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Climate change and management intensity alter spatial distribution and abundance of P mineralizing bacteria and arbuscular mycorrhizal fungi in mountainous grassland soils

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ABSTRACT

In mountainous grasslands management adaptations are required to maintain soil functions. We investigated climate change (CC) and management effects on the abundance and potential activity of microbiota catalyzing the major steps of P transformation which are still unknown in these grasslands.

Soil samples were taken from intact plant-soil mesocosms managed extensively or intensively (two vs. five mowing and slurry applications, respectively). These mesocosms were previously translocated from high to lower altitudes to simulate two CC scenarios (CC1: +1 °C warming and mean annual precipitation (MAP) of 1347 mm and CC2: +3 °C warming and MAP of 956 mm), while control mesocosms (CC0) were relocated at their original site (6 °C and MAP of 1400 mm). Specific marker genes for P-solubilization (*gcd*), P-mineralization (*phoN*, *phoD*, *phnX* and *appA*), P-uptake (*pitA* and *pstS*), total bacteria and arbuscular mycorrhizal fungi (AMF) were quantified by quantitative real-time PCR. Spatial distributions of phosphatase activities were analyzed in situ by zymography analysis and total organic C, N and P contents were measured.

Gene abundances and enzymatic activities were comparable for both managements under CC0, except for phytase-harboring (*appA*) microbiota which decreased under intensive management. The abundance of microbiota which catalyzes organic P (Po) mineralization (*phoN* and *appA*) and those harboring quinoprotein glucose dehydrogenase (*gcd*) for P solubilization significantly dropped by interacting effects of CC2 and extensive management. The same effect was found for microbes harboring specific P transporters (*pitA* and *pstS*). Under intensive management, microbiota catalyzing Po mineralization (*phoN* and *appA*), and alkaline phosphatase activities tended to increase in CC2. Noteworthy, the AMF abundance was reduced at 0–5 cm soil depth under CC. Our results suggest that CC and extensive management reduced microbial P solubilization, mineralization and uptake, while management intensification may increase P availability, which leads to shifts in nutrient stoichiometry and decreased AMF abundance.

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1. Introduction

Nutrient turnover in grasslands of the Northern Limestone Alps in Europe is particularly important for fodder production and biodiversity conservation as these grasslands are characterized by high soil organic carbon and total nitrogen (TN) stocks, which are highly vulnerable to climate change (CC; Wang et al., 2016; Garcia-Franco et al., 2021). The development of sustainable management practices of these grasslands is therefore of prime importance to avoid significant losses of nutrients, which negatively affect soil functions and ecosystem services including positive feedback for CC mitigation.

These mountainous grasslands are used for fodder production where the dominant N input and output is cattle slurry fertilization and grass mowing, respectively. However, the intensity of management may differ and often depends on farm size/type and elevation. An extensive management with one to two fertilizer applications and two mowing events per year is typically used at high altitudes, while an intensive slurry application, up to five times, and five mowing events are commonly used at lower altitudes (Zistl-Schlingmann et al., 2020). The differences in management intensities may induce altered stoichiometry of nutrients and hence influences microbial processes and activities in soil, which in the long run also affects soil organic matter (SOM) pools (Abbas et al., 2020; Apostolakis et al., 2022). While many studies have shown the importance of the carbon to nitrogen (C:N) ratio for soil functioning, the consequences of changes in the nutrient stoichiometry for phosphorous (P) turnover and the C:P ratio are less known, although P is considered the second most important nutrient for plant net primary production (Vitousek et al., 2010).

Microorganisms play a key role for P release in soil by mineralization and solubilization from organic P (Po) and inorganic P (Pi) pools which can increase the concentration of dissolved orthophosphate readily available for plant uptake. In this sense, AMF are important symbiotic partners for plants, which increase P uptake efficiency (Van Der Heijden et al., 2006; Kobae, 2019). Regarding the provision of phosphate, microbes can mineralize Po by releasing several hydrolytic enzymes, such as acid (e.g. *phoN*), and alkaline phosphatase (e.g. *phoD*), phytases (e.g. *appA*) or phosphonatases (*phnX*) (Rodríguez et al., 2006) or they solubilize adsorbed Pi by secreting organic or inorganic acids, protons and siderophores. A well described marker gene for P solubilization is the *gcd* gene which codes for a periplasmic quinoprotein glucose dehydrogenase that catalyzes the formation of gluconic acid by glucose oxidation leading to soil acidification and consequently solubilization of mineral bound P (Babu-Khan et al., 1995; Goldstein, 1995). Further, many bacteria harbor genes coding for different P transporters in their genome, which are differently regulated, which leads to, at least temporally, immobilization of P in the microbial biomass (Hsieh and Wanner, 2010). For example, the low affinity Pi transporter (Pit) is a cation symporter, while the high-affinity P-specific transporter (Pst) is upregulated at low Pi availability in soil (Santos-Beneit et al., 2008). Several studies have suggested that the abundance and diversity of P-solubilizing bacteria is enhanced by low P availability in grasslands (Mander et al., 2012; Widdig et al., 2019). Additionally, changes in the abundance of P mineralizing microbes, P-solubilizing microbes, and AMF have been shown in a number of fertilization studies where N was added, indicating a link between P and N cycling (Unger et al., 2016; Aanderud et al., 2018; Jilling et al., 2018; Hestrin et al., 2019; Wagg et al., 2019).

The application of cattle slurry that contains not only N but also C and P may alter the C:N:P stoichiometry of soil, especially under an intensive management. It is assumed that storage of C in soil can be altered by cattle slurry fertilization promoting SOM mineralization (Schlingmann et al., 2020) and may lead at the long-term to mineralization of Po via increased phosphatase activity (Vitousek et al., 2010; Peñuelas et al., 2012; Dong et al., 2019). However, for mountainous and alpine grasslands, these effects are not well described mainly considering also interacting effects of management intensity and CC.

To simulate different scenarios of CC in mountainous grasslands, we transplanted intact plant-soil mesocosms from a high-altitude site (CC0) to lower altitudes, simulating moderate (CC1) and strong (CC2) CC effects of warming and reduced precipitation and applied different grassland management intensities by mowing and cattle slurry fertilization (Zistl-Schlingmann et al., 2019). We used quantitative real time PCR (qPCR) and in situ soil zymography of acid and alkaline phosphatases to follow changes in abundance, potential activity and distribution of bacteria involved in P transformation in two depth of the mesocosms (0–5 cm and 5–15 cm). We previously found that an exposure of mountainous grassland soils to CC1 and CC2 effects induced shifts in total dissolved N concentrations which reduced the C:N ratio already one year after the treatments were established (Andrade-Linares et al., 2021). These shifts of C:N ratio under CC decreased the microbial biomass mainly when intensive agricultural management was applied (Andrade-Linares et al., 2021). These effects may alter the N:P ratio in the long run, which may induce changes in the overall nutrient stoichiometry and affect microbial functions, and thus SOM decomposition rates (Spohn, 2016; Heyburn et al., 2017).

Thus, we hypothesize that (i) as a result of the increased availability of N, due to higher N mineralization rates under extensive management, the change to low soil C:N and high N:P ratios promote bacteria to invest more into acquisition of P from organic and inorganic pools in soils. The AMF abundance will decrease due to the lower plant biomass under extensive management at CC2. (ii) Intensive management will increase phosphatase activity and the abundance of microbes harboring genes that catalyze P mineralization in soil which can decrease the N:P ratio and thus negatively affects the abundance of P-solubilizing bacteria and AMF.

