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N_2 fixation is less sensitive to changes in soil water content than carbon and net nitrogen mineralization

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ABSTRACT

Biogeochemical processes catalyzed by soil microorganisms depend on the soil water content (SWC). Yet, little is known about the sensitivity of non-symbiotic N_2 fixation to changes in SWC in comparison to carbon (C) and net nitrogen (N) mineralization. Therefore, we determined the rates of N_2 fixation, C mineralization, and net N mineralization in soils at five SWCs that were created by water addition to the field-moist soil (rewetting), resulting in SWCs of 20, 40, 60 and 80% of the soil water holding capacity (WHC) which correspond to 14, 28, 42, and 57% water-filled pore space (WFPS). The soils originated from an arid, semiarid, Mediterranean, and humid site located in the Coastal Cordillera of Chile. The N_2 fixation rate was measured immediately after the adjustment of the SWC and additionally after a five-day pre-incubation period. We found that the rates of all three processes where higher in the rewetted soils compared to the field-moist soils, but the sensitivity to changes in SWC differed between the processes. N_2 fixation tended not to increase with increasing SWC beyond 20% WHC. Furthermore, the N_2 fixation rate increased faster after rewetting in the soils of the semiarid and Mediterranean zone than in the soil of the humid zone. In contrast, C mineralization reached the highest rate at 80% WHC in the soil of the humid zone and at 60% and 80% WHC in the soils of the other three climate zones. The net N mineralization rate was highest at 40% and 60% WHC in the soils of the semiarid and Mediterranean zone, and was significantly decreased at 80% WHC compared to 60% WHC. Our study shows, first, that N_2 fixation is less sensitive to changes in SWC than C and net N mineralization and tends to be already at its maximum in a given soil at low SWC, i.e., 20% WHC. This is likely because the diffusion of N_2 in soil does not increase with the SWC, in contrast to the diffusion of organic solutes which are the substrate of C and N mineralization. Second, our results indicate that the N_2 fixation rate increases more quickly in response to rewetting of soils of the more arid climate zones than of the humid zone, which might indicate a microbial adaptation to the climate conditions. The results have important implications since they suggest that the N_2 fixation rate is likely less affected than the C and net N mineralization rates by decreases in the SWC that might occur more frequently in the future due to climate change.

1. Introduction

The soil water content (SWC) will likely decrease during the next decades in many parts of the world due to climate change (Trenberth et al., 2014). Therefore, it is important to understand how the SWC affects biogeochemical reactions in soils. Water is essential for all reactions that are catalyzed by microorganisms as it is required for the movement of (1) dissolved organic matter and inorganic nutrients, (2) enzymes that catalyze biochemical reactions, and (3) microbial cells that intracellularly catalyze reactions, such as the mineralization of carbon (C) and nitrogen (N) and the fixation of N_2 (Moyano et al., 2013). When soils dry out, water-filled pores become disconnected and water films become thinner and increasingly discontinuous. As a result, diffusion path lengths become more tortuous and the rate of solute diffusion declines, which decreases substrate supply to heterotrophic microorganisms (Skopp et al, 1990; Schjønning et al., 2003). In contrast to the movement of solutes, the diffusion of gas decreases with increasing water-filled pore space (WFPS) (Cook and Knight, 2003; Moyano et al., 2013; Román et al., 2021). Thus, oxygen (O_2) can become limiting for aerobic organisms as soil-pore space filled with water approaches

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saturation levels, and as a result, the metabolic activity of aerobic organisms decreases. Thus, optimal WFPS for respiration of aerobic microorganisms (called C mineralization hereafter) must allow for diffusion of solutes on the one hand, and for O_2 diffusion on the other hand (Manzoni et al., 2012; Moyano et al., 2013; Yan et al., 2016).

N_2 fixation by free-living bacteria (diazotrophs) in aerobic soils also depends on the SWC as cells need organic C to gain energy for N_2 fixation. However, in contrast to the movement of organic compounds, that are the substrate of the C and N mineralization reaction, the diffusion of the gas N_2 decreases with increasing SWC (Cook and Knight, 2003). Consequently, it can be hypothesized that N_2 fixation is less sensitive to changes in the SWC because the diffusion of the substrate or educt of the N_2 fixation reaction does not increase with SWC. Yet, there are only few empirical studies that explored the dependence of N_2 fixation on the SWC in soils. Most studies that investigated the response of N_2 fixation by free-living microorganisms to moisture have focused on N_2 fixation of bacteria associated with mosses (cyanobacteria-bryophyte association) in high latitude ecosystems, finding increased N_2 fixation rates at increased SWC (Gundale et al., 2009; Whiteley and Gonzalez, 2016; Rousk et al., 2017). There is only one study that explored the soil water-dependence of N_2 fixation by free-living bacteria that are not associated with mosses (Li et al., 2018), to our knowledge. This study found that N_2 fixation in calcareous soils in subtropical forests increased with SWC (Li et al., 2018). However, there is no study that systematically explored the sensitivity of N_2 fixation in comparison to the sensitivity of C or N mineralization to changes in SWC, as far as we know. Such a study is required because all three reactions are central to the biogeochemical cycling of elements, and we need to understand the sensitivity of these processes to variation in SWC, since the SWC responds to changes in climate (Trenberth et al., 2014). It can be expected that the O_2 concentration has no effect on the N_2 fixation by diazotrophs in well-aerated, aerobic soils since they have developed mechanisms to protect the oxygen-sensitive nitrogenase enzyme complex against O_2 (Gallon, 1992).

Frequent and extreme drying-rewetting may select for microbial taxa that are more tolerant to desiccation stress (Evans and Wallenstein, 2012). Accordingly, microbial communities previously exposed to disturbances, such as drought, have proven to be more resistant to this stress than communities that have not been exposed to the stress before (Fierer et al. 2003; Waldrop and Firestone, 2006; Evans and Wallenstein, 2012; Canarini et al., 2021). Furthermore, it was shown that soil microorganisms in relatively dry ecosystems with only occasional precipitation events respond more quickly to soil rewetting in terms of respiration than in ecosystems with more frequent precipitation events, which might also be mediated by the vegetation (de Nijs et al., 2019). Hence, it can be hypothesized that N_2-fixing microorganisms in relatively dry ecosystems with infrequent precipitation events also react more rapidly after rewetting than in humid ecosystems where the SWC varies less strongly throughout the year.

