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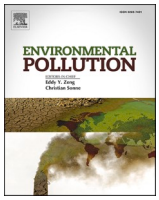

Rising temperature and atmospheric CO_2 combine to antagonistically alter Cd mobility and biogeochemistry in an agricultural soil☆

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ABSTRACT

Soil cadmium (Cd) contamination threatens ecosystems and crop safety. Understanding how individual climate change factors influence soil Cd bioavailability is essential for mechanistic understanding and future risk assessments. This study examined individual and combined effects of elevated temperature (+4 °C) and doubled atmospheric CO_2 (800 ppmv) on soil Cd bioavailability, biogeochemistry, and greenhouse gas emissions in agricultural soils with native (0.13 mg Cd kg^{-1}) and high Cd (1.5 mg Cd kg^{-1}). Elevated temperature increased porewater Cd up to 50 % relative to ambient, while doubled atmospheric CO_2 did not alter porewater Cd. Combined future conditions increased porewater Cd by 30 % relative to ambient indicating an antagonistic interaction. Doubled atmospheric CO_2 enhanced microbial nitrogen fixation and reduced ammonium oxidation, increasing ammonium concentrations up to 10-fold relative to ambient. Elevated temperature stimulated microbiome activity and ammonium oxidation, leading to 1.7-fold more CO_2 and 5.5-fold more N_2O compared to ambient, both exceeding levels observed under combined future climate. These contrasting single-factor responses highlight the non-additive nature of combined climate factor effects. Warming alone overestimated and CO_2 alone underestimated the combined impact on Cd mobility and soil biogeochemistry. Simulating multiple climate drivers is therefore essential for accurate environmental prediction and sustainable Cd management under climate change.

1. Introduction

Anthropogenic climate change, driven by rising atmospheric CO_2, is altering soils' physico-biogeochemical properties, impacting soil health (Rustad et al., 2001; Oliverio et al., 2017; Jansson and Hofmockel, 2020; Lehmann et al., 2020). The worst-case climate scenario (RCP 8.5 (IPCC, 2013),) is now considering a plausible trajectory, with temperatures projected to rise 2.1–3.9 °C by 2100 (Le Quéré et al., 2018; Schwalm et al., 2020; Liu and Raftery, 2021). Studies on soil responses often assess single climate factors in isolation, such as temperature (Rustad et al., 2001; Price and Sowers, 2004; Oliverio et al., 2017; Dai et al., 2020), moisture (Orchard and Cook, 1983; Wang et al., 2023a), or CO_2 (van Groenigen et al., 2011; Kelly et al., 2013; Qiu et al., 2019). However, single-factor approaches can misrepresent real-world soil system responses (Rillig et al., 2019). This highlights the need to explicitly investigate combined climatic factors to understand if they amplify, mitigate, or modify individual effects.

Elevated atmospheric CO_2 has minor effects on soil respiration (van Groenigen et al., 2011; Du et al., 2022), but alters the expression of microbial genes involved in nitrogen-cycling, thereby affecting the diversity and activity of microorganisms responsible for nitrogen transformations. For example, higher abundance of denitrifying genes under elevated CO_2 (Qiu et al., 2019) may increase N_2O emissions (Kammann et al., 2008). Conversely, the abundance of the bacterial *ammonia monooxygenase* gene decreased under elevated CO_2, resulting in decreased N_2O emissions (Kelly et al., 2013).

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Temperature is a key determinant for microbial activity and respirational output in soils (Rustad et al., 2001; Jansson and Hofmockel, 2020) due to its influence on enzyme activities determining rates for nitrogen cycling, metal im- and exporting, or essential cell-maintenance (Gadd, 1990; Price and Sowers, 2004; Steinweg et al., 2013; Dai et al., 2020). Therefore, an increase in atmospheric temperature within the range projected by the year 2100 (approximately +2 to +4 °C) can enhance microbial respiration in the future (Rustad et al., 2001; Jansson and Hofmockel, 2020). However, excessive temperature increases can surpass the activity optima, leading to cell inactivation or even cell death (Lloyd and Taylor, 1994; Pietikäinen et al., 2005). Substantial cell death reduces soil respiration, whereas organic matter released by moderate cell death may stimulate respiration of surviving microorganisms (Zhang et al., 2023b). Higher temperatures also affect soil moisture and salinity, which causes osmotic stress to soil life and enhances nutrient adsorption to soil minerals (Butcher et al., 2016). These changes can limit nutrient diffusion in the pore space, diminishing microbial activity and respiration (Schindlbacher et al., 2012; Sun et al., 2018; Jansson and Hofmockel, 2020). Additionally, reduced soil moisture can increase porewater metal concentrations, potentially amplifying their toxic effects on microbial processes (Acosta et al., 2011).