2. Materials and methods

2.1. Site description and sampling

We selected three grassland sites along an altitudinal gradient in the Northern Limestone Alps in Southern Germany: Esterberg (47.52°N; 11.16°E; 1260 m a.s.l), Graswang (47.57°N; 11.03°E, 860 m a.s.l) and Fendt (47.83°N; 11.07°E, 600 m a.s.l). The soil at the high elevation site (Esterberg) has been classified as a *Rendzic Phaeozem* (IUSS Working Group WRB 2015) rich in soil organic carbon (129.8–188.9 mg g^{-1}) and TN content (13.8–18.8 mg g^{-1}), resulting in a C:N ratio of 9.4–10.0 (Garcia-Franco et al., 2021). The inorganic C content ranged between 2.8 and 5.9 mg g^{-1} and a pH of 6.1 has been measured (Garcia-Franco et al., 2021).

The environmental conditions, soil and plant characteristics, as well as the applied forms of grassland management intensities have been described in detail in previous studies (Zistl-Schlingmann et al., 2019; Garcia-Franco et al., 2021; Andrade-Linares et al., 2021). In brief, 54 intact plant-soil mesocosms (30 cm inner diameter and 30 cm height) were transplanted either (1) within the sampling site Esterberg (where 18 mesocosms were re-transplanted as control; CC0; current climate conditions of 6 °C and MAP of 1400 mm); (2) to Graswang (CC1; 18 mesocosms under moderate CC of +1 °C warming and MAP of 1347 mm) or (3) to Fendt (CC2; 18 mesocosms under strong CC of +3 °C warming and MAP of 956 mm) to simulate CC effects within a space for time approach. Overall, a total number of 54 intact plant-soil mesocosms were transplanted in summer 2016 as described by Andrade-Linares et al. (2021). From spring 2017 on half of the mesocosms were subjected to an extensive (Ext) management that included two cattle slurry applications and two mowing events per year, and the other half to an intensive management (Int) which included four to five slurry applications and three to four mowing events per year. This management is corresponding to local farmers' practice. Cattle slurry was obtained from the local farmer at the CC2 site. Further information on management is provided in Schlingmann et al. (2020). Table S1 summarizes annual C, N, and phosphate-P addition with cattle slurry for the different

treatments. At the sampling time in autumn 2018, all extensively managed mesocosms had received four cattle slurry applications and four mowing events in total, while the intensively managed mesocosms were treated with eight to nine cattle slurry applications and a similar number of mowing events in 2017 and 2018. After two years of exposure triplicate soil samples (six mesocosms per site, three per Int and three per Ext management) were collected in autumn (November 2018) from 0 to 5 cm and 5–15 cm depth and stored at 4 °C and −80 °C for chemical and molecular analyses, respectively. For soil zymography assays, intact soil blocks (15 cm × 15 cm x 4 cm wide) were extracted from the middle of the mesocosm by using metallic stainless-steel cubes with sharp edges to easily penetrate the soil.

2.2. Nucleic acid extraction from soil

DNA was extracted from 0.5 g of frozen soil (Lueders et al., 2004; Töwe et al., 2011) following the modifications previously described in Andrade-Linares et al. (2021). DNA was quantified by a fluorometer (Qubit 4, Invitrogen) using a Qubit dsDNA BR Assay kit (Invitrogen, USA). DNA quality was estimated photometrically by the ratios of absorbance at 260 nm–280 nm, and 260 nm–230 nm. Negative controls were included using the same reagents in empty tubes, to check for contamination during the extraction. DNA extracts were stored at −80 °C for further use.

2.3. Quantitative real-time PCR assay

Absolute abundance of microorganisms catalyzing selected processes of P transformation and uptake were assessed based on the quantification of respective marker genes using SYBR Green® based quantitative real-time PCR (qPCR) assays which were carried out in a 7300 real-time qPCR machine (Applied Biosystems). The extracted DNA was used to quantify bacteria that carry the genes *phoN, phoD, pnhX,* and *appA* that encode phosphatases, bacteria that have a quinoprotein glucose dehydrogenase (*gdc*) and bacteria harboring the low affinity Pi transporter (*pitA*) and the high-affinity P-specific transporter (*pstS*). Total bacterial abundance was quantified by qPCR using the 16S rRNA gene as a marker. The conditions of qPCR reactions and primers are described in Table S2. Abundance of AMF was assessed by using 0.5 pmol μl^{-1} of primers FLR 3 and FLR4 which amplified specifically the large subunit (LSU) rRNA genes (ca. 380 bp) of Glomeraceae, Gigasporaceae, Archaeosporaceae and Acaulosporaceae (Gollotte et al., 2004; Mummey and Rillig, 2007). The qPCR conditions for AMF were as follows: 95 °C for 10 min, 40 cycles of 93 °C for 30 s–60 °C for 40 s–72 °C for 45 s, followed by one extension cycle of 95 °C for 15 s–60 °C for 30 s–95 °C for 15 s. All qPCR efficiencies were higher than 80% with coefficient of determination (R^2) of the standard curves above 0.99. The specificity of the amplified products was checked by melting curves of the amplicons and on 2% agarose gels of randomly selected samples.

2.4. Soil zymography

Intact soil blocks from the translocated and re-located mesocosms were transported to Bayreuth and stored at 20 °C overnight. The next three days, the spatial distribution of acid and alkaline phosphatase activity was analyzed by soil zymography following Spohn and Kuzyakov (2013) (with modifications) using nylon membranes (Filter Bioscience Membrane Technology Co., Ltd., China) and 4-methylumbelliferyl phosphate (Sigma-Aldrich) as substrate (for details see Suppl. Material). These membranes were photographed and phosphatase activity was calculated based on a linear correlation between different 4-methylumbelliferone concentrations and the corresponding gray values of the images (Spohn and Kuzyakov, 2013).

2.5. Analyses of C, N, P and concentrations of microelements in soil

Total C (TC) and TN concentrations were determined by an Elemental Analyzer (Euro EA, Eurovector, Milano, Italy) using 2–5 mg of dried (55 °C during 24 h) and milled soil material. Inorganic carbon (IC) was determined after heating the milled samples in a muffle (Heraeus – electronics, Germany) at 550 °C during 4 h, while the organic carbon (OC) was calculated by subtracting the IC from the TC content. C and N concentrations were determined in triplicates in two independent measurements and their means were calculated. Total content of phosphorous (P), potassium (K), aluminium (Al), calcium (Ca), iron (Fe), magnesium (Mg), manganese (Mn) and zinc (Zn) in soil were measured in triplicate by ICP-OES (inductively coupled plasma optical emission spectrometry; Optima 8300, Perkin Elmer, USA) after digestion with HNO_3 and H_2O_2 (Vitow et al., 2021). The plant-available P, Mg and K were analyzed using the double lactate (DL) method according to Riehm (1943, mod. by VDLUFA, 1991). Two grams of air-dried fine soil (<2 mm) were placed in a 200 mL shaker bottle with 100 mL extract solution (0.02 M calcium lactate (Ca[CH3CH(OH)COO]2) and 0.02 M HCl). The samples were shaken by end-over-end rotation and the extract was filtered using P-free filters (MN 616 G, Machery-Nagel). The element concentrations in the extract were measured by the ICP-OES.