Soils that experience pronounced drying and rewetting often respond to rewetting by strongly increased rates of C and N mineralization, which has been termed “Birch effect” (Birch, 1964; Jarvis et al., 2007; Schimel, 2018). It is not entirely understood which processes cause the Birch effect (Schimel, 2018). Most likely, the effect results from several processes, including the activation of dormant microorganisms, the mineralization of microbial biomass that died during the preceding drought phase, and mineralization of osmolytes that are released by microbial cells upon soil rewetting (Schimel, 2018).

The aim of this study was to determine the sensitivity of N_2 fixation, C mineralization, and net N mineralization to changes in SWC. We tested the hypothesis that N_2 fixation increases less strongly with increasing SWC than C and net N mineralization. For this purpose, we selected sites from different climate zones because the sensitivity of the three processes to changes in SWC might differ between different climate zones due to an adaptation of the soil microbial community to the prevalent SWC. In order to allow comparison of the soils, we adjusted the SWC relative to water holding capacity (WHC) and WFPS of each soil. In addition, we incubated the soils also at field moisture. We determined the rates of N_2 fixation, C mineralization, and net N mineralization in soils of four climate zones at five SWCs. This approach allowed us to gain results about the sensitivity of these three processes to changes in SWC in soils from different climate zones with different properties. The soils originated from an arid, semiarid, Mediterranean, and humid site located in the Coastal Cordillera of Chile which have a comparable bedrock and experience little direct anthropogenic disturbance.

2. Material & methods

2.1. Study sites and soil sampling

Soils were collected at four sites located between 26 and 38° S in the Coastal Cordillera of Chile in March 2020. The sites form a precipitation sequence covering arid, semiarid, Mediterranean, and humid climate with rainfall increasing from 10 to 1,084 mm yr^{-1} and mean annual temperature ranging from 18 to 14 °C (Table 1). The investigated soils developed on plutonic rocks of similar granitoid lithology. All sites were free of glaciation during the last glacial maximum and have not received inputs of volcanic material (Oeser et al., 2018). The arid site Pan de Azúcar is located in the southern range of the Atacama Desert, and is part of the national park Pan de Azúcar. The soil was classified as a Regosol (Bernhard et al., 2018). The arid to semiarid site Santa Gracia is located in a private reserve. In order to distinguish it from the previous site we will call it semiarid in the following. The soil was classified as a Cambisol and the vegetation is formed by shrubs and cacti, while herbaceous plants are sparse. The Mediterranean site is located in the national park La Campana. The soil was classified as Cambisol and the site hosts a sclerophyll forest. The humid site is located in the national park Nahuelbuta. The soil was classified as an Orthodystric Umbrisol. The main tree species are southern beech (*Nothofagus antarctica*) and monkey puzzle trees (*Araucaria araucana*) with an understory of shrubs, bamboo, and grasses. At the arid site, there are no higher plants. Thus, non-symbiotic N_2 fixation is the only form of N_2 fixation that potentially occurs in this ecosystem. At the semiarid site, there are only cacti and a few non-N_2 fixing plant species. Thus, non-symbiotic N_2 fixation is the only form of N_2 fixation that occurs in this ecosystem. At the Mediterranean site, there are Acacia trees (*Acacia caven*). Thus, it is very likely that here also symbiotic N_2 fixation takes place. In the humid forest, no N_2 fixing plant species was identified. Thus, symbiotic N_2 fixation likely plays only a subordinate role here, if any. Details about climate, parent material, soils, vegetation, and soil CO_2 concentrations are given in Bernhard et al. (2018), Oeser et al. (2018), Brucker and Spohn (2019), and Spohn and Holzheu (2021). At each of the four sites, four soil samples (of about 2000–3000 g field moist soil each) were collected from the mineral soil in a depth of 0–10 cm within a square of 10 m × 10 m using a shovel. The soil was sieved (<2 mm) and roots were removed. Both during and after sieving, the four soil samples from each site were thoroughly mixed to gain one composite soil sample for each of the four sites. These composite soil samples are called “field-moist soil” in the

Table 1
Location and climate properties of the four study sites in four climate zones in the Coastal Cordillera in Chile (for more information see Oeser et al., 2018; Bernhard et al., 2018), including mean annual temperature (MAT) and mean annual precipitation (MAP).

Climate zone	Site	Southern latitude	Western longitude	MAT [°C]	MAP [mm yr^{-1}]
Arid	Pan de Azúcar	26.11015	70.54916	18.1	10
Semiarid	Santa Gracia	29.75726	71.16571	16.1	87
Mediterranean	La Campana	32.95599	71.06340	14.9	436
Humid	Nahuelbuta	37.80801	73.01514	14.1	1084

following until the SWC is experimentally manipulated.

2.2. Soil physical and chemical analyses

The SWC of the field-moist soil was determined gravimetrically for each soil sample with four replicates. In addition, the WHC (also called maximum water holding capacity elsewhere) was determined for all four soils in four replicates. For this purpose, soils were placed on filters in metal cylinders and saturated with water for 24 h. Afterwards, the samples remained on a sand bath for 24 h for drainage before being weighed again. Subsequently, the samples were dried at 105 °C in an oven for 24 h and cooled down in a desiccator before the weight of the dried samples was recorded. Based on these measurements, the SWC at WHC was determined as follows.

$$\text{SWC at WHC} = (\text{saturated soil } [g] - \text{dry soil } [g])/\text{dry soil } [g]\cdot 100\%$$

A part of the soil was air-dried and the soil pH was measured in three replicates in a soil:solution ratio of 1:5 (in H_2O and 0.01 M $CaCl_2$). For this purpose, 5 g of soil were mixed with either 25 ml of distilled water or with 25 ml of 0.01 M $CaCl_2$. The suspension was shaken every 15 min over the course of an hour before using a pH meter (pH 340, WTW GmbH, Weilheim, Germany) with a Sentix 51 electrode for measurement.

Another part of the soil was dried at 105 °C and the total soil organic C (TOC) and total N (TN) concentrations were analyzed using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS; delta S, ConFlo III, Finnigan MAT Bremen, Germany) after samples had been milled. The soil of the arid site contained carbonates (Bernhard et al., 2018). Therefore, the total C content of this soil was corrected by the carbonate-C content (as given in Bernhard et al. 2018).