Cadmium, ranked among the most hazardous soil contaminants, is ubiquitous and highly mobile in soils (Kubier et al., 2019; Sauvé et al., 2000) and holds no known metabolic function. Still, Cd toxicity to soil life depends on its concentration and, more crucially, the fraction of Cd found in porewater or loosely associated with mineral surfaces and available for biota uptake, i.e. here termed bioavailable (Vig et al., 2003; Shahid et al., 2017). Recent global assessments emphasize that bioavailable fractions of toxic metals, including Cd, better reflect actual ecological and health risks compared to total concentrations alone (Hou et al., 2025; Sharma and Muehe, 2025). In fact, bioavailable Cd directly determines its environmental mobility, crop uptake potential, and overall ecosystem toxicity (Zhang et al., 2025). At high concentrations, cadmium decreases microbial activity, disrupts nutrient uptake, and compromises cell membrane integrity, leading to leakage of cellular contents and loss of essential ions (Wyszkowska and Wyszkowski, 2002; Shentu et al., 2008; Lu et al., 2013a). Cadmium can also damage DNA, affecting replication or transcription, potentially causing cell death in microbes without metal resistance genes (Ron et al., 1993; Abbas et al., 2017). Some microorganisms adapt to Cd stress by producing metal-chelating compounds, enzyme adaptation, and activating efflux pumps to remove Cd (Bravo and Braissant, 2022). In contrast, and despite its inhibitory effects, low bioavailable concentrations of Cd can stimulate microbial activity through nutrient mobilization or the activation of stress response systems, enhancing microbial growth, N-utilization, and basal respiration (Lu et al., 2013a; Shi and Ma, 2017; Xu et al., 2019). However, climate-induced increases in Cd bioavailability may shift stimulating microbiome effects to adverse ones.

In mechanistic soil adsorption experiments, elevated temperature decreased Cd bioavailability in soils by enhancing endothermic adsorption of Cd to mineral surfaces (Adhikari and Singh, 2003; Li et al., 2011), adsorption capacity of soil minerals, and complexation capacity of organic matter to Cd (Cornu et al., 2016; Hu et al., 2022). Simultaneously, higher temperatures may increase Cd bioavailability through enhanced dissolution of Cd-bearing minerals (Morse and Arvidson, 2002). Our previous work demonstrated that future climatic conditions using the combination of elevated temperature, increased atmospheric CO_2, and passively reduced soil moisture increased porewater Cd in agricultural soils by decreasing porewater pH through enhanced microbial ammonium oxidation and organic acid production (Drabesch et al., 2024). Similarly, Wang et al. (2020a) showed that concurrent elevated temperature and atmospheric CO_2 conditions can increase Cd bioavailability and its uptake by crops, posing additional risks to food safety under climate change (Wang et al., 2020b). This enhanced mobilization of Cd holds especially true for soils with a pH below 7 (Drabesch et al., 2024). However, it remains unclear which specific climatic parameters (temperature or atmospheric CO_2) affect the interaction between microorganisms, nutrient dynamics, and Cd bioavailability in the soil and to which degree. We hypothesize that combined climatic parameters interact in a non-additive manner to influence microbial nutrient cycling in soils and potentially resulting in altered Cd bioavailability. These interactions of individual climatic parameters may amplify, diminish or modify the individual effects of each factor, leading to outcomes that cannot be predicted by their individual contribution.

The objective of this study, therefore, was to link the impacts of the individual climate parameters elevated temperature and doubled atmospheric CO_2 to changes in the bioavailability of soil Cd by understanding changes in geochemistry (pH, dissolved organic carbon (DOC), nutrient-specifically nitrogen-cycling) and microbiome responses. To do so, we exposed an agricultural soil to either an ambient climate, a 4 °C increased atmospheric temperature, doubled atmospheric CO_2, or the combination of these two climatic parameters to represent future climatic conditions based on the Representative Concentration Path 8.5 (RCP 8.5) of the 5th Assessment Report (IPCC, 2013). The used soil with a native Cd content of 0.1 mg Cd kg^{-1} represents uncontaminated agricultural soils. A second Cd treatment involved the amendment with 1.4 mg Cd kg^{-1}, a concentration similar to that found in 20 % of contaminated topsoils in the US and Europe. According to local regulations, soils with such Cd content can still be used for biomass energy production and are found in Europe, China, and North America (Birke et al., 2017; Shi et al., 2019; Smith et al., 2019). This study may serve as the foundation to better understand to which degree and in which direction elevated temperature and doubled atmospheric CO_2 may affect Cd mobility and soil biogeochemical cycles individually in contrast to their combined effects.

2. Material & methods

2.1. Soil sampling, characterization, and preparation for incubation experiments

Soil for incubation experiments was obtained from the topsoil (0–20 cm depth), of agricultural farmland near Tuebingen, Germany (48°26′39.6 "N 9°02′43.3"E) in December 2019. Classified as a cambisol, it had a circumneutral pH of 6.7 ± 0.1, a native Cd concentration of 125 ± 6 µg $kg^{-1}_{dry\ soil}$ and a total organic carbon content of 3.95 ± 0.15 % (Table S1).