2.6. Statistical analyses

All data were checked for normal distribution by applying the Shapiro-Wilk normality test and linear models "lm". Homoscedasticity was tested by Levene's test (Fox, 2015). If data were not normally distributed or with no homogeneous variance, the values were log- or Box-Cox-transformed (Yeo and Johnson, 2000). The effects of climate change, agricultural management and their interaction on gene abundances and total C, N, P, K and microelements concentrations were tested, using the R package "nlme" for mixed effect models (Pinheiro et al., 2019). For the mixed effect models, CC (CC0, CC1 and CC2) and management (Ext and Int) 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). In addition, a relative change (RC) for gene abundances and activities of acid and alkaline phosphatases in soil zymography compared to the respective control CC0 was calculated as:

$$RC = \left(\frac{Sample - \bar{x}CC0}{\bar{x}CC0}\right) \quad (1)$$

In Eq. (1) *Sample* denotes a single translocated mesocosm and $\bar{x}CC0$ the mean of all reinserted mesocosms in CC0 (Berauer et al., 2019; Andrade-Linares et al., 2021). 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 the RC at a single elevation and management type was significantly different from zero, we used one sample *t*-test under the null hypothesis $\mu = 0$. The spatial distribution of the enzymatic activity on the zymography was also analyzed at a scale of 50 × 50 pixels by comparing each normalized image. For each zymography block, the distribution of the activities was checked in the space (x, y) and the mean matrix from similar distributions was computed and normalized by subtracting each grid from the mean and dividing by the standard deviation for each treatment. Normalized image matrices were compared to test for equal distributions between the management and CC gradients by Chi square test with a 5% of significance level. In addition, principal component analyses (PCA) were done using the packages "FactoMineR" and "factoextra"; in case of missing values (n = 3) the "imputePCA" function of the "missMDA" package was applied. Correlations were tested by "corrplot" package. 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 P turnover in mountainous grasslands

The abundance of bacteria, measured as 16S rRNA gene copies, was not affected by CC and management intensities at 0–5 cm. Only in CC1, the Int management application resulted in an increase in bacterial abundance (Fig. 1a). In contrast, a reduction of bacterial abundance was found in the deeper soil depth (5–15 cm) in all mesocosms subjected to CC2 independent of management (Fig. S1a). The AMF abundance, based on the measurement of gene copy numbers of the LSU region, significantly decreased as a result of CC2 exposure in the upper soil depth of the mesocosms with no additional management effect (Fig. 1b). In CC1 mesocosms, a reduction of AMF abundance was observed only in combination with Int management (Fig. 1b). In samples obtained from the deeper soil sampling depth (5–15 cm), no effects of CC or management or the combination of these both factors were observed for AMF abundance (Fig. S1b).

When comparing Ext vs. Int management under the current climate conditions (CC0), the Int management significantly reduced ($p < 0.05$) the abundance of bacteria harboring phytases (*appA*) at 0–5 cm, while the abundance of *phoN*- *phoD*- and *phnX*-harboring bacteria did not change significantly in the two investigated soil depths (Fig. S2). Surprisingly, CC2 reduced the abundance of Po mineralizing bacteria (*phoN*, *appA*) at 0–5 cm and at 5–15 cm (*phoN, phoD*) only in the Ext managed mesocosms where a reduced fertilization was applied (Fig. 2, Fig. S2 and Fig. S4). Bacteria which carry the *gcd* gene were negatively affected in their abundance in both CC2 soil depths under Ext management as compared to CC0 (Fig. S3). However, the RC analysis only showed a significant impact for the deeper soil depth (Fig. S4).

Similar patterns were observed for bacteria which carry the low affinity P-transporter (*pitA)* and the P-specific transporter (*pstS)*. CC2 had a negative impact on the abundance of these microbial functional groups, mostly in those mesocosms where low fertilization regimes (Ext management) were performed. This impact was independent of the sampling depth (Fig. 2, Fig. S3 and Fig. S4).

3.2. Potential acid and alkaline phosphatase activity as well as their distribution at the microscale level

Under CC0, the RC of potential acid and alkaline phosphatase activities was not significantly different from zero for both forms of management intensities, irrespective of soil depth, indicating that under the current climatic conditions, the application of different intensities of management did not influence potential phosphatase activity (Fig. S5). In addition, the potential activities of phosphatases were not significantly affected by Ext management in any soil depth (Table 1, Fig. 2 and Fig. S4). In contrast, increased fertilizer inputs, as a result of Int management, enhanced marginally ($p = 0.09$) the potential activity of alkaline phosphatases in soil mainly at 0–5 cm in CC1 as well as CC2 (Fig. 2 and Fig. S4).

Overall, the potential acid phosphatase activity in soil was lower than potential alkaline phosphatase activity in both soil depths (Table 1), irrespective of treatment. Acid phosphatase activities ranged between 5% and 36% compared to alkaline phosphatases, which were at least three-fold higher (Table 1).

In addition, we analyzed the enzyme distribution in soil samples by comparing each image split in two spatial matrices (x, y) separately for management and CC scenarios. The distribution of soil phosphatase activity in the mesocosms did not differ significantly ($\alpha > 0.05$) between the Ext and Int management and across the CC scenarios (Fig. 3 and Fig. S6). The potential enzyme activities were not significantly correlated to the abundance of bacteria carrying *phoN* and *phoD* genes (data not shown).

3.3. Total C:N:P stoichiometry and element concentrations in soil

Overall, the soil OC:TN ratio ranged from 8.8 (CC1 at 5–15 cm) to 9.7 (CC1 at 0–5 cm) for Ext- and from 9.6 (CC1 at 0–5 cm) to 10 (CC0 at 5–15 cm) for Int management (Fig. 4a and Fig. S7a). The application of Ext management resulted in a slightly reduced OC:TN ratio in CC2 (9.3) as compared to CC0 at 0–5 cm soil depth, while in CC1 this ratio was significantly higher (Fig. 4a). Significantly lower ratios of OC to TN were observed only in CC1 by the application of an Int management (9.6) as compared with CC0 (Fig. 4a). In contrast, in the deeper soil layer, differences in OC:TN were not significant (Fig. S7a).

Regardless of the management intensity, OC and TN were not different between CC0 and CC1 in the two investigated soil depths

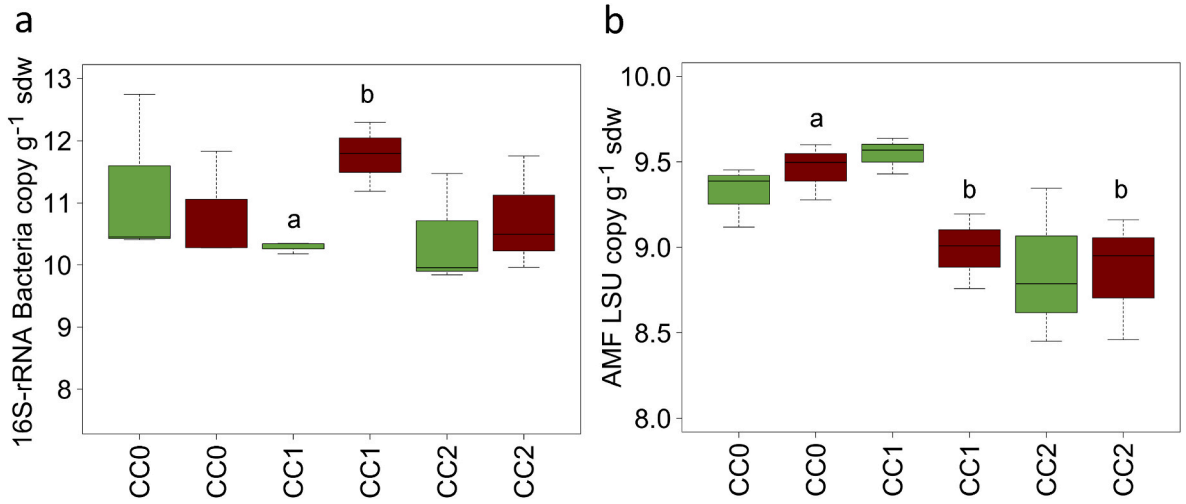

Fig. 1. Gene abundances of total bacteria (16s rRNA; a) and total arbuscular mycorrhizal fungi (AMF; b) per gram of soil dry weight (sdw) at 0–5 cm in soil mesocosms treated by an extensive (green) and intensive (brown) agricultural management from 2017 at the different climate change scenarios CC0 (the respective control), CC1 (moderate climate change scenario) and CC2 (strong climate change scenario). Boxplots represent soil gene copy numbers (on a log10 scale), medians (black lines inside the box) and maximal and minimal values (n = 3). Significant differences ($p < 0.05$) among the different climate change scenarios*treatment are represented by different letters separately for bacteria and AMF.