2.3. Incubation experiments

We determined the rates of N_2 fixation, C mineralization, and net N mineralization in the four soils at five different SWCs (including field moist) in four replicates for C and net N mineralization, and in six replicates for N_2 fixation, as detailed below. In order to allow comparison of the soils, we adjusted the SWC relative to WHC of each soil, i.e., 20, 40, 60, or 80% WHC. Thus, we avoid the pitfall of studies that explore soils with different texture and organic matter content at the same volumetric water content, which results in very different water availabilities for the different soils. We also determined the process rates in the samples at field moisture (i.e., SWC of the fresh samples), which reflects a typical SWC of each of the soils. We calculated the rates of all three processes over the first five days of incubation, for the sake of comparison. The measurement of all three processes started immediately after the SWC was adjusted by adding the respective volume of distilled water dropwise with a pipette to the soils that were all relatively dry at the time of sampling (Table 2). In addition, the N_2 fixation rate in all soils at all five SWCs was also assessed a second time after a five-day pre-incubation period that started with the adjustment of the SWCs, in order to assess how quickly the rate changes after rewetting.

2.3.1. N_2 Fixation

The fixation rate of atmospheric N_2 by free-living microorganisms (diazotrophs) was determined using an artificial atmosphere with ^{15}N-labeled N_2 gas according to Zechmeister-Boltenstern (1996). For this purpose, 10 g of soil (dry-mass equivalent) were weighed into 20 ml serum flasks (USB Type I, DWK Life Sciences GmbH, Germany). The SWC was adjusted to either 20, 40, 60%, or 80% WHC with six replicates for each SWC, while another six replicates were kept field moist. The SWC was adjusted dropwise with distilled water using a pipette. For the treatment with pre-incubation, the water addition step was performed five days prior to the adjustment of the artificial atmosphere. During pre-incubation, the samples were incubated in the dark at 20 °C. For treatments with no pre-incubation, water was added 45 min before the adjustment of the artificial atmosphere to allow for water distribution within the soil.

For the adjustment of the artificial atmosphere, samples were connected to a gas line. The serum flasks were first evacuated with a vacuum pump for about 2 min (min. −828 mbar; max. −840 mbar), then flushed with argon for 10 min, and finally evacuated again for at least 3 min. The pressure in the sample flasks was adjusted with the vacuum pump to −825 mbar. An artificial atmosphere was created in the evacuated serum flasks by containing 80.5 (v/v) % ^{15}N-N_2 (98 atom% enriched; Lot No.: MBBC5404, Sigma-Aldrich Inc., USA), 5.5 (v/v) % O_2, and 17.5 (v/v) % Ar. After the adjustment of the artificial atmosphere was completed, the samples were immediately incubated at 20 °C in the dark for five days. Controls were kept in flasks without ^{15}N at 20 °C in the dark. At the end of the incubation, the flasks were opened under the fume hood and the soil was freeze dried for 24 h. The freeze-dried soil samples were milled and in addition also non-labeled natural abundance control soil samples were milled on a different ball mill. The isotopic signature of the N in the soil samples was determined using an elemental analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS; delta S, ConFlo III, Finnigan MAT Bremen, Germany). The N_2 fixation rate (in ng N g soil^{-1} d^{-1}) was estimated using an isotope mixing model (Zechmeister-Boltenstern, 1996):

$$^{15}N_2\ fixation\ \left[ng\ N\ g\ soil^{-1}\ d^{-1}\right] = TN\ \left[mg\ N\ g\ soil^{-1}\right]\cdot\frac{\left(^{15}N_{2,L} - {}^{15}N_{2,N}\right)\ [atom\%]}{100\cdot incubation\ time\ [d]}\cdot 10^6$$

where TN is the total soil N [mg N per g soil dry weight], $^{15}N_{2,L}$ [atom%] the percentage of ^{15}N atoms in the labeled sample, $^{15}N_{2,NL}$ [atom%] the percentage of ^{15}N atoms in the non-labeled sample (control), and incubation time [d] the time between the beginning and end of the incubation with $^{15}N_2$ (i.e., 5 days).

Table 2
Soil physical properties, including soil texture, soil water content (SWC) of the field-moist soil, SWC at 100% water holding capacity (WHC), and percentage of the WHC found in the field-moist soils from the arid, semiarid, Mediterranean, and humid climate zone. Data on soil texture is taken from Bernhard et al. (2018).

Climate zone	Texture			SWC [%]	SWC at 100% WHC [%]	%WHC of field-moist soil
	Sand [%]	Silt [%]	Clay [%]			
Arid	69	20	11	0.64 ± 0.02	24.04 ± 0.14	3
Semiarid	77	16	7	0.72 ± 0.02	26.16 ± 0.67	3
Mediterranean	72	17	11	5.52 ± 0.10	84.86 ± 0.59	7
Humid	52	22	26	14.96 ± 0.07	82.67 ± 0.79	18

2.3.2. C mineralization

To determine the C mineralization (respiration) rate, 20 g of soil (dry-mass equivalent) were weighed into 350-ml glass flasks. The caps of the flasks had septa to allow for the collection of gas samples using a syringe. The experiment was started by adjusting the SWC and closing the flask. The SWC was adjusted to either 20, 40, 60%, or 80% WHC with four replicates for each SWC, while another four replicates were kept field moist, as detailed above. The samples were stored in a climate chamber in the dark at 20 °C. The CO_2 concentrations in the flasks were measured at six time points; immediately, after the SWC was adjusted, and then 2, 5, 10, 20, and 40 days after the SWC had been adjusted. The CO_2 concentrations were determined using a gas chromatograph (GC; SRI 8610C Gas Chromatograph, SRI Instruments, Inc., USA). At the point of gas sample collection, air pressure, air temperature, and the pressure inside the glass flask was noted for each sample at each measurement time point. The CO_2 concentration in ppm was then used to calculate the volume [m^3] of CO_2 (V_CO_2) in the headspace of the flasks. The headspace was determined by subtracting the soil and water volume from the total volume of the flask (350 ml) by considering the respective soil densities. By taking into account the sum of air pressure and pressure in the glass flask p [Pa], V_CO_2, the air temperature T [K], the conversion of [mol] into [µg], the universal gas constant $R = 8.314$ kg m^2 s^{-2} mol^{-1} K^{-1}, and the ideal gas law, the amount of CO_2 was calculated using the following equation:

$$CO_{2\_gas}\ [\mu g] = \frac{p\ [kg\ m^{-1}\ s^{-2}] \cdot V\_CO_2\ [m^3]}{R\ \left[kg\ m^2\ s^{-2}\ mol^{-1}\ K^{-1}\right] \cdot T\ [K]} \cdot 12 \cdot 10^{-6}$$

To allow direct comparison with the rate of N_2 fixation, we calculated the C mineralization rate over the first five days of the incubation experiment by dividing the difference in CO_2 between day 0 and day 5 by the soil dry mass and the incubation time. Immediately at the end of the 40-day incubation experiment, the microbial biomass C and N concentration of the soils were determined (see below).