Prior to experiments, soil was air-dried at 20 °C in the dark, sieved through a 2 mm mesh, and visually inspected to manually remove plant residues and rock fragments. The homogenized soil was evenly split into subsamples using a sample splitter (RT 50, Retsch, Germany) to ensure uniform physicochemical properties. Before incubation, sieved soil was spiked with either $CdCl_2$ for Cd-bearing setups ("High Cd") or with $CaCl_2$ for native soils ("Native Cd") to account for chlorine toxicity effects (Darrah et al., 1987; Rath et al., 2016) and maintain ionic strengths (Cavallaro and McBride, 1978). The following amendments were applied: native Cd with 0.4 mg Ca $kg^{-1}_{dry\ soil}$ ($CaCl_2$, trace metal grade, Sigma Aldrich), and high Cd with 1.4 mg $kg^{-1}_{dry\ soil}$ ($CdCl_2$, trace metal grade, Sigma Aldrich). Following amendment, soils underwent three dry–wet cycles (5 %–20 % gravimetric water content) over 14 weeks for metal acclimatization, allowing Cd equilibration within the soil matrix and microbial adaptation to Cd exposure (Simpson et al., 2004; Khan et al., 2010)(see *Supplementary Information*).

2.2. Experimental setup

Black PVC-U tubes (inner diameter: 7.3 cm; height: 33 cm), washed with 1 M HCl, MilliQ water, and 80 % ethanol, were filled with 800 g of air-dried soil. Tubes were randomly distributed in climate-controlled chambers (110 × 50 × 50 cm) made of poly(methyl methacrylate) (front/top) and polyvinylchloride (ground/back/sides) (Fig. S1A). Each

tube had a butyl stopper with a gas sampling port for headspace gas sampling without disturbing the incubation atmosphere (Fig. S1B and C). Rhizonsampler (RhizonFlex, 5 mm, 0.22 μm, Rhizosphere Research Products, Netherlands) were installed horizontally, 8 cm below the soil surface. Four climatic conditions were established based on the IPCC RCP 8.5 climate scenario (IPCC, 2013), using climatic-controlled chambers. Chambers were placed outside to maintain natural day/-night cycles: (1) ambient (ambient temperature and CO_2 ~430 ppmv), (2) elevated temperature (+4 °C above ambient, ambient CO_2), (3) doubled CO_2 (800 ppmv CO_2, ambient temperature), and (4) combined future conditions (+4 °C and 800 ppmv CO_2). Temperature in each chamber was monitored at 1-min intervals using DS18B20 sensors (Analog Devices, USA) connected to Raspberry Pi 3b + units (Raspberry Trading, United Kingdom). Elevated temperature conditions (+4 °C above ambient) were maintained by heater fans (HP8232, Phillips, Germany). Elevated temperature (+4 °C) conditions refer explicitly to increased air temperature within the chambers; soils were indirectly warmed from the atmosphere, without any direct soil heating applied. Elevated CO_2 was achieved by mixing ambient air with pure CO_2 (industrial grade 99.5 %, Westfalen Gas, Germany) using a two-tube gas proportioner (Cole-Parmer, USA), resulting in air exchange approximately eight times per hour (flow rate: 36.6 L min^{-1} ambient air, 14.66 mL min^{-1} CO_2). For detailed setup description, see (Drabesch et al., 2024). Replicate numbers were four for ambient and future conditions in both native and high Cd. Due to logistical constraints, replicates were reduced to three for individual elevated T and CO_2 treatments. Air temperature and CO_2 levels were monitored continuously with three DSB18B20 temperature sensors (Maxim integrated, USA) and two AirCO2ntrol logger (TFA Dostmann, Germany) (Fig. S1D). Soil gravimetric water content (Fig. S1E) was checked every five days and weight-adjusted to 25 % with autoclaved artificial rainwater. Chambers were placed outside to maintain natural day/night cycles (detailed conditions in Fig. S1).

2.3. Porewater analysis

Porewater was extracted from each column through rhizons using 20 mL syringes (Braun, Germany). Porewater pH was measured in triplicate with an InLab Easy pH probe (Mettler Toledo, USA). The remaining porewater was filtered (0.22 μm PES, pre-washed with 10 mL ultra-pure water to remove organic impurities), before Cd quantification by ICP-MS (see *Supplementary Information)*. Porewater organic carbon (DOC) and nitrogen species (nitrate, ammonium) were quantified on a multi N/C 2100 (Analytik Jena, Germany) and a continuous flow analyzer (AA3, Seal analytical, USA), respectively.

2.4. Gas analysis

For determining GHG emissions, columns were sealed with butyl stoppers (Deutsch Neumann, Germany) from outside the chambers by leveling down screw rods. A cannula connected with Tygon tubing (ID 1 mm, Ismatec) and a 3-way valve (Braun, Germany) allowed sampling. A 3 mL syringe flushed the tubing, followed by collection of 10 mL headspace gas at 0, 15, and 30 min. Samples were injected into helium-flushed 20-mL crimped headspace vials, and analyzed via gas chromatography (see *Supplementary Information)*.