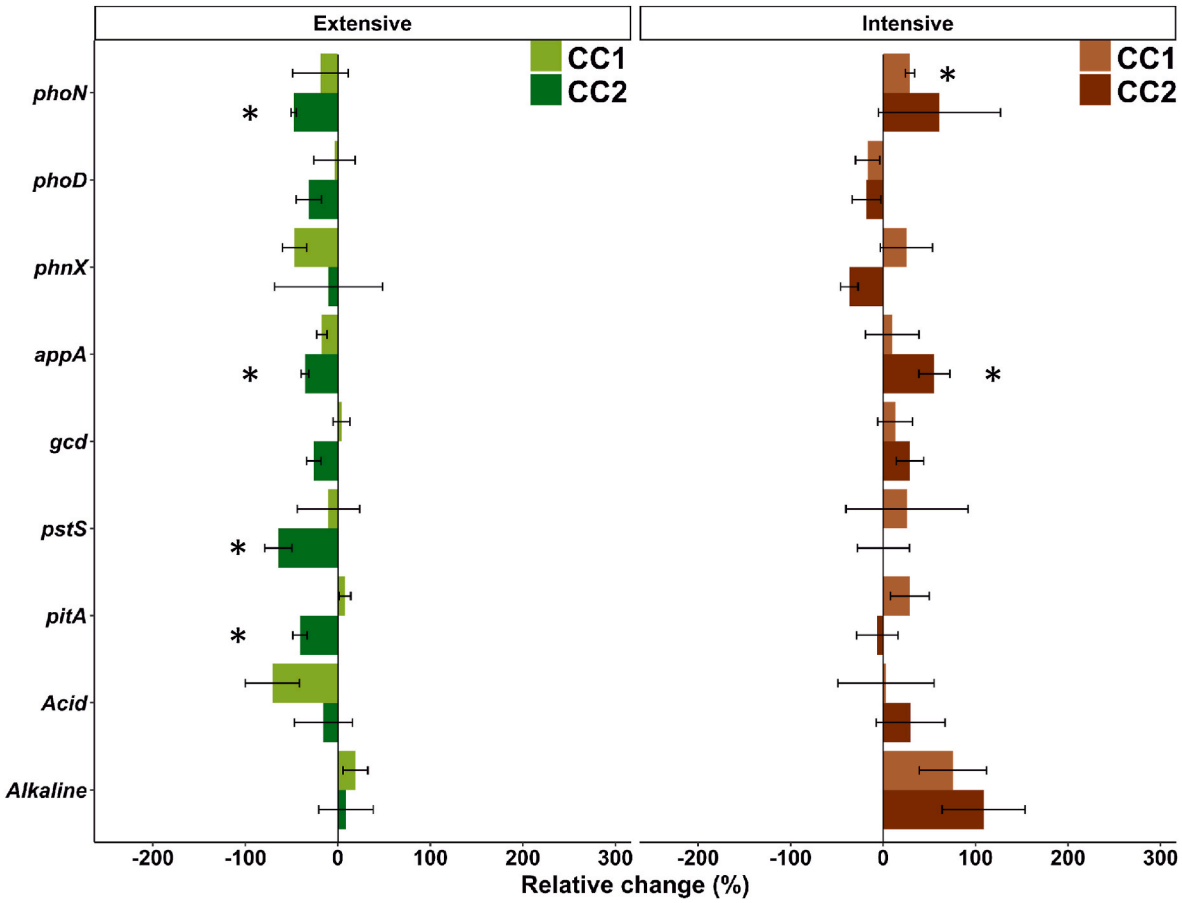

Fig. 2. Relative change (represented in percentage) of P cycling involved gene abundances at 0–5 cm depth in CC1 (moderate climate change scenario) and CC2 (strong climate change scenario) compared to CC0 (the respective control) for extensive and intensive agricultural management (n = 3). Mean values (± standard error) significantly different from zero ($p < 0.05$) are indicated by asterisk. P-cycling processes: Genes: *phoN* - acid phosphatase, *phoD* - alkaline phosphatase, *phnX* - phosphonoacetaldehyde hydrolase and *appA* – phytase (P mineralization), *gcd* - quinoprotein glucose dehydrogenase (P solubilization), *pstS* - starvation-induced transporter (Pi uptake) and *pitA* - low-affinity Pi transporter. Enzymes: Acid: acid phosphatase activity and Alkaline: alkaline phosphatase activity.

Table 1
Acid and alkaline phosphatase activities calculated from the zymography analysis in soil mesocosms treated by an extensive and intensive agricultural management at current climate conditions (CC0, control), moderate (CC1) and strong climate change (CC2) scenarios. Values are means ± standard error (n = 3). Significant differences ($p < 0.05$) are indicated by different letters.

Depth	0–5 cm						5–15 cm					
Management	Extensive			Intensive			Extensive			Intensive		
Climate effect	CC0	CC1	CC2	CC0	CC1	CC2	CC0	CC1	CC2	CC0	CC1	CC2
Acid phosphatase (pmol mm^{-2} h^{-1})	1.9 ± 0.7 a	0.6 ± 0.6 a	1.6 ± 0.6 a	1.9 ± 1.2 a	1.9 ± 1.0 a	2.5 ± 0.7 a	2.4 ± 0.8 a	0.5 ± 1.0 a	1.6 ± 0.4 a	2.4 ± 1.3 a	2.3 ± 0.8 a	2.8 ± 0.9 a
Alkaline phosphatase (pmol mm^{-2} h^{-1})	10.1 ± 1.9 a	12.0 ± 1.3 a	11.0 ± 3.0 a	7.0 ± 1.9 a	12.4 ± 2.6 a	14.7 ± 3.1 a	10.0 ± 2.2 a	10.7 ± 3.0 a	11.5 ± 3.7 a	6.6 ± 2.4 a	11.3 ± 3.6 a	13.8 ± 3.8 a

(Table 2). However, a decrease of OC and TN was observed at 0–5 cm depth with increasing CC effects (CC2 scenario), and at 5–15 cm soil depth for only Ext management. Overall, soil OC showed a strong positive correlation with soil N ($R^2 = 0.98$, $p < 0.0001$).

Concerning the total P concentrations which were comparable between CC0 and CC1 for the two soil depths, a 1.5-fold decrease was observed at 0–5 cm in CC2 under Ext management (Table 3). The OC:P mean ranged from 31 (CC2 at 5–15 cm) to 62 (CC1 at 0–5 cm) in the two investigated soil depths when an Ext management was applied, whereas OC:P ratios ranged from 46 (CC2 at 5–15 cm) to 89 (CC2 at 0–5 cm) after the application of an Int management (Fig. 4b and S7b). The TN:P mean ratios in Ext managed soils were between 3.4 (CC2 at 5–15 cm) and 6 (CC0 at 0–5 cm and CC1), while they ranged from 4.9 (CC2 at 5–15 cm) to 9 (CC2 at 0–5 cm) in Int managed mesocosms (Fig. 4c and Fig. S7c). OC:P and TN:P ratios increased under Int management (Fig. 4b and c). However due to the high variance of these data in CC2, these ratios were not significant ($p = 0.24$ for OC:P and $p = 0.23$ for TN:P). Available P concentrations (mg kg^{-1} of soil dry weight (sdw) ± standard deviation) ranged from 66.5 ± 13.4 mg kg^{-1} sdw (Ext management) to 79.9 ± 12.4 mg kg^{-1} sdw (Int management) at 0–5 cm soil depth in CC1. These values were not significantly different from those measured in CC0 (59.7 and 71.3 mg kg^{-1} sdw, respectively for Ext and Int management; Table 3). Available P was significantly ($p < 0.05$) reduced (41.7 ± 12.4 mg kg^{-1} sdw) in CC2 when Ext management was applied (Table 3). In addition, a significant interaction between CC2 and Int management was observed, as P availability increased to 64.1 ± 9.2 mg kg^{-1} sdw