2.3.3. Net N mineralization

Net N mineralization is defined as the net release of inorganic N by microorganisms, thus it equals the gross N mineralization minus microbial N immobilization. For the determination of the net N mineralization rate, 20 g soil (dry-mass equivalent) were weighed into 250-ml polyethylene flasks. The SWC was adjusted to five different SWCs, as explained above, with 24 experimental units per SWC for every soil. Thus, there was a total of 480 experimental units (four soils × five SWCs × 24 replicates). The samples were stored in a climate chamber in the dark at 20 °C. Four replicates per SWC for every soil were extracted at six different times; immediately after the adjustment of the SWC, and 2, 5, 10, 20, and 40 days after the SWC had been adjusted. For this purpose, 100 ml of a 1.0 M KCl solution were added to the soil and the flasks were shaken on an overhead shaker for 1 h. Subsequently, the samples were filtered <0.45 µm (Cellulose Acetate Filter, Sartorius Stedim Biotech GmbH, Germany) using vacuum (min. −150 mbar; max. −450 mbar) at 5 °C in a climate chamber. The extracted soil solution was collected in 100-ml PE flasks and frozen until analysis. The concentrations of ammonium (NH_4^+) and nitrate (NO_3^-) were determined using a flow injection analyzer (FIA; FIA-LAB, MLE Dresden, Germany) according to the instructions of the manufacturer. After addition of two different reagents the concentration of NH_4^+ was determined photometrically at 590 nm and the NO_3^- concentration at 546 nm. To allow direct comparison with the rate of N_2 fixation, we calculated the net N mineralization rate over the first five days of the incubation experiment by dividing the difference in the amount of inorganic N (the sum of N in NH_4^+ and NO_3^-) between day 0 and day 5 by the soil dry mass and the incubation time.

2.4. Microbial biomass C and N

Immediately at the end of the incubation for the determination of the C mineralization rate, the microbial biomass C and N concentrations (MBC and MBN) were determined using the chloroform fumigation-extraction method (Vance et al., 1987). Fumigation was performed for 24 h in a desiccator. Fumigated and non-fumigated samples were extracted in 0.5 M K_2SO_4 in a ratio of 1:5 (soil:extractant). Samples were diluted in a ratio of 1:20 before measuring dissolved C and N using a TOC/TN analyzer (multi N/C 2100, Analytik Jena, Jena, Germany). Microbial biomass C and N were calculated by dividing the difference in the concentrations of the extracts of the non-fumigated samples and the fumigated samples by the soil dry mass and multiplying it with a conversion factor of 2.22. The C and N concentrations of the non-fumigated samples are considered labile organic C and N.

2.5. Model and statistical analyses

The relationship between the measured rates (of N_2 fixation, C mineralization, and net N mineralization) and the SWC were modelled using a Michaelis-Menten-kinetics, similar as in Davidson et al. (2012), as follows.

$$Rate = Rate_{max} \cdot \frac{SWC}{k + SWC}$$

$Rate_{max}$ is the maximum rate of the respective microbial process, and k is a constant that corresponds to the SWC required to reach half R_{max}. The Michaelis-Menten model is usually used to describe enzyme activity as a function of substrate concentration. Here we used it to model the rates of N_2 fixation, C mineralization, and net N mineralization as a function of SWC. The rationale behind this is that all three processes, and particularly C and net N mineralization, depend to some extent on exoenzyme activity that is affected by the SWC in a similar way as by the substrate concentration because the SWC affects the diffusion of the exoenzymes (Holz et al., 2019).

Further, we converted the SWC given in %WHC to SWC given in % WFPS by dividing the values by 1.415, following Franzluebbers (2020). Thus, SWCs of 20, 40, 60 and 80% of the soil WHC correspond to 14, 28, 42, and 57% WFPS. In addition, we normalized the rates of N_2 fixation, C mineralization, and net N mineralization by dividing all rates measured in one soil at different SWCs by the highest rate determined for this soil in order to adjust for the inherent differences in the magnitude of the processes among the soils. For the N_2 fixation rates, this normalization was done separately for the pre-incubated and non-pre-incubated soils.

Data were proven to be normally distributed using the Shapiro-Wilk test and homogeneity of variance was proven using Levene's test. The significance of differences between treatments and sites was tested using ANOVA. The Tukey test was used as a post-hoc test. The significance of differences between the N_2 fixation rates were detected using the Kruskal-Wallis test and the Wilcoxon test as post-hoc test, as the criterion of normality was not met. In all analyses, $p < 0.05$ was considered for the threshold of significance. All analyses were performed using R, version 3.6.1 (R Core Team, 2019).

3. Results

3.1. Soil properties

The SWC in the field-moist samples was between 3% (Mediterranean zone) and 18% (humid zone) of the soil WHC (Table 2). The pH_{H2O} ranged between 8.59 (arid zone) and 6.04 (humid zone; Table 3). The TOC content increased in the order arid < semiarid < humid < Mediterranean. The molar TOC:TN ratio increased along the precipitation gradient from 7.0 in the soil of the arid zone to 34.5 in the soil of the humid zone, and the ratio of organic carbon-to-inorganic nitrogen (DOC:

Table 3
Soil chemical properties, including pH measured in water and $CaCl_2$, total organic carbon (TOC), total nitrogen (TN), dissolved organic carbon (DOC) and inorganic nitrogen (DN) as well as microbial biomass carbon (MBC) in the K_2SO_4 extracts of the field-moist soils from the arid, semiarid, Mediterranean, and humid climate zone, b.d.l.: below detection limit.