2.5. Soil sampling during incubation

Soil was sampled from the top 5 cm in each column, homogenized with a sterile spatula (baked at 300 °C for 3 h), and allocated as follows: sterile 2-mL microcentrifuge tube for molecular ecology analysis, 15-mL centrifuge tube for geochemical analysis, and aluminum cups for water content determination. Geochemical samples were stored at −20 °C if not processed immediately, while molecular ecology samples were stored at −80 °C.

2.6. Microbial community composition and activity

DNA/RNA were co-extracted from 0.6 g of frozen soil with a phenol/chloroform/isoamyl alcohol phase separation (Lueders et al., 2004), and dissolved in a 100 μL TE-buffer. DNA/RNA contents were quantified with a Qubit 2.0 fluorometer (Thermo Fischer Scientific), and quality checked for 260/280 ratios on NanoDrop (Thermo Fisher Scientific). Aliquots were stored at −80 °C. RNA samples were purified from DNA with the Invitrogen™ TURBO DNA-free™ Kit (Thermo Fisher Scientific) and transcribed into complementary DNA (cDNA) with Invitrogen™ SuperScript™ III Reverse Transcriptase (Thermo Fischer Scientific). DNA removal and cDNA synthesis were verified on 1 % (w/v) ethidium-bromide-stained agarose gel.

Microbial 16S rRNA transcripts were sequenced (see *Supplementary Information)*. Nitrogen-cycle-related genes and transcripts (ammonium oxidizing archea (AOA) *amoA*, ammonium oxidizing bacteria (AOB) *amoA*, *nirK*, *nirS*, *nifH*, *nosZ*, and 16S rRNA) were quantified via quantitative PCR (qPCR, 1xSsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, USA). Transcripts were below quantification limits. A 10 μL reaction contained 1 μL of template DNA (~4 ng μL^{-1}) or a tenfold dilution series of standard plasmids, with gene-specific primers *(*Table S2*)*. qPCR was run on a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories, USA), and data analyzed with the CFX Maestro software (Bio-Rad Laboratories, USA).

2.7. Data analysis

Mean and standard errors were calculated for all datasets. Effects of climate, Cd content, and their interaction were evaluated with two-factorial ANOVA for end-point data, and Linear Mixed Models (LMMs) for time-resolved data, considering hierarchical data structure and replicate variability. Estimated marginal means were compared to evaluate treatment differences. Model fit was assessed using Akaike's Information Criterion. Repeated Measures ANOVA tested the effects of time, treatment, and their interaction, with Mauchly's Test checking sphericity assumptions. Students t-tests assessed differences between treatments at specific time points. Linear Regressions analyzed trends over time, with relationships evaluated using R^2 and p-values. Significance was set at $p < 0.05$.

3. Results

3.1. Temperature and atmospheric CO_2 induced alterations of porewater cadmium

One of the determinants for mobile Cd is pH, ranging between 7.1 and 7.5 (Table S3). Temperature-based climates (elevated temperature alone and future climate) significantly decreased porewater pH by at least 0.1 pH units from day 12 onward relative to ambient climate.

Initial porewater Cd in the native Cd soil under ambient conditions was stable at around 0.11 ± 0.03 μg L^{-1} (Fig. S2A). Doubled atmospheric CO_2 almost significantly affected porewater Cd concentration, verified by a pairwise comparison to ambient ($p = 0.06$, Table S4a, Fig. 1A). Elevated temperature increased porewater Cd up to 50 % after 12 days compared to ambient conditions ($p = 0.05$, Table S4a). The results of the LMM further emphasize the strong effect of elevated temperature on Cd concentrations, as shown by the significant difference between ambient and elevated temperature (Table S5). The combination of elevated temperature and doubled CO_2 of the future scenario increased porewater Cd by 30 % in the native Cd soil compared to ambient from day 12 onward ($p = 0.08$, Table S4a). LMM indicates a significant interaction between native and elevated Cd treatments responding differently to climate conditions.

In the high Cd treatment under ambient conditions, initial porewater Cd was 3.6 ± 0.2 μg $L^{-1,}$ which decreased to 1.7 ± 0.3 μg L^{-1} towards the end (Fig. S2B). Similar to the native Cd soil, a doubling of