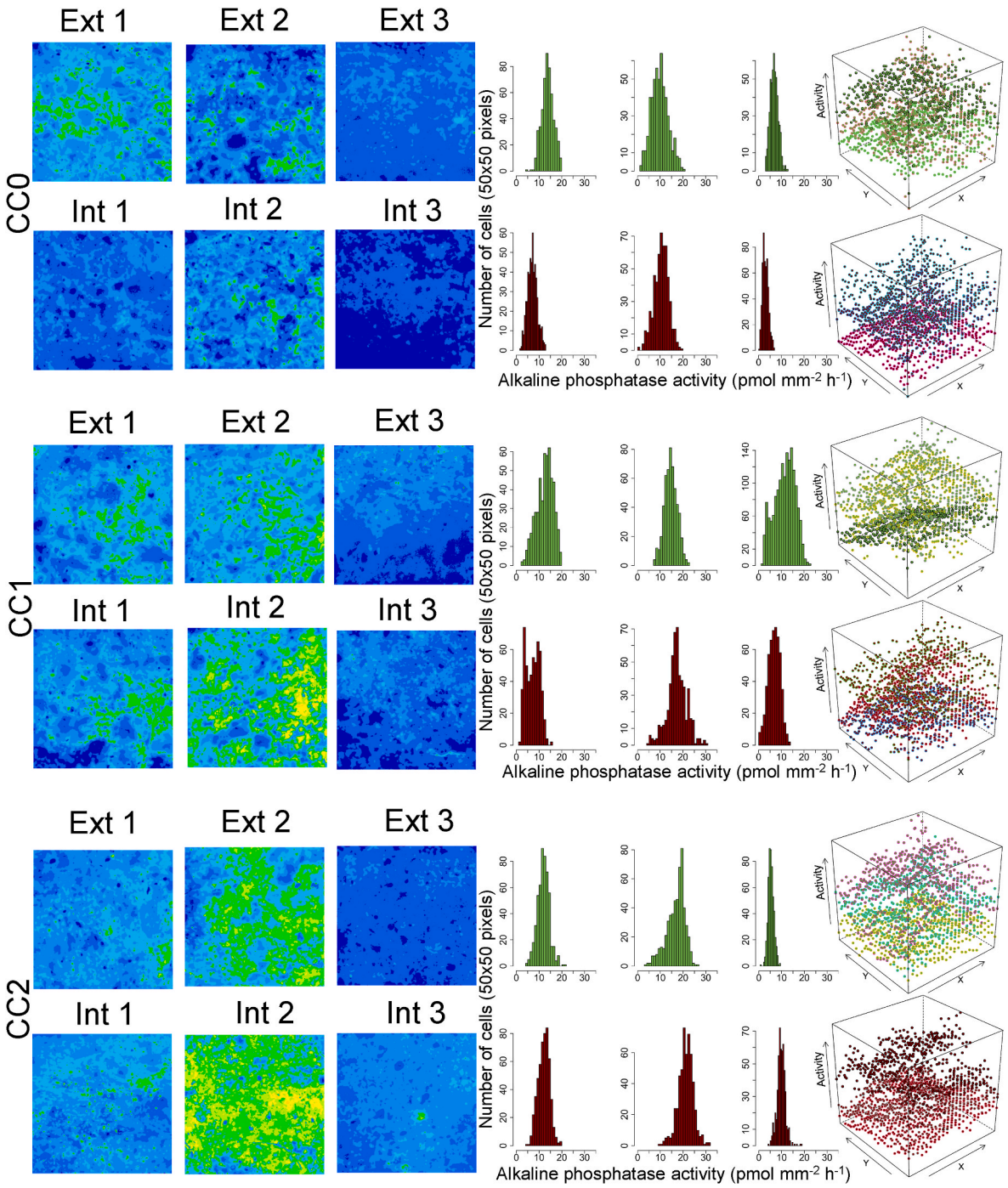

Fig. 3. Soil zymography representation of the alkaline phosphatase activity in situ in soil mesocosms treated by an extensive (Ext) and intensive (Int) agricultural management (left; n=3), with their respective frequency histograms (middle) and activity distribution on a bidimensional XY plane (right). X represent activities from left to the right and Y from bottom to top on the zymography image at the different scenarios CC0 (the respective control), CC1 (moderate climate change scenario) and CC2 (strong climate change scenario).

(Table 3). At 5–15 cm soil depth, available P ranged from 22.5 mg kg^{-1} sdw (CC2 in both Ext ± 14.6 and Int ± 5.7 managements) to 36.6 ± 11.8 mg kg^{-1} sdw (Int managed CC0) without significant differences (Table 3).

The total concentrations of elements capable of P sorbtion in soil showed that Al and Fe concentrations were the most abundant among the elements in both depths (Table 3). While Al and Fe increased respectively 41.5% and 20.4% in CC1, and 22.7% and 15.4% in CC2 at 0–5 cm depth under Int management, Zn decreased 30.4% in CC2 regardless of the agricultural management (Table 3). Al and Fe concentrations were significantly ($p < 0.05$) higher under Int management as compared with those in CC0 soil, whereas Zn concentration was significantly lower in CC2 soil under Ext management (Table 3). In addition, K concentration in soil increased significantly up to 26% in Int managed CC1 mesocosms in comparison to that in CC0 (Table 2). While plant-available K concentrations were significantly lower ($p < 0.05$) regardless to the management in CC2 at 5–15 cm soil depth, the available Mg concentrations decreased under an Ext management application at 0–5 cm in CC2 compared to CC0 and CC1 (Table 3).

To link the soil parameters with the abundances of bacteria driving

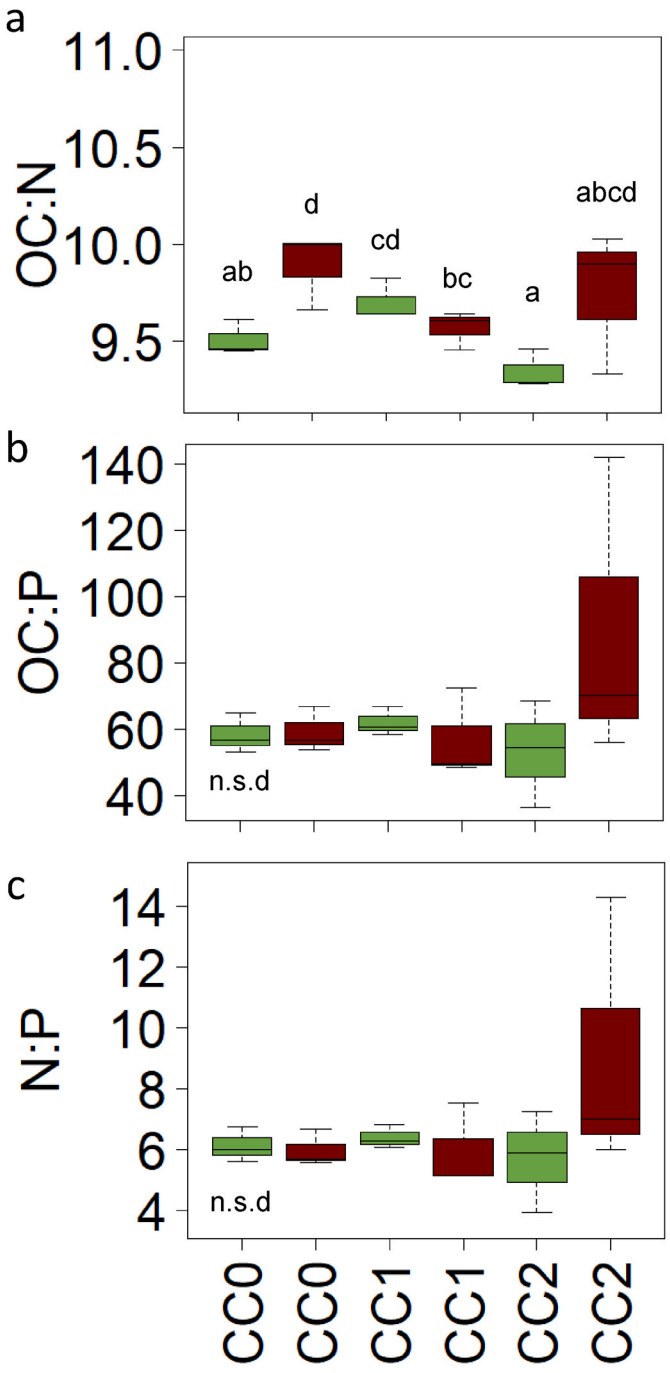

Fig. 4. Ratios on a mass basis of organic C (OC) to total N (N) (a), OC to total P (P) (b) and N to P (c) at 0–5 cm depth in soil mesocosms treated by an extensive (green) and intensive (brown) agricultural management from 2017 at current climate conditions (CC0, control), moderate (CC1) and strong climate change scenario (CC2). Values are means ± standard error (n = 3). If significant differences ($p < 0.05$) among climate change scenarios*treatment were found, these are indicated by different letters separately by macronutrient or ratio while no differences by n.s.d.