Climate zone	pH_{H20}	pH_{CaCl2}	TOC [g kg^{-1}]	TN [g kg^{-1}]	Molar TOC:TN ratio	DOC [g kg^{-1}]	DN [g kg^{-1}]	DOC:DN ratio	MBC [g kg^{-1}]
Arid	8.59	7.89	2.8	0.32	7.00	0.04	0.03	1.70	0.08
Semiarid	7.67	6.79	18.25	1.60	13.30	0.33	0.05	7.71	0.15
Mediterranean	7.28	6.64	141.45	6.04	26.82	1.17	0.12	11.16	0.91
Humid	6.04	4.67	110.52	3.74	34.47	0.17	b.d.l.	NA	0.71

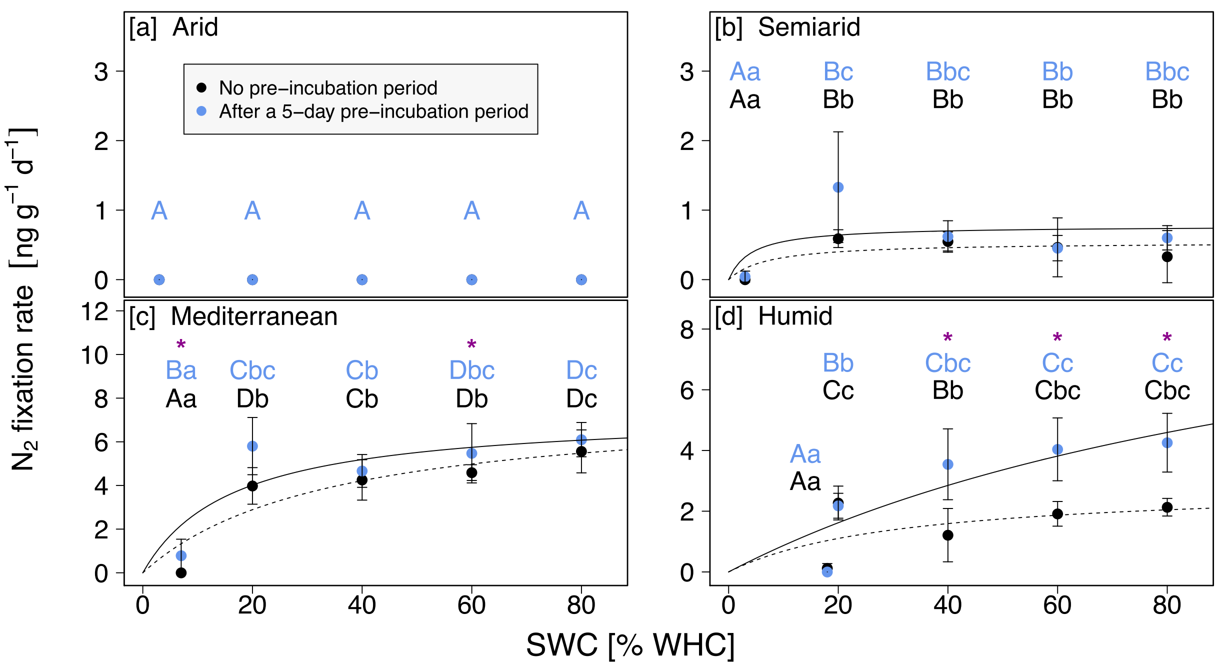

Fig. 1. N_2 fixation rate as a function of the soil water content (SWC) given as % water holding capacity (WHC) for soils of the [a] arid, [b] semiarid, [c] Mediterranean, and [d] humid climate zone determined with and without a 5-day pre-incubation period. Error bars depict standard deviations ($n = 6$). Different capital letters indicate significant differences ($p < 0.05$) between the climate zones at a given SWC, and different small letters indicate significant differences ($p < 0.05$) between the different SWC in one climate zone. Asterisks indicate significant differences between the pre-incubated and the non-pre-incubated soils. An asymptotic concave function was fitted to the N_2 fixation rates, indicated by the dashed and the continuous line for the non-pre-incubated and pre-incubated soils, respectively.

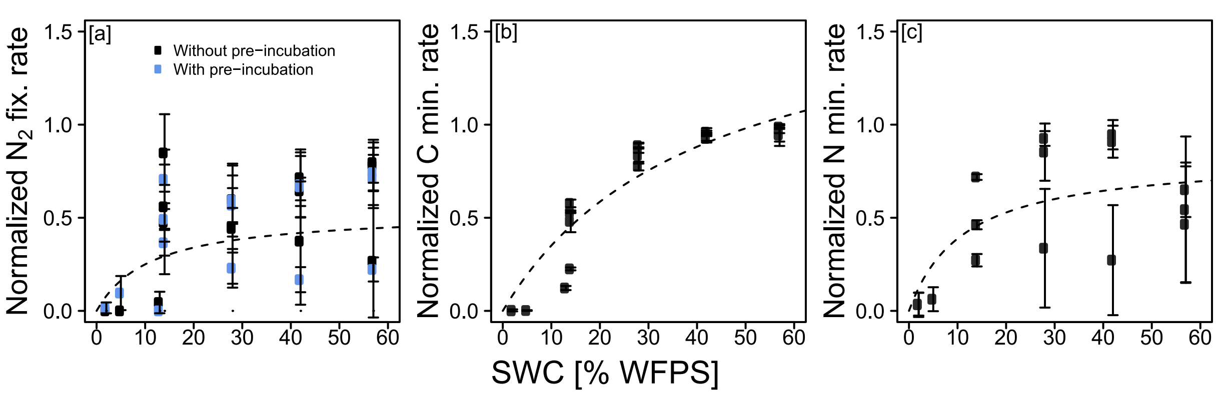

Fig. 2. Normalized rates of N_2 fixation [a], C mineralization [b], and net N mineralization [c] as a function of the soil water content (SWC) given as %water-filled pore space (WFPS) for all sites with a rate > 0. Normalization was done by dividing all rates measured in one soil at different SWCs by the highest rate determined for this soil. Please notice that the normalization of the N_2 fixation rate was done separately for the samples with and without pre-incubation, which cancels the effect of pre-incubation out. An asymptotic concave function (Michaelis-Menten model) was fitted to the rates (indicated by the dashed line).