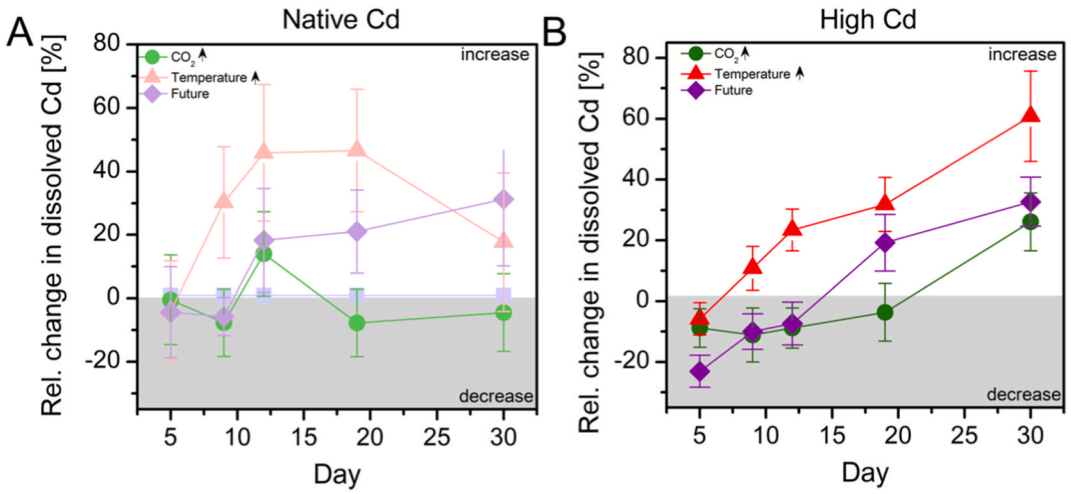
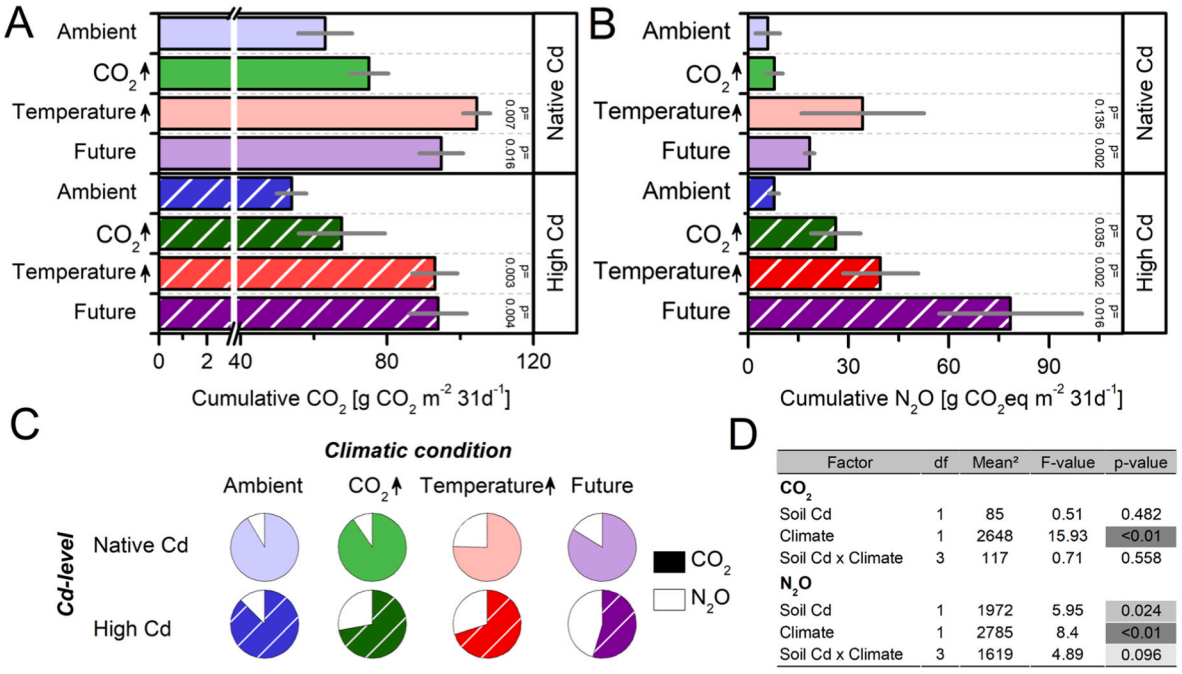


Fig. 1. Temporally resolved changes in porewater cadmium in an agricultural soil exposed to different climate variables: Change in porewater Cd concentrations relative to ambient climatic conditions during incubation of an agricultural soil with native (0.13 mg Cd kg^{-1} dry soil; light colors) (A) and high Cd contents (1.5 mg Cd kg^{-1} dry soil; dark colors) (B). During incubation, soils were exposed to either doubled atmospheric CO_2 concentrations (800 ppm_v, green), elevated temperature (+4 °C relative to ambient temperature, red), or future climatic conditions (the combination of doubled atmospheric CO_2 and elevated temperature, purple). Grey-shaded areas of each graph depict a decrease in porewater Cd relative to the ambient climate at each time point, while the white upper areas of each graph feature an increase in porewater Cd relative to the ambient climate. Three or four biological replicates are presented as mean values ± standard errors. Actual Cd concentrations in the porewater under each of the four climatic incubations are presented in Fig. S2. Specific *t*-test results are presented in Table S4 and associated linear mixed model in Table S5. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

atmospheric CO_2 did not affect porewater Cd (Fig. 1B), which aligns with the non-significant pairwise comparison between ambient and doubled CO_2 conditions (p = 0.06, Table S4a). At day 30, an increase may be observable. Elevated temperatures increased porewater Cd from day 9 onwards ending with a 60 % increase relative to ambient. LMM highlights a significant influence of elevated temperature on porewater



