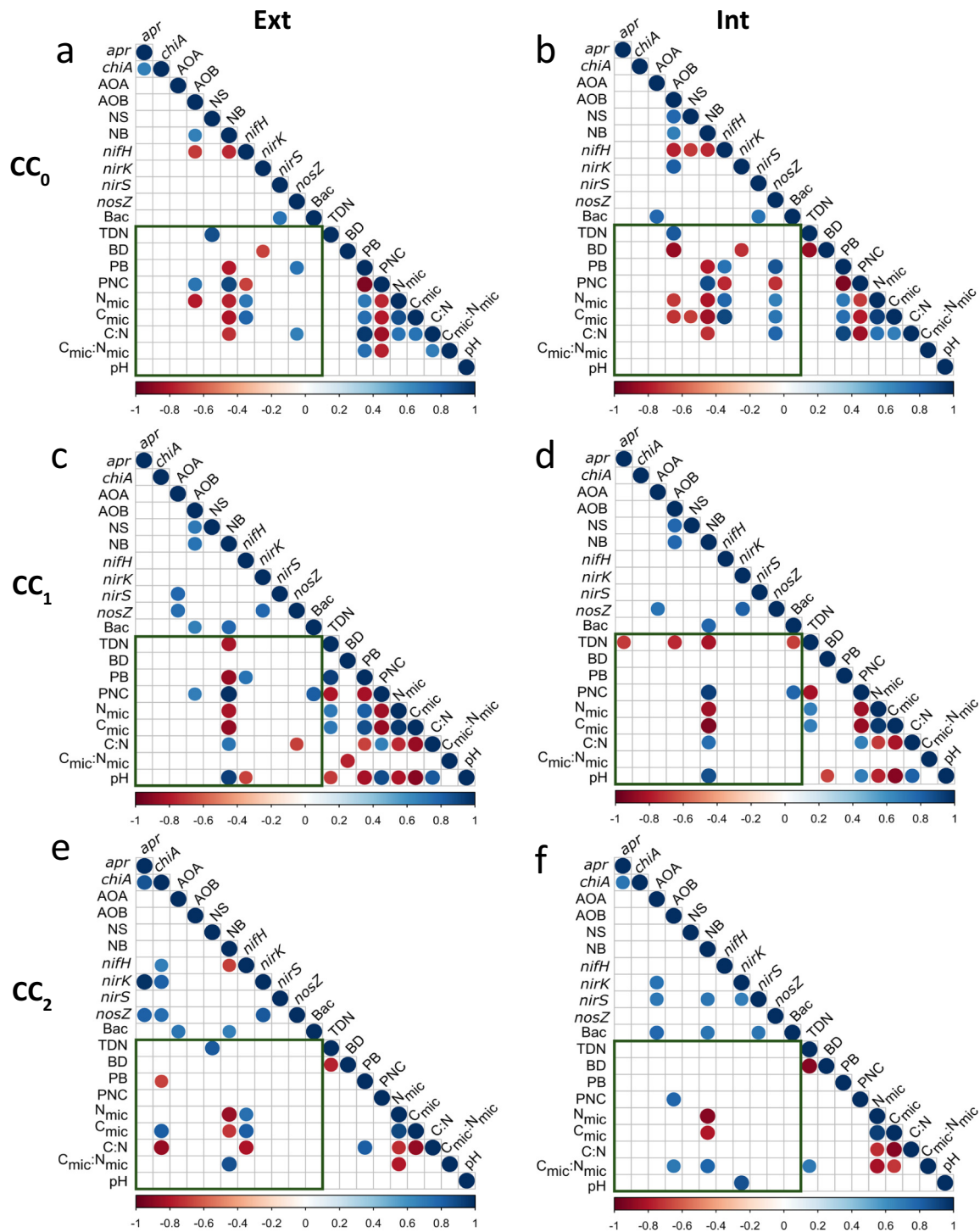
## 4. Discussion

The montane grassland soil used in this study (Esterberg, 1260 m a.s.l.) is an extensively managed soil which presents higher concentrations of SOC and N compared to grassland soils at lower altitudes (Bardgett and van der Putten, 2014; Garcia-Franco et al., 2020; Wiesmeier et al., 2013). The translocation of intact plant-soil mesocosms from this high-altitude site to lower altitudes, naturally simulated the effects of climate change with an increase of soil temperature and at the same time a decrease of precipitation (Gobiet et al., 2014; Schlingmann et al., 2020). We used these settings to investigate the short-term impact of climate change combined with the application of an extensive and an intensive agricultural management, on the abundance of microbiota, which catalyze different steps in N turnover and on a number of soil and plant parameters as indicators of ecosystem functioning in a mesocosm study.

### 4.1. Shifts in TDN and DOC as driver for $N_2$ -fixing and N-mineralizing bacteria

Our results indicate that the short-term exposure to simulated climate change negatively impacts the abundance of free-living  $N_2$ -fixing bacteria under intensive management (Figs. 1a and 2c). Although, the reduction of  $N_2$ -fixing bacteria as a result of higher fertilization has been already proven in several studies (Berthrong et al., 2014; Coelho et al., 2009; Revellini et al., 2019), we observed already a decrease of the  $N_2$ -fixing bacteria at  $CC_2$  in spring before management intensity increased as indicated by negative RC values (Fig. 2a). This might indicate that  $N_2$ -fixing bacteria may respond faster or be less resilient to warming and reduced precipitation. However, it needs to be noted that  $N_2$  fixation by free living microorganisms might not substantially contribute to total biological  $N_2$  fixation in the investigated grasslands, which is dominated by symbiotic fixation associated to roots of legumes, which contributes up to  $15 \text{ kg N ha}^{-1} \text{ year}^{-1}$  (Zistl-Schlingmann et al., 2020). Consequently, a decrease in biological nitrogen fixation by free-living microorganisms in these soils is not expected to lead to nutritional limitations for plants.

The higher concentrations of TDN in spring under  $CC_2$  could be explained 1) by the reduction of the snow cover at this altitude