DN) in the extracts showed a similar increase (Table 3). In the soil of the humid zone, inorganic nitrogen (DN) in the extracts was below the detection limit (Table 3).

3.2. N_2 Fixation

Across all soils, the N_2 fixation rate was significantly highest in the soil of the Mediterranean zone (Fig. 1). In the soil of the arid zone, N_2 fixation did not occur (Fig. 1a). The N_2 fixation rate showed a similar sensitivity to changes in SWC at all sites (see normalized data in Fig. 2a and S1). N_2 fixation tended to be highest at 20% WHC at a given soil and did not increase much further with increasing SWC, except for the non-pre-incubated samples of the Mediterranean soil (Figs. 1 and 2a).

In the soil of the semiarid zone, no difference was found in the N_2 fixation rate between the samples that were pre-incubated for five days and the samples that were not pre-incubated (Fig. 1b). In contrast, for the soil of the humid climate zone, the pre-incubated samples had significantly higher N_2 fixation rates than the non-pre-incubated samples at 40, 60, and 80% WHC (Fig. 1d), indicating that the N_2 fixation rate in this soil increased only slowly after rewetting. In the soil of the Mediterranean zone, there was also a significant difference between the pre-incubated and non-pre-incubated samples at two SWCs (Fig. 1c).

3.3. C mineralization

The cumulated amount of CO_2-C increased strongly during the first two days of incubations and more slowly thereafter in all soils (Fig. S2). The initial increase in CO_2-C to rewetting ("Birch effect") was highest in the soil of the semiarid and Mediterranean site. Considering the first five days of the incubation, the C mineralization rate increased in the order arid $\ll$ humid $<$ semiarid $<$ Mediterranean (Fig. 3). The C mineralization rate showed the same sensitivity to changes in SWC at all sites (see normalized data in Fig. 2b and S3). The C mineralization rate was lowest at field-moisture content in all soils and increased with SWC. The C mineralization rate was highest at 80% WHC in the soil of the humid zone and at both 60% and 80% WHC in the other three soils (Fig. 3).

3.4. Net N mineralization

Net N mineralization did not occur in the soil of the humid climate zone (Fig. 4) which had the largest TOC:TN ratio (Table 3). In the other three soils, the cumulated amounts of inorganic N increased strongly during the first two days of incubation and more slowly thereafter (Fig. S4). The initial increase in the inorganic N content in response to rewetting ("Birch effect") was highest in the soil of the semiarid and Mediterranean site. Considering the first five days of the incubation, the net N mineralization rates increased in the order humid $<$ arid $<$ semiarid $<$ Mediterranean (Fig. 4). The net N mineralization rate showed a similar sensitivity to changes in SWC at all sites (see normalized data in Fig. 2c and S5). Net N mineralization was lowest at field-moisture content in all soils and increased with SWC, except for the soil of the arid zone in which the rate did not change significantly with SWC (Fig. 4). The net N mineralization rate was highest at 40% and 60% WHC. The rate was significantly decreased at 80% WHC compared to 60% WHC in the soils of the semiarid and Mediterranean zone.

3.5. Microbial biomass C and N

The soil MBC content increased in the order arid $<$ semiarid $<$ humid $<$ Mediterranean (in the field-moist samples; Table 3 and Fig. S6). The MBN content increased in the order arid $<$ semiarid $<$ Mediterranean in the field-moist samples, while MBN was below the detection limit at the humid site (Fig. 5). Rewetting, i.e., the increase in the SWC from field moisture to a SWC $\geq$ 20% WHC, led to a significant increase in the MBN content in the soils of the semiarid and Mediterranean zone (Fig. 5). This increase in MBN caused a small, statistically significant reduction in the molar microbial biomass C:N ratio from 8.5 to 4.0 in the semiarid soil and from 13.5 to 5.0 in the Mediterranean soil (Fig. S7).

4. Discussion

Here we found that the N_2 fixation rate tended to be highest (in a

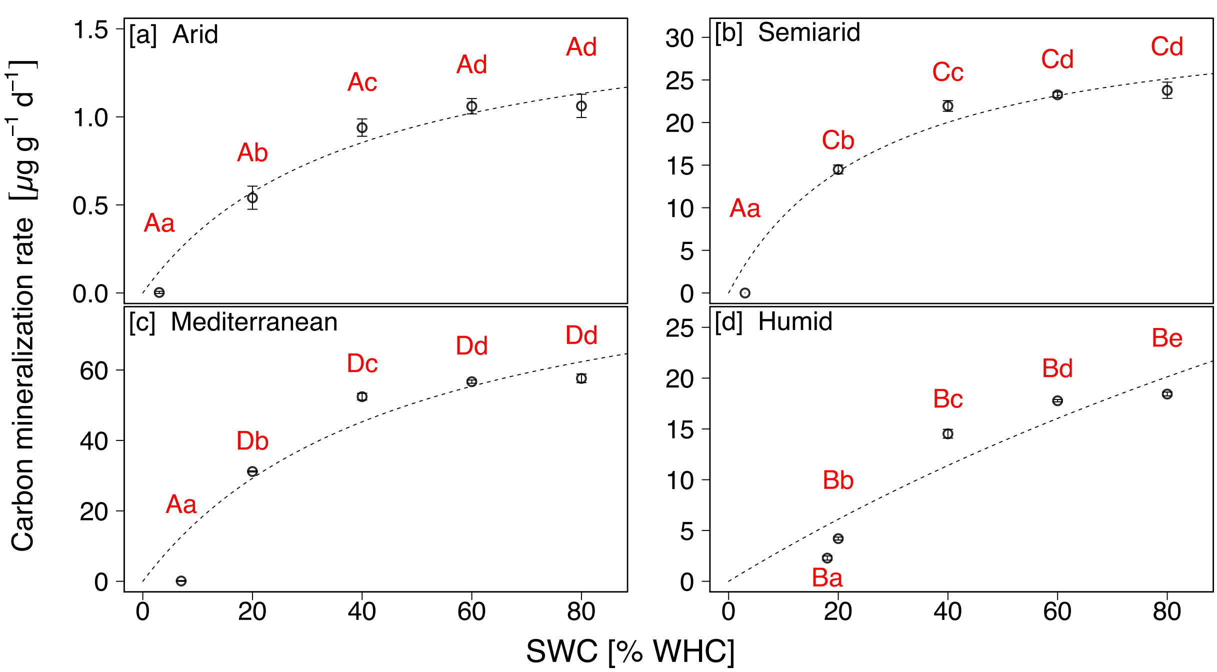

Fig. 3. Carbon mineralization rate as a function of the soil water content (SWC) given as % water holding capacity (WHC) for soils of the [a] arid, [b] semiarid, [c] Mediterranean, and [d] humid climate zone. The mineralization rates were calculated across 5 days of incubation. Error bars depict standard deviations (n = 4). Different capital letters indicate significant differences ($p < 0.05$) between the climate zones at a given SWC, and different small letters indicate significant differences ($p < 0.05$) between the different SWC in one climate zone. An asymptotic concave function was fitted to the C mineralization rates (indicated by the dashed line).