Factor	df	Mean²	F-value	p-value
CO_2				
Soil Cd	1	85	0.51	0.482
Climate	1	2648	15.93	<0.01
Soil Cd x Climate	3	117	0.71	0.558
N_2O				
Soil Cd	1	1972	5.95	0.024
Climate	1	2785	8.4	<0.01
Soil Cd x Climate	3	1619	4.89	0.096

Fig. 2. Cumulative greenhouse gas emissions from agricultural soil exposed to different climate variables. Emissions of CO_2 (A) and N_2O (B) were cumulated over 31 days of incubation of agricultural soil with a native (0.13 mg Cd kg^{-1} dry soil, light colors) and high Cd content (1.5 mg kg^{-1} dry soil, striped symbols). The relative contribution of CO_2 and N_2O to the warming potential of this soil during incubation is given for each climate variable. (C). During incubation, soils were exposed to either ambient climatic conditions (blue), doubled atmospheric CO_2 concentrations (800 ppm_v, green), elevated temperature (+4 °C relative to ambient temperature, red), or future climatic conditions (the combination of doubled CO_2 and elevated temperature, purple). A two-factorial ANOVA analysis for the interaction of soil Cd (native or elevated) and climatic conditions (ambient, doubled atmospheric CO_2, elevated temperature, and future climate) of cumulative emission data is presented (D), statistical significance is highlighted with grey-shaded p-values. Supportive t-tests assessing direct comparisons of treatments are presented in Table S4b and c, and linear mixed model analyses for time-series data in Tables S6 and S7 respectively for CO_2 and N_2O. Three or four biological replicates are presented as mean values ± standard errors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Cd compared to ambient conditions (Table S5). Combined doubled atmospheric CO_2 and elevated temperature for the future treatment, increased porewater Cd compared to the ambient climate with a lag time of 12 days ending up with significantly 30 % more porewater Cd.

3.2. Temperature, atmospheric CO_2 and Cd-induced alterations in greenhouse gas emissions

Emitted CO_2 was consistent over time, thus cumulative data are presented (Fig. 2). Native Cd incubations emitted 63 ± 7 g CO_2 m^{-2} under ambient conditions. While doubling atmospheric CO_2 did not increase cumulative CO_2, elevated temperature significantly increased CO_2 by 70 % relative to ambient (Table S4b). Combining both climatic parameters in the future treatment also significantly increased cumulative CO_2 to 96 ± 6 g CO_2 m^{-2} 31 d^{-1}, representing a 50 % increase compared to ambient. Therefore, the cumulative CO_2 emissions under future climatic conditions were found to be between those observed for scenarios with either doubled atmospheric CO_2 levels and elevated temperatures.

From the soil with a high Cd content, 54 ± 4 g CO_2 m^{-2} 31 d^{-1} was emitted within 31 days under ambient conditions (Fig. 2). Doubling atmospheric CO_2 resulted in a non-significant 30 % increase in cumulative CO_2 emissions to 68 ± 12 g CO_2 m^{-2} 31 d^{-1}. Increases in temperature resulted in a significant 70 % increase to 93 ± 6 g CO_2 m^{-2} 31 d^{-1} relative to ambient (Table S4b). Combining doubled CO_2 and elevated temperature for the future climate, the high Cd soil emitted the same amount of CO_2 as under elevated temperature. This finding aligns with the LMM, indicating significant climatic effects on CO_2 emissions though the interaction between Cd and climate conditions was not statistically significant. A significant impact of climatic parameters on final cumulative CO_2 is supported by a two-way ANOVA (Fig. 2D). This aligns with the LMM (Table S6), which accounts for incubation period and confirms no significant effects of soil Cd content or its interaction with climate conditions but a significance of only climatic conditions.

Nitrous oxide emissions from soil with native Cd accumulated to 6 ± 4 g CO_2eq m^{-2} 31 d^{-1} under ambient conditions (Fig. 2B–Table S4c). Doubling atmospheric CO_2 did not affect cumulative N_2O. Elevated temperature alone increased the emissions to 34 ± 18 g CO_2eq m^{-2} 31 d^{-1}, however not significantly, as N_2O emissions varied considerably among biological replicates. Under future conditions with combined doubled CO_2 and elevated temperature, N_2O emission increased significantly to 18 ± 1 g CO_2eq m^{-2} 31 d^{-1} relative to ambient climatic conditions.