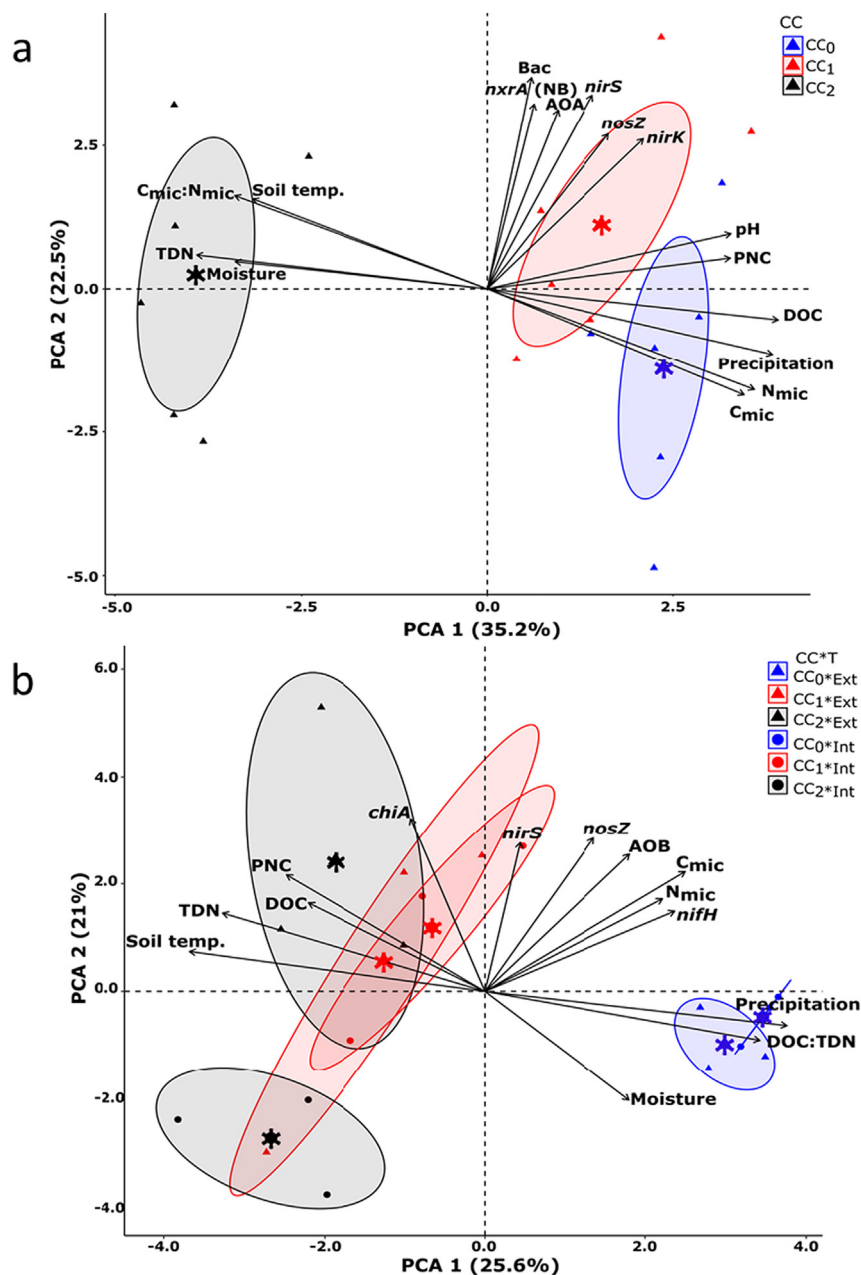


**Fig. 3.** Spearman correlations (positive: blue and negative: red;  $P < 0.05$ ) integrating data of gene abundances and soil properties in the translocated mesocosms  $CC_0$  (a, b),  $CC_1$  (c, d), and  $CC_2$  (e, f) treated by using an extensive (Ext) and intensive (Int) agricultural management ( $n = 9$ ). The squares shows the relationships between gene abundances (names description on Fig. 1; Bac: bacterial 16s rRNA) and soil properties (TDN: total dissolve nitrogen; BD: bulk density; PB: plant biomass; PNC: plant N content; Nmic and Cmic: microbial N and C biomass; C:N: dissolved organic carbon to TDN ratio).

that can lead to more frequently freeze-thaw cycles which physically disrupts soil aggregates, roots and microorganisms and thus releasing available nutrients into the soil (Budge et al., 2011; Garcia-Pausas et al., 2017; Guan et al., 2018) and/or 2) by higher mineralization rates due to warming (Song et al., 2017; Wang et al., 2016). Additionally, a significant increase in aboveground plant biomass (>2-fold) in spring was observed at the lower altitudes ( $CC_1$  and  $CC_2$ ). The

increased levels of N availability together with the soil/air temperatures and soil moisture in spring, contributed to a higher plant growth (Cannone et al., 2008). Similar short-term responses of the aboveground biomass were observed in previous studies on down-slope translocated plant communities or mesocosms without fertilization inputs (Berauer et al., 2019; Wang et al., 2016). Therefore, plant fitness may be not affected at the short-term which could