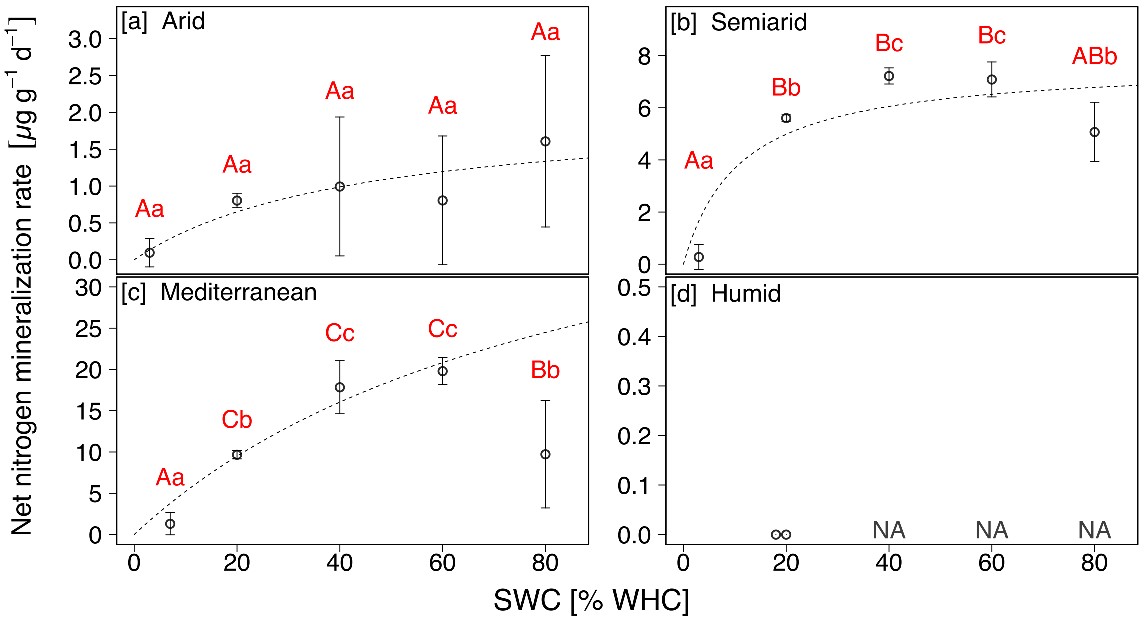

Fig. 4. Net nitrogen mineralization rate as a function of the soil water content (SWC) given as % water holding capacity (WHC) for soils of the [a] arid, [b] semiarid, [c] Mediterranean, and [d] humid climate zone. The mineralization rates were calculated across 5 days of incubation. Error bars depict standard deviations (n = 4). Different capital letters indicate significant differences ($p < 0.05$) between the climate zones at a given SWC, and different small letters indicate significant differences ($p < 0.05$) between the different SWC in one climate zone. An asymptotic concave function was fitted to the net N mineralization rates (dashed line).

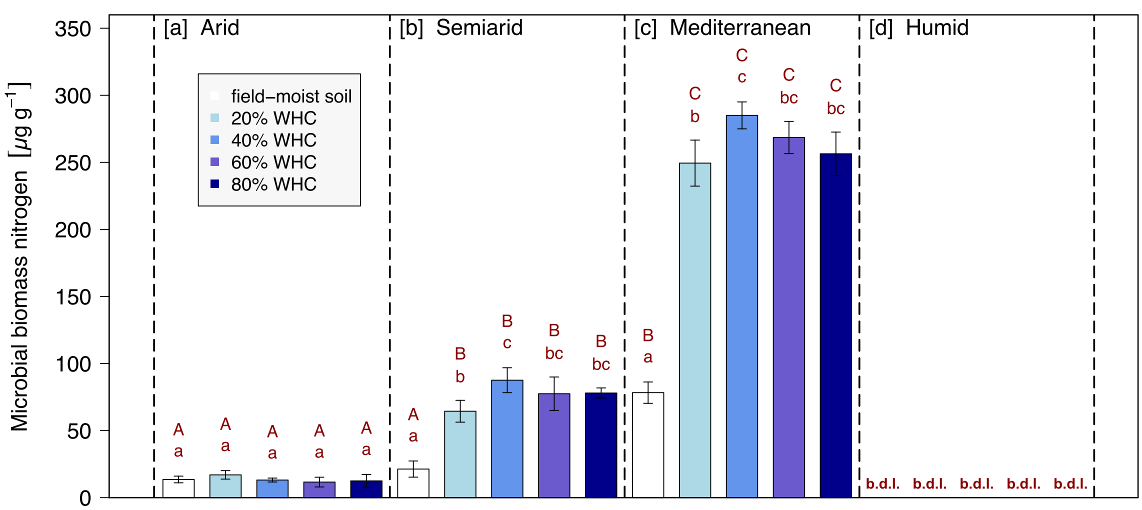

Fig. 5. Microbial biomass nitrogen in the four soils from four climate zones ([a] arid, [b] semiarid, [c] Mediterranean, and [d] humid climate zone) at five soil water contents (field-moist soil (fm), 20, 40, 60, or 80% WHC). Error bars depict standard deviations (n = 4). Different capital letters indicate significant differences ($p < 0.05$) between the climate zones and different small letters indicate significant differences ($p < 0.05$) between the different SWC in one climate zone. b.d.l.: below detection limit.

given soil) at only 20% WHC and did not significantly increase with increasing SWC. In contrast, the C mineralization rate was significantly highest at 60–80% WHC, while the net N mineralization rate was highest at 40–60% WHC, and did not increase further with increasing SWC.