Soil with a high Cd content emitted N_2O in a similar range as soils with native Cd under ambient conditions (Fig. 2B). Doubling atmospheric CO_2 or increasing temperature significantly enhanced N_2O emissions to 26 ± 7 g CO_2eq m^{-2} 31 d^{-1} and 39 ± 11 g CO_2eq m^{-2} 31 d^{-1} (Table S4c), respectively, compared to ambient. Under future conditions, significantly more N_2O was emitted from the high Cd soil relative to ambient conditions amounting to 78 ± 21 g CO_2eq m^{-2} 31 d^{-1}. LMM confirmed significant effects of both Cd content and climate on N_2O emissions, as well as a strong interaction between these factors (Table S7). These findings indicate that elevated Cd levels amplify the effect of future conditions on N_2O emissions, particularly under the combined influence of elevated CO_2 and temperature. A two-way ANOVA of the endpoint cumulative N2O emissions confirmed the significant effects of individual climatic parameters and soil Cd content on cumulative N_2O emissions. While the ANOVA suggested a potential interaction between doubled atmospheric CO_2 and elevated temperatures under future conditions (Fig. 2D), the LMM, which accounts for time dependency across incubation period, provided stronger evidence for this interaction.

3.3. Temperature and atmospheric CO_2 impacts on dissolved organic carbon dynamics in soil

Porewater OC in the native Cd soil under ambient conditions was stable over time (linear regression: R^2 = 0.007, Table S8) averaging around 60.2 ± 0.7 mg L^{-1} (Fig. 3A). During incubation, doubling atmospheric CO_2 did not affect DOC, while elevated temperature from day 9 onwards resulted in significantly higher DOC, up to 66.5 ± 1.2 mg L^{-1} (Table S4d). Combining doubled CO_2 and elevated temperature for future climatic conditions resulted in a significant 10 % increase throughout the incubation to 66.1 ± 1.9 mg L^{-1}.

The high Cd soil exhibited higher porewater OC than the native soil Cd (Fig. 3B–Table S4d). For all treatments, DOC concentrations increased in the first 12 days of incubation and decreased thereafter. Under ambient conditions, the initial DOC concentration was 65.4 ± 1.8 mg L^{-1} and increased least compared to other climates before decreasing to 63.9 ± 1.8 mg L^{-1}. Under doubled atmospheric CO_2 the DOC did not differ relative to the ambient condition, shown by a repeated measures ANOVA for the interaction of time and DOC (Table S9). Elevated temperature resulted in a 10 % increase in DOC at day twelve, peaking at 76.6 ± 4.5 mg L^{-1}. The combined climatic conditions resulted in a 10 % increase in DOC to a concentration of up to 78.0 ± 0.6 mg L^{-1} during the first two weeks of incubation and decreased to the same level as under ambient around day 21 of incubation.

3.4. Alterations of soil nitrogen dynamics under future temperature and atmospheric CO_2

Porewater ammonium decreased across all treatments throughout incubation (Fig. 4). This decline was not uniform, and the extent varied across climatic conditions and soil Cd content. For the soil with native Cd levels under ambient climatic conditions, the ammonium concentrations averaged 0.23 ± 0.04 mg L^{-1} and remained stable from day nine (linear regression: R^2 = 0.280, p = 0.094, Table S10) (Fig. 4A). Under doubled atmospheric CO_2, porewater ammonium consistently exceeded 2 mg L^{-1}, which was on average 7.2-fold higher compared to ambient conditions. Under elevated temperature, porewater ammonium remained stable above 1.5 mg L^{-1} until day 12, after which it decreased to 0.40 ± 0.10 mg L^{-1}. Under combined future climatic conditions, porewater ammonium decreased to 0.27 ± 0.02 mg L^{-1} by day 12 and remained stable thereafter.

In the soil with a high Cd content, porewater ammonium remained low at 0.22 ± 0.03 mg L^{-1} under ambient climatic conditions (Fig. 4B). Under doubled atmospheric CO_2, porewater ammonium was stable at 2.43 ± 1.14 mg L^{-1} until day 12 and decreased to 1.01 ± 0.27 mg L^{-1} around day 21. Under elevated temperature, porewater ammonium decreased constantly until day 21, reaching the same concentration as under doubled atmospheric CO_2. When subjected to combined future climatic conditions, porewater ammonium displayed a prominent decline, nearly reaching depletion by day 21.

In contrast to the porewater ammonium, nitrate concentrations did not change considerably over time within all treatments, hence seasonal whisker plots are presented (Fig. 4C and D, temporally resolved data in Fig. S3). In the soil with a native Cd content, porewater nitrate averaged 40.4 ± 9.2 mg L^{-1} across season, not being significantly different to the 40.0 ± 5.7 mg L^{-1} observed under doubled atmospheric CO_2 (Fig. 4C–Table S4e). Notably, in the elevated temperature scenario porewater nitrate was significantly increased with an average around 54.3 ± 8.2 mg L^{-1}. Under the combined future climatic scenario, porewater nitrate was also increased compared to the ambient climate with 46.7 ± 6.0 mg L^{-1}, albeit not as high as under the elevated temperature climate. For the soil with a high Cd content, climate impacts on porewater nitrate were not as pronounced as those with a native Cd content (Fig. 4D). Under the ambient climate, porewater nitrate averaged 41.7 ± 6.2 mg L^{-1}. Under the doubled atmospheric CO_2 climate,