**Fig. 4.** Principal component analysis (PCA) integrating gene abundance and soil/plant properties measured in the translocated mesocosms CC<sub>0</sub>, CC<sub>1</sub> and CC<sub>2</sub>. PCA was performed for spring (a; n = 6) and summer (b; n = 3) sampling. Climate change effects (CC) are differentiated by colors (blue: CC<sub>0</sub>, red: CC<sub>1</sub> and black: CC<sub>2</sub>), while agricultural management by symbol (triangle for extensive (Ext) and dot for intensive (Int) management). The ellipses display the 95% confidence interval and are drawn around the group mean (indicated by asterisk). Bac: 16S rRNA of total bacteria, *nxrA* (NB): nitrite oxidizing bacteria, *nirS* and *nirK*: nitrite reducing bacteria, AOA: ammonia oxidizing archaea, *nosZ*: N<sub>2</sub>O reducing bacteria, AOB: ammonia oxidizing bacteria and *chiA*: N mineralizing bacteria. Soil temp.: soil temperature, TDN: total dissolved nitrogen, DOC: dissolved organic carbon, PNC: plant N content, C<sub>mic</sub>: N<sub>mic</sub>: microbial C biomass (C<sub>mic</sub>) to microbial N biomass (N<sub>mic</sub>).

contribute to increased input of labile C and N via root exudation suitable for N mineralization.