4.1. N_2 Fixation less sensitive to changes in SWC than C and N mineralization

The N_2 fixation rate was less dependent on the SWC than the C and net N mineralization rates likely because N_2 fixation does not only depend on the diffusion of solutes (which increases with increasing SWC) but also on the diffusion of N_2 (which decreases with increasing SWC; Cook and Knight, 2003; Moyano et al., 2013). Thus, N_2 fixation is likely less sensitive to changes in the SWC than mineralization processes because the diffusion of N_2 in soil does not increase with the SWC, in contrast to the diffusion of organic solutes. In general terms, our results agree with the finding of previous studies that N_2 fixation increases with increasing SWC (Gundale et al., 2009; Whiteley and Gonzalez, 2016; Rousk et al., 2017; Li et al., 2018). Yet, our study is the first to show that the N_2 fixation rate is less sensitive to changes in SWC than C and net N mineralization rates, and that the N_2 fixation rate tends to be already at its maximum (in a given soil) at relatively low SWC, i.e., 20% WHC (Fig. 1), which corresponds to 14% WFPS (Fig. 2a). Thus, our study supports the conclusion of a recent meta-analysis that increases in drying and rewetting might lead to a divergence in the rate of different element cycling processes (Gao et al., 2020).

The sensitivity of the C mineralization rate to changes in the SWC has been very intensively investigated (Borken and Matzner, 2009; Manzoni et al., 2012; Moyano et al., 2013; Sierra et al., 2015), and previous studies also found a maximum C mineralization rate at 60% and 80% WHC (Moyano et al., 2013), as we did here. However, this is the first study that compared the sensitivity of C mineralization to changes in the SWC with the sensitivity of N_2 fixation and net N mineralization to changes in the SWC. The rate of net N mineralization was significantly lower at 80% WHC than at 60% WHC in some soils (Fig. 4). It can be speculated that the reason for this might be related to a higher sensitivity of microfauna than of bacteria and fungi to a high SWC. Microfauna, such as protozoa and nematodes, are important for net N mineralization because they have a relatively high biomass C:N ratio compared to bacteria and release inorganic N when grazing on bacteria (Clarholm, 1985).

4.2. Activation of soil microorganisms due to water addition and the Birch effect

The activity of all three processes in the field-moist soils was lower than predicted by the model based on the empirical data (Figs. 1–4). The reason for this is most likely that the field-moist soils, collected at the end of summer, were all relatively dry (Table 2), and addition of water activated dormant bacteria in all soils, even when the amount of added water was small. Thus, the higher rates in the rewetted soils are likely not only the result of increased diffusion of solutes, but also activation of dormant bacteria (Schimel et al., 2007; Schimel, 2018). The increase in C and net N mineralization during the first two days after water addition ("Birch effect") was largest in the semiarid and Mediterranean soil and lower in the soil of the humid climate zone (Figs. S2 and S4). This might be related to the fact that the humid soil experiences fewer and less severe drought events than the soils in the more arid climate zones (Brangarí et al., 2021).

4.3. The response of N_2 fixation to water addition

For the soil of the semiarid zone, the N_2 fixation rate in the pre-incubated samples did not differ significantly from the rate determined in the non-pre-incubated samples, in contrast to the soil of the humid zone for which significant differences between the pre-incubated samples and the non-pre-incubated samples were observed (Fig. 1). Thus, diazotroph microorganisms in the semiarid soils upregulate the rate of N_2 fixation faster after rewetting than diazotrophs in the soil of the humid zone. This might indicate a microbial adaptation to the climatic conditions at the semiarid study site which allows microorganisms to use moist periods in the otherwise dry soils very effectively. In more general terms, our findings are in accordance with previous studies demonstrating that frequent drying-rewetting may select for microbial taxa that are tolerant to desiccation stress (Fierer et al. 2003; Waldrop and Firestone, 2006; Evans and Wallenstein, 2012) and that soil microorganisms in relatively dry ecosystems respond more quickly to soil rewetting (in terms of respiration) than in ecosystems with more frequent precipitation events (de Nijs et al., 2019). Furthermore, the finding is in accordance with the observation that the Birch effect was smaller in the soil of the humid zone than in the soils of the semiarid and Mediterranean zone (see 4.2).

4.4. The effect of soil properties on process rates

As in any climate sequence, the properties of the soils studied here co-vary with the climate, which has to be taken into account when discussing the effect of SWC on rates of element cycling. The differences in soil properties likely explain differences in the process rates observed between the soils at a given SWC. In the soil from the arid zone, rates of C and net N mineralization were low due to the low contents of TOC, TN, and microbial biomass C (Table 3), which is typical for desert soils (Noy-Meir, 1973; Steven, 2017). In the soil from the humid zone, no net N mineralization was detectable (Fig. 4), and also microbial biomass N and soil DN were below the detection limit (Fig. 5, Table 3), indicating a strong N limitation in this soil. These findings can be mainly attributed to the high TOC:TN ratio of the soil (the molar TOC:TN ratio was 34.47, see Table 3), which is likely beyond the critical element threshold ratio. It has been shown based on contrasting soils of temperate forests that the critical element threshold TOC:TN ratio for N mineralization is 28 (Heuck and Spohn, 2016). Above this critical element threshold ratio, microorganisms in temperate forests are N limited and N immobilization but not net N mineralization occurs (Heuck and Spohn, 2016; Spohn, 2016).

4.5. Conclusion

We found support for our hypothesis since the N_2 fixation rate was less sensitive to changes in SWC than the C and net N mineralization rates, which is likely because the diffusion of N_2 in soil does not increase with the SWC, in contrast to the diffusion of organic solutes. Furthermore, we showed that diazotroph microorganisms in semiarid and Mediterranean soils upregulate the rate of N_2 fixation faster after rewetting than diazotrophs in the humid zone, which likely allows them to use moist periods in the otherwise dry soils very effectively. Our study has important implications since it suggests that N_2 fixation is likely less affected than C and net N mineralization if soil moisture content decreases, for instance due to climate change.

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.geoderma.2022.115973.

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