the different processes in P turnover and uptake across all CC scenarios, we used PCA to investigate the relationships separately for management and depth (Fig. 5 and Fig. S8). A clear separation of CC2 from CC1 and CC0 at 0–5 cm was observed for Ext management as a result of lower nutrient availability, Zn and available Mg concentrations in soils subjected to CC2 which was correlated with a low abundance of bacteria capable for Po mineralization (*phoN*, *phoD*, *appA*), P solubilization (based on *gcd* gene abundance) as well as bacteria which harbor the P-

Table 2
Carbon and nitrogen in soil mesocosms treated by an extensive and intensive management from 2017 under the different climate change scenarios to simulated respectively current climate conditions (CC0, control), moderate (CC1) and strong climate change (CC2) scenarios. Values are means ± standard error (n = 3). Different letters indicate significant differences between the treatments ($p < 0.05$). Total carbon (TC), total nitrogen (TN), organic carbon (OC), and inorganic carbon (IC) were measured at 0–5 and 5–15 cm soil depth.

	Extensive			Intensive		
0–5 cm	CC0	CC1	CC2	CC0	CC1	CC2
TC (mg g^{-1})	212.0 ± 1.1 d	201.8 ± 8.1 bcd	125.9 ± 13.5 a	208.1 ± 1.2 c	202.8 ± 4.8 cd	182.0 ± 7.0 b
TN (mg g^{-1})	20.7 ± 0.4 c	19.9 ± 1.0 bc	12.4 ± 1.5 a	20.1 ± 0.2 c	20.1 ± 0.4 c	17.7 ± 0.7 b
OC (mg g^{-1})	196.9 ± 5.2 c	192.9 ± 9.4 bc	116.9 ± 14.7 a	199.3 ± 0.6 c	192.7 ± 3.8 c	172.4 ± 4.9 b
ON (mg g^{-1})	19.9 ± 0.6 c	19.2 ± 0.7 c	11.9 ± 1.5 a	19.8 ± 0.2 c	19.4 ± 0.5 c	17.0 ± 0.7 b
IC (mg g^{-1})	13.3 ± 4.5 a	10.4 ± 6.4 a	6.7 ± 3.7 a	7.1 ± 3.2 a	7.7 ± 3.3 a	7.4 ± 5.0 a
IN (mg g^{-1})	0.33 ± 0.0 b	0.31 ± 0.0 b	0.10 ± 0.0 a	0.30 ± 0.0 b	0.33 ± 0.1 ab	0.32 ± 0.0 b
5–15 cm	CC0	CC1	CC2	CC0	CC1	CC2
TC (mg g^{-1})	162.7 ± 12.9 b	157.4 ± 19.3 b	82.7 ± 7.1 a	166.2 ± 7.8 b	157.3 ± 17.4 b	138.1 ± 22.1 b
TN (mg g^{-1})	16.6 ± 1.3 b	15.9 ± 2.0 b	8.2 ± 0.8 a	16.8 ± 0.7 b	16.3 ± 1.8 b	13.9 ± 2.1 b
OC (mg g^{-1})	155.4 ± 11.0 b	142.0 ± 20.6 b	75.8 ± 8.1 a	160.3 ± 7.6 b	151.1 ± 17.7 b	132.9 ± 19.1 b
ON (mg g^{-1})	15.8 ± 1.2 b	14.6 ± 2.1 b	8.1 ± 0.8 a	16.4 ± 0.9 b	15.5 ± 1.9 b	13.7 ± 2.1 b
IC (mg g^{-1})	4.1 ± 2.2 a	6.3 ± 3.7 a	7.8 ± 3.9 a	4.2 ± 2.9 a	2.4 ± 1.1 a	5.3 ± 2.3 a
IN (mg g^{-1})	0.28 ± 0.0 bc	0.34 ± 0.0 c	0.08 ± 0.0 a	0.26 ± 0.0 b	0.27 ± 0.1 abc	0.29 ± 0.0 bc

transporter *pitA* (Fig. 5a). A similar PCA separation was observed at deeper soil depth, however here stronger correlation of bacteria capable for Po mineralization and P solubilization on PC1 were found (Fig. S8a). For Int management, the three CC scenarios clearly separated at 0–5 cm depth (Fig. 5b). The higher potential for Po mineralization by phytase (*appA*) and alkaline phosphatase activities, as well as P solubilization (*gcd*) were correlated with higher concentrations of Al, K and Fe and lower concentrations of C and N (total and organic) in CC2 (Fig. 5b). Here CC1 was separated from CC0 due to the higher abundance of bacteria harboring the *phoN* gene and the marginal increase of bacteria carrying *pitA,* Mn, available Mg, and inorganic C and N (Fig. 5b). At deeper soil depth (5–15 cm) the CC scenarios were not clearly differentiated by the effects of abiotic and biotic soil parameters (Fig. S8b).

4. Discussion

Mountainous grasslands fulfill important ecosystem functions, including C sequestration, nutrient retention, and biodiversity preservation (Leifeld et al., 2009; De Deyn et al., 2011; Wiesmeier et al., 2013; Egarter Vigl et al., 2016; Garcia-Pausas et al., 2017). However, warming can lead to mineralization of SOM and therefore organic C, P and N concentrations might decrease (Cannone et al., 2008; Budge et al., 2011;

Table 3
Total P, elements and plant-available P, K and Mg concentrations in soil mesocosms treated by an extensive and intensive agricultural management at current climate conditions (CC0, control), moderate (CC1) and strong climate change (CC2) scenarios. Values are means ± standard error (n = 3). Significant differences ($p < 0.05$) are indicated in bold and by different letters.