However, the abundance of N-mineralizing bacteria (*apr* and *chiA*) was not significantly increased in spring compared to CC<sub>0</sub> (Fig. 2a; Table S2). We propose that N-mineralizing bacteria were not promoted, because TDN and DOC increased to the same extent, as indicated by stable DOC:TDN ratios. The increase of N mineralizing bacteria in summer at CC<sub>2</sub> under extensive management might be the consequence of increasing root exudation and DOC release by plants, which increased the demand for N subsequently and likely stimulates microbial chitinase and protease response (Edwards et al., 2006), which was also shown for other alpine grasslands with low nutrient input (Brankatschk et al., 2011). Our results are also in line with other studies investigating

climate change effects in alpine ecosystems reporting increased gross N-mineralization rates and available N with warming (Dawes et al., 2017; Wang et al., 2016). Recently, it has been shown that plant N nutrition in grasslands is largely based on N mineralization rather than on the use of recently added slurry-N (Schlingmann et al., 2020; Zistl-Schlingmann et al., 2020). Therefore, the increased N mineralization may further promote productivity especially under extensive management, which however also bears the risk of soil N mining because plant N outputs exceed fertilizer N inputs that are stabilized in soil (Schlingmann et al., 2020). Therefore, complex interactions of soil properties and climate change effects might affect key processes in N turnover including N mineralization and denitrification at the long-term exposure (Chen et al., 2015; Hammerl et al., 2019; Keil et al., 2015).

#### 4.2. The role of functional redundancy of nitrifiers for resilience

Against our hypothesis the N input by manure fertilization and mowing did not increase ammonia-oxidizing bacteria and archaea but resulted in a contrary response of both functional redundant groups. Compared to ammonia oxidizing bacteria, ammonia oxidizing archaea were higher in abundance and were not affected by climate change and agricultural management in our study, which was also underlined by stable and low RC values (Fig. 2). This suggests that ammonia oxidizing archaea are more tolerant to climate change and agricultural management. It is known that ammonia oxidizing archaea can tolerate drought conditions (Gleeson et al., 2010; Hammerl et al., 2019), dominate many ecosystems (Leininger et al., 2006; Schleper, 2010) and use organic N as nutrient source (Alves et al., 2013; Prosser and Nicol, 2012). In contrast, ammonia oxidizing bacteria dynamics have been usually related to N mineralization and nitrification rates (Wang et al., 2016), and also to fertilizer applications (Stempfhuber et al., 2014; Szukics et al., 2019). We observed a negative impact of Int management on ammonia oxidizing bacteria abundance in summer which was obvious by the negative RC values and might be related to the marginal higher bulk density (BD) found in Int managed mesocosms that could lead to a reduction of the O<sub>2</sub> availability which limit the oxidation of ammonia by ammonia oxidizing bacteria (Hooper et al., 1997). Hampered nutrient transport because of low soil moisture (Niboyet et al., 2011; Stark and Firestone, 1995), which is most likely at CC<sub>2</sub> during summer, could additionally impact ammonia oxidizing bacteria. As the abundance of N-mineralizing bacteria increased in summer a reduction of the AOB abundance under warming and Int management involved the risk of increased ammonia (NH<sub>3</sub>) accumulation and volatilization. In this regard, the calculation of fertilizer N losses in these grasslands indicated that NH<sub>3</sub> accounted for 6.9% of the N losses, while N<sub>2</sub> production accounted for 31–42% (Zistl-Schlingmann et al., 2019). Moreover, a high abundance and activity of arbuscular mycorrhizal fungi can be expected in those grasslands, which may be able to successfully compete with slow-growing nitrifiers for NH<sub>4</sub><sup>+</sup> and thus prevent its accumulation (Storer et al., 2018; Veresoglou et al., 2012).