Depth	0–5 cm						5–15 cm					
Management	Extensive			Intensive			Extensive			Intensive		
Climate	CC0	CC1	CC2	CC0	CC1	CC2	CC0	CC1	CC2	CC0	CC1	CC2
P (mg g^{-1})	3.4 ± 0.1 b	3.1 ± 0.2 b	**2.2 ± 0.1 a**	3.4 ± 0.2 b	3.5 ± 0.5 b	2.2 ± 0.5 ab	3.3 ± 0.3 a	2.7 ± 0.5 a	2.8 ± 0.9 a	3.2 ± 0.3 a	3.1 ± 0.0 a	2.9 ± 0.5 a
K (mg g^{-1})	7.4 ± 0.8 ab	6.9 ± 0.4 b	6.7 ± 0.7 b	**5.8 ± 0.1 a**	**9.5 ± 1.0 c**	7.3 ± 1.1 abc	6.0 ± 1.2 a	7.4 ± 0.6 a	7.3 ± 2.3 a	7.8 ± 0.8 a	8.3 ± 0.6 a	8.0 ± 0.7 a
Al (mg g^{-1})	36.4 ± 2.8 ab	34.6 ± 0.2 b	35.4 ± 1.7 ab	**31.3 ± 0.8 a**	**44.3 ± 3.7 c**	38.4 ± 2.0 bc	35.6 ± 4.5 a	42.3 ± 6.0 a	40.0 ± 9.1 a	42.7 ± 2.6 a	42.3 ± 5.2 a	44.4 ± 1.1 a
Ca (mg g^{-1})	26.5 ± 5.3 a	25.0 ± 7.5 a	21.6 ± 5.8 a	23.5 ± 7.5 a	18.0 ± 1.7 a	14.7 ± 5.6 a	20.5 ± 5.2 ab	20.7 ± 12 ab	28.9 ± 1.3 b	18.5 ± 4.0 a	19.7 ± 3.9 a	18.3 ± 4.7 ab
Fe (mg g^{-1})	24.5 ± 0.9 ab	23.7 ± 0.6 b	25.1 ± 1.7 ab	24.0 ± 0.9 b	28.9 ± 3.1 ab	**27.7 ± 1.3 a**	28.6 ± 1.6 a	32.0 ± 5.3 a	29.1 ± 3.7 a	30.1 ± 1.2 a	29.3 ± 5.2 a	32.3 ± 1.6 a
Mg (mg g^{-1})	13.8 ± 1.9 a	12.4 ± 2.7 a	11.1 ± 1.5 a	11.8 ± 2.0 a	10.7 ± 0.9 a	9.9 ± 1.9 a	11.2 ± 1.7 a	13.0 ± 3.5 a	12.9 ± 0.6 a	11.3 ± 1.5 a	11.4 ± 0.8 a	11.8 ± 1.6 a
Mn (mg g^{-1})	0.99 ± 0.1 a	0.91 ± 0.1 a	0.90 ± 0.02 a	0.98 ± 0.2 a	1.3 ± 0.2 a	0.98 ± 0.03 a	1.1 ± 0.2 ab	1.3 ± 0.2 ab	1.2 ± 0.2 ab	1.1 ± 0.2 ab	**0.99 ± 0.1 a**	**1.3 ±0.03 b**
Zn (mg g^{-1})	0.23 ± 0.02 b	0.22 ± 0.02 b	**0.16 ±0.01 a**	0.23 ± 0.02 b	0.26 ± 0.04 b	0.16 ± 0.03 ab	0.23 ± 0.03 a	0.20 ± 0.02 a	0.21 ± 0.05 a	0.24 ± 0.03 a	0.20 ± 0.01 a	0.22 ± 0.03 a
Available P (mg g^{-1})	0.07 ± 0.01 b	0.07 ± 0.02 b	**0.04 ± 0.01 a**	0.06 ± 0.01 b	0.08 ± 0.01 b	0.06 ± 0.01 b	0.03 ± 0.00 a	0.03 ± 0.00 a	0.02 ± 0.01 a	0.04 ± 0.01 a	0.03 ± 0.01 a	0.02 ± 0.00 a
Available K (mg g^{-1})	0.19 ± 0.01 a	0.19 ± 0.02 a	0.16 ± 0.07 a	0.19 ± 0.06 a	0.26 ± 0.04 a	0.24 ± 0.02 a	0.09 ± 0.01 abc	**0.11 ± 0.01 b**	**0.05 ± 0.02 a**	**0.12 ± 0.01 c**	0.10 ± 0.03 abc	**0.08 ± 0.01 a**
Available Mg (mg g^{-1})	3.24 ± 0.32 c	3.03 ± 0.35 c	**1.64 ± 0.41 a**	2.37 ± 0.18 b	2.90 ± 0.17 bc	2.45 ± 0.55 ab	2.34 ± 0.51 a	2.35 ± 0.58 a	1.56 ± 0.46 a	2.68 ± 0.90 a	2.16 ± 0.37 a	2.21 ± 0.58 a

Wang et al., 2016; Watanabe et al., 2019). 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 (Garcia-Franco et al., 2021; Wiesmeier et al., 2013). We found significant changes not only in the abundance of bacteria which catalyze major steps of P transformation but also in the abundance of AMF, mainly in treatments where a strong CC scenario was applied (CC2).

4.1. P solubilizing bacteria are suppressed by climate change mainly under extensive management

In contrast to our hypothesis, the abundance of bacteria able to produce gluconic acid decreased significantly under Ext management in CC2 as compared to CC0. A previous study showed that the *gcd* expression is repressed by high dissolved phosphate concentrations readily available for plants and microbes (Zeng et al., 2016). Thus, low P availability in soil may increase the abundance of *gcd*-harboring bacteria, and the abundance of P-solubilizing bacteria in general (Pastore et al., 2020; Spohn et al., 2020). Nevertheless, it is also possible that other organic acids are produced, or other P solubilizing strategies became abundant like siderophore production, which is especially relevant for iron-bound P. However, our results about plant-available P concentrations indicated that primary production in mountainous grassland might be limited by plant-available P and that the input of organic fertilizer might increase P solubilization by bacteria that use organic C as source to produce organic acids which can solubilize P.

In our study, the soil under CC2 and Ext management showed a decreased content of OC and N in both depths as compared with CC0, however a decrease in total P and available P was only observed in the upper soil depth. In addition, total P and available P concentrations were significantly reduced under the combination of strong climate change (CC2) and Ext management. Thus, decreased OC, N and P resulted in a molar OC:N:P stoichiometry of 53:6:1 and a lower OC:N ratio of 9.3 as compared to CC0 and CC1 in the upper soil layer. The OC:N ratios in CC0 and CC1 were close to that previously reported for this grassland soil by Franco-García et al. (2021). However, an OC:N:P stoichiometry of 27:3:1 was found in the deeper soil layer (5–15 cm), where the OC:N ratio was similarly low (9.2) as that in the upper soil depth, but the N:P ratio was much lower than the upper layer. Low OC:N and N:P ratios may promote SOM mineralization and not P solubilization under this CC scenario (Andrade-Linares et al., 2021). Berauer et al. (2021) found that while the plant C:N ratio (17.8–26.6) increased with warming in mesocosms under Ext management, the N:P ratios did not change significantly (7–9.2) across the CC scenarios. The difference between the soil C:N:P ratio and the plant C:N:P ratio suggests that due to warming higher

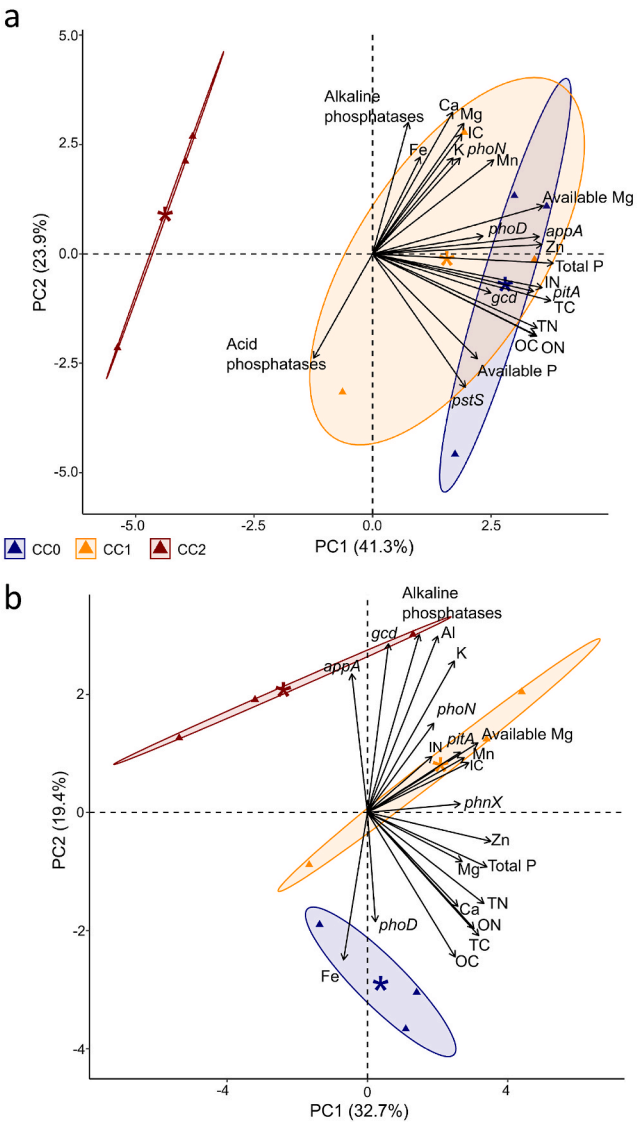

Fig. 5. Principal component analyses (PCA) integrating P cycling gene abundance, phosphatases activity and C, N, P and element concentrations measured at 0–5 cm soil depth in the translocated mesocosms CC0 (control; current conditions), CC1 (moderate climate change scenario) and CC2 (strong climate change scenario). PCA was performed for Extensive (a; n = 3) and Intensive (b; n = 3) agricultural management. The ellipses display the 95% confidence interval and are drawn around the group mean (indicated by asterisk). P mineralizing bacteria (*phoD*, *phnX* and *appA*), P solubilizing bacteria (*gcd*) and P uptake genes (*pitA* and *pstS*). TC: total carbon, OC: organic carbon, IN: inorganic carbon, TN: total nitrogen, ON: organic nitrogen, IN: inorganic nitrogen.

N concentration in soil might be utilized first by microbes than by plants and thus plants depend on microbial activity and interactions to get access to available N and P more than under intensive management. However, the yearly plant productivity was marginally higher in CC2 (1122 ± 86 g m^{-2}) as compared to CC0 (869 ± 80 g m^{-2}) which was due to the changes in plant community composition towards fast-growing species, i.e. graminoids which were dominant in CC2 (Berauer et al., 2021). Nevertheless, it is unclear if plant community shifts influence soil microbial community composition and activity in soil or if microbial community changes induce different above ground biodiversity.

Overall, the low P plant-availability in CC2 under Ext management may be related to low abundance of both bacteria that harbor genes catalyzing P mineralization (*appA*, *phoN* and *phoD*) and P solubilization (*gcd)*. PCA results indicated that P mineralization and P solubilization were correlated to C, N and element concentrations in these grasslands and that the intensive addition of nutrients by slurry application and mowing alleviate the low plant-available P under CC effects.

4.2. Increased abundance of P mineralizing bacteria as result of climate change and intensive management

Concerning our second hypothesis, the increased management intensity in combination with CC2 effects increased the abundance of bacteria carrying acid phosphatase (*phoN*) and phytase (*appA*) genes at 0–5 cm in soils in which a trend towards higher N:P ratios was observed. However, acid phosphatase activities in soil were very low as indicated by zymography analysis and significant differences of their spatial distribution were not detected in CC2.

Zymography analysis showed increased potential alkaline phosphatase activity at CC2 where the abundance of bacteria carrying *phoD* gene was higher than that of bacteria carrying *phoN* which did not change across the different scenarios under Int management. PhoN is a periplasmic and nonspecific phosphatase produced by several microbial taxa that is able to hydrolyze both phosphomonoesters and phosphodiesters from SOM at an optimal pH of 6.5 (Rossolini et al., 1998). PhoD, a monomeric alkaline phosphatase with similar activity as PhoN, is activated by Ca^{2+} (Ragot et al., 2015). The production of these enzymes, that is metabolically expensive, is tightly regulated by means of a two-component system of the Pho regulon which control a cross-talk between Pi and N regulatory pathways (Santos-Beneit, 2015).

Additionally, the N:P ratio in soil can be altered by Int management in grasslands which is crucial to consider for application strategies. This specifically applies, if pH is considered, which decreased at the short-term exposure (Andrade-Linares et al., 2021) combined with significant increases of Al and Fe found in CC2 soil which might derive from the slurry. These increases may lead to P adsorption by their soluble forms (Al^{+3} and Fe^{+2}), and in the long term to low microbial P immobilization and limited plant P availability. The increase in potential phosphatase activity may indicate that microbes are P limited by additional N inputs as increased N availability might increase P demand (Spohn, 2016; Margalef et al., 2017; Neal et al., 2017).

4.3. Decreased abundance of AMF by climate change and management

The abundance of AMF in Ext managed soil was not significantly affected by CC effects as hypothesized. However, it could be explained due to the plant productivity was not decreased at the short term and therefore AMF was not affected by low C exudation from the plant (Berauer et al., 2021). Species of *Acaulospora*, *Glomus*, *Diversispora* and a number of new AMF species have been described in alpine grasslands, which are able to colonize the typical plant species found in the grasslands studied here (Read and Haselwandter, 1981; Oehl et al., 2003, 2011; Vandenkoornhuyse et al., 2003; Ceulemans et al., 2019). AMF can transfer not only Pi but also N to the plant (Govindarajulu et al., 2005) and in return take C from the plant host, however, this symbiosis may depend on the OC:N:P stoichiometry and P availability (Thirkell et al., 2016). Thus, under CC2, the low amount of OC and N as compared to CC0 may lead to a decrease in extraradical mycelia of AMF in soil. Nevertheless, the abundance of AMF was negatively affected already at CC1 by the application of an Int management where the OC:N:P stoichiometry was 55:6:1 in comparison to that found in the Int managed soil of 62:6:1 at CC0. Therefore, it is important to consider the potential negative effect of N inputs on AMF, which can impact their diversity as it has been shown that already 7.7 kg N ha^{-1} yr^{-1} decreased AMF richness (Ceulemans et al., 2019) A decrease in AMF biodiversity may impact plant richness and productivity which may lead to negative feedbacks effecting both above and below ground biodiversity (Van Der Heijden et al., 2006; Jiang et al., 2021; Zhang et al., 2022).

5. Conclusions

Management intensity of mountainous grasslands strongly impacts the stoichiometry of elements in soil mainly under CC. Our study suggests that grassland management under CC should consider the impact of interacting effects of CC and management on AMF and bacterial activities related to plant nutrition for diversity stabilization, plant productivity and ecosystem functioning. Our findings indicate that prealpine grasslands that are rich in SOC and N can be affected by extensive management under climate change triggering low soil C to N ratio due to high microbial activity. This, in turn, can reduce organic P mineralization and P solubilization, resulting in a decreased P plant-availability. In the long run this reduction in plant-available P may decrease plant growth. However, intensive management which involves inputs of C, N and P can promote plant growth. The inputs can increase the activity of P-mineralizing enzymes and the abundance of P solubilizing bacteria under climate change. As a result, the plant-availability of P can be elevated which might be beneficial for plant growth and productivity. However, it may also drive SOM decomposition which may reduce OC stocks in these grasslands. Further, intensive management may decrease the AMF abundance and diversity, and select for fast growing plants and microorganisms. However, effects found in this study just reflect short-term adaptation processes and may differ in the long run. In addition, this study mainly focused on the assessment of microbial potentials, while activity pattern was not measured. Thus, future studies may take these issues into account, which might be essential to predict impacts of CC and management on P transformation and P stocks in mountainous grasslands, which could be the basis for the development of targeted mitigation strategies.

Author contributions

D.R. A-L took and prepared soil samples, conducted the molecular work, prepared and analyzed results, and wrote the original draft. U.S. conducted the zymography analysis and reviewed the draft. S.S. supervised the molecular results and reviewed the draft. M.D. conceptualized the experimental work, reviewed the draft. R.G. coordinated the mesocosm management and slurry analyses. M. Sp. supervised zymography analysis and reviewed the draft. C.B. conducted the available P and microelement analyses and reviewed the draft. M.W. reviewed the draft. N.F-G. reviewed the draft. M.S conceptualized the experimental work, edited and reviewed the draft.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2023.109175.

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