Compared to CC<sub>0</sub> NB was only lower in Ext management in summer (Fig. 2c), suggesting that NB can be influenced not only by N-inputs (Han et al., 2018; Han et al., 2017) but also by seasonal dynamics (Bell et al., 2010; Hammerl et al., 2019). In contrast, NS was not significantly affected by climate change and agricultural management (Figs. 1b, 2). A stronger decrease of nitrite oxidizers at CC<sub>2</sub> in the long-run may lead to high levels of NO<sub>2</sub><sup>-</sup> in soil and consequently to the potential increase of N<sub>2</sub>O emission (Caranto and Lancaster, 2017). A situation that could be critical, considering that the *nosZ* abundance tended to be reduced under the same condition in summer. However, after short-term exposure, no significant increase of N<sub>2</sub>O emissions induced by climate change and Int management has been observed, though in tendency slightly higher mean emission rates were observed under Int management. The average N<sub>2</sub>O emissions found in this study (Fig. S3) were still on a low level and comparable to those reported previously for translocation experiments from CC<sub>1</sub> to CC<sub>2</sub> (Unteregelsbacher et al., 2013), which might be explained by: 1) strong spatio-temporal variations, and 2) higher N<sub>2</sub> emissions than N<sub>2</sub>O emissions (Zistl-Schlingmann et al., 2019), which were correlated to high *nosZ* expression rates previously (Chen et al., 2015). As both the abundance of both *nosZ* and *nirS* decreased (Fig. 1), the production and reduction of N<sub>2</sub>O might remain on a stable level.

N uptake by microbes and plants clearly differed between seasons and across elevations. While microbial biomass (N<sub>mic</sub> and C<sub>mic</sub>) was significantly higher in summer as compared to spring, the N content in plants showed the opposite trend. This could indicate that competitions for substrate between nitrifying microbes and plants may occur under these conditions due to the stimulated plant growth, soil moisture, temperature and therefore potential higher DOC availability (Jaeger et al., 1999). This competition may occur most

intensively between plants and heterotrophic microbes like fungi and bacteria that usually depend on the supply of available organic C (Hodge et al., 2000), including those *Nitrospira* species able to perform complete ammonia oxidation (comammox) (Daims et al., 2016; Hayatsu et al., 2008). The higher concentrations of TDN that were found regardless of the management application at CC<sub>1</sub> and CC<sub>2</sub> compared to CC<sub>0</sub> may indicate a potential mineralization of soil organic nitrogen (SON). As the abundance of N-mineralizing bacteria was only promoted by Ext management (Fig. 2c), it is also possible that saprotrophic fungi with proteolytic and chitinolytic activities (Semchenko et al., 2018) or the symbiosis of arbuscular mycorrhizal fungi and plants are improved. Fungal diversity in general (Millard and Singh, 2010) but also the diversity of arbuscular mycorrhiza (De Deyn et al., 2011; Urcelay and Díaz, 2003) is positively related to plant diversity and in addition soil fungal community affect bacterial composition and might increase competition under lower N availability. Nonetheless, Int management under climate change may reduce, for instance plant-nitrifier competition for NH<sub>4</sub><sup>+</sup>, which may lead to a potential contribution of N<sub>2</sub>O emissions by nitrification (Caranto and Lancaster, 2017). Therefore, the analysis of <sup>15</sup>N-isotope based gross N turnover rates and plant N uptake rates in alpine grasslands can help to determine which and how N-pool fluxes are involved in N plant-microbe competition at a longer-term of exposure to climate change and agricultural management and under which circumstances the detected microbial potentials are exploited (Dannenmann et al., 2009; Hodge et al., 2000; Zistl-Schlingmann et al., 2020).

Nevertheless, significant differences between CC<sub>1</sub> and CC<sub>2</sub> were evident in each season. While according to our hypothesis responses of TDN, plant biomass, plant N content and N<sub>mic</sub> were higher at CC<sub>2</sub>, the different management intensity in summer interfered with the climate change effects and responses differed between CC<sub>1</sub> and CC<sub>2</sub> for some parameters. (Fig. 2d). For example, TDN and plant N content were higher at CC<sub>1</sub>. These higher N concentrations may either promote nitrification due to potentially higher NH<sub>4</sub><sup>+</sup> concentrations in soil, which could contribute to N losses by leaching of accumulated NO<sub>3</sub><sup>-</sup> or increased N<sub>2</sub>O emissions and to changes in overall nutrient turnover (Bowles et al., 2018; Klaus et al., 2018). In contrast, a higher potential for N-mineralization, reduced bacterial oxidation of ammonia and fixation of N<sub>2</sub>, and decreased plant and microbial biomass were observed at CC<sub>2</sub>, which may lead to a reduced pool of labile C and N in the long run (Chen et al., 2015).

#### 5. Conclusion

This study represents a first step in integrating potential short-term responses of microbial functions to changes in soil and plant properties as impacted by climate change and agricultural management in montane grasslands. Our data demonstrates a strong interaction of climate change and management intensity on the abundance of microbiota catalyzing key steps of the N-cycle and related N-pools in soil after a short-term exposure. It is an open question if the observed resilience of the functional groups of soil microbiota under investigation towards the climate change treatments can be also observed in the long run. Despite it is an open question how the investigated systems will develop on the long-run, shifts in the short-term are of significant importance as they might determine the long-term development according to classical ecological theories (Pickett and White, 1985).

The high elevation site Esterberg is a typical extensively used montane grassland. The management included one fertilization in spring, one biomass harvest in July followed by cattle grazing until the end of the growing season (20 animal per ha<sup>-1</sup> during summer). Thus based on the extensive management, the soil at this site harbors a high diversity of microbiota (data not shown). All investigated functional groups show a high degree of functional redundancy and thus a stable pattern on the overall abundance after a short-term exposure might be a result

of shifts in the diversity of each functional group. The differing response pattern of ammonia oxidizing bacteria and archaea is a good indication for this. Taking this issue of functional redundancy into account it becomes obvious that future studies should also consider the role of fungi in such systems. Partly our data already emphasizes that fungi may play an important role mostly the role of arbuscular mycorrhizal fungi in taking up mineral N as indicated already partly by our data would be important to understand in more detail.

Based on the fact that interacting effects of management intensity and climate change were already visible after a one year's period of transplantation, there is a strong need to consider mitigation strategies to avoid negative feedback loops between above- and belowground biota. Therefore, agricultural management strategies need to consider: 1) preventing the mining of soil organic nitrogen, 2) maintaining the balance of C and N stoichiometry, which can affect microbial C and N biomass, 3) a reduction of potential N losses after manure application by considering local weather events and 4) stabilization of plant productivity and diversity, as a reduction of those negatively impacts below-ground diversity and functioning.

Our study focused on the microbial N turnover potential as an indicator for ecosystem functioning and resilience. However, it remains unclear under which conditions this potential is recalled. Thus, follow up studies are needed, which combine in situ fluxes and gene expression pattern of the microbiome to identify the trigger for the actual N turnover processes under climate change and different management intensity in this fragile ecosystem. However, such an experiment requires a different design, because of the high fluctuations in gene expression and emission rates like more spatially resolved replicates taken in higher frequency or related to specific fertilization or weather events. Additionally, the interplay of fungi and bacteria should be further investigated in future studies as they are competing for the same resources and niches.

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## CRedit authorship contribution statement

Diana Andrade-Linares: Experimental work, results preparation and analysis, writing - original draft preparation, writing review & editing. Marcus Zistl-Schlingmann: Experimental work & reviewing draft. Baerbel Foessel: Experimental work & reviewing draft. Michael Dannenmann: Conceptualization, reviewing & editing. Stefanie Schulz: reviewing & writing draft. Michael Schloter: Conceptualization, reviewing, writing & editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.146672>.

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