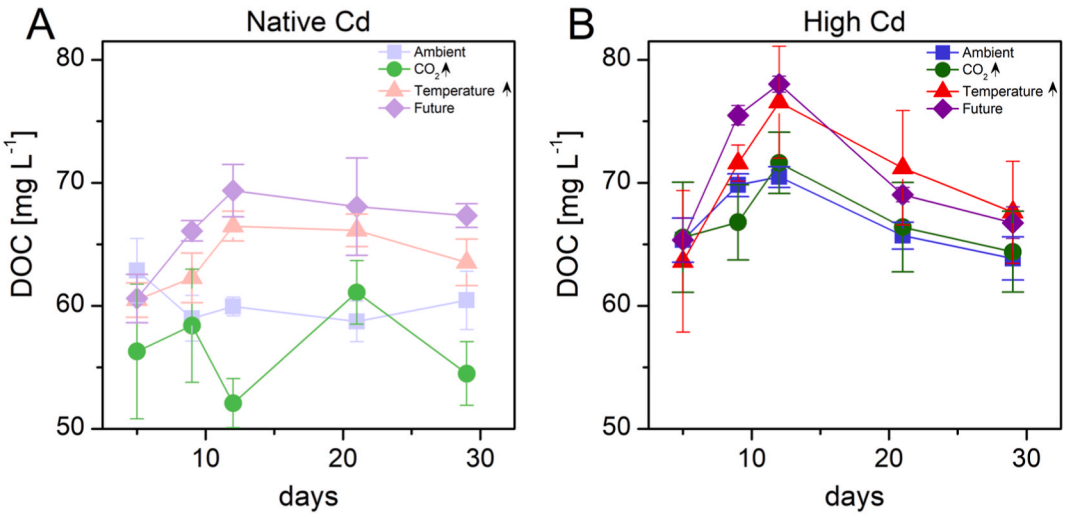


Fig. 3. Temporally resolved dissolved organic carbon in an agricultural soil exposed to different climatic variables: Dissolved organic carbon concentrations in porewater during incubation of an agricultural soil with native (0.2 mg Cd kg^{-1} dry soil, light colors) (A) and high Cd content (1.5 mg Cd kg^{-1} dry soil, dark colors) (B). During incubation, soils were exposed to either an ambient climate (blue), doubled atmospheric CO_2 concentrations (800 ppm_v, green), elevated temperature (+4 °C relative to ambient temperature, red), or future climatic conditions (the combination of doubled atmospheric CO_2 and elevated temperature, purple). Three or four biological replicates are presented as mean values ± standard errors. P-values of t-tests is presented in Table S4 and repeated Measures ANOVA interpretation in Table S9. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

porewater nitrate, with on average 40.7 ± 6.7 mg L^{-1}, did not decrease and a potential increase (p = 0.099, Table S4e) for porewater nitrate under the elevated temperature climate to an average of 48.6 ± 7.4 mg L^{-1}. Under combined future climatic conditions, porewater nitrate varied most, with an average decrease to 35.3 ± 7.8 mg L^{-1}.

3.5. Cadmium and climate impacts on bacteriome/archeome composition

The majority of identified bacterial and archaeal members in the agricultural soil were facultative aerobic microorganisms, based on 16S rRNA gene sequencing using universal primers (Fig. S4). Acclimatization of the soil to spiking with 1.3 mg Cd $kg^{-1}_{dry\ soil}$ for 14 weeks did not considerably alter the composition and diversity of the microbial community on a phylum level (Fig. S4). However, the *16S* rRNA gene copy number increased by 30 % from $1.2 \times 10^{11} \pm 6.4 \times 10^{9}$ to $1.6 \times 10^{11} \pm 1.5 \times 10^{10}$ copies $g^{-1}_{dry\ soil}$ (Fig. S5A). Nonetheless, the AOA *amoA* gene, responsible for anaerobic ammonium oxidation, decreased 70 % from $2.3 \times 10^{7} \pm 1.0 \times 10^{6}$ to $1.6 \times 10^{7} \pm 2.9 \times 10^{6}$ copies $g^{-1}_{dry\ soil}$ (Fig. S5).

During incubation under ambient climate, high Cd in the soil increased the absolute abundances of the 16S rRNA bacterial and archaeal gene and the *nifH* gene compared to the native Cd soil. In contrast, *nosZ* and *nirK* gene abundances were not affected by the presence of higher soil Cd (Fig. 5). In comparison, the absolute abundance of the AOA *amoA* gene decreased from $1.4 \times 10^{7} \pm 1.3 \times 10^{6}$ to $1.0 \times 10^{7} \pm 2.2 \times 10^{6}$ copies $g^{-1}_{dry\ soil}$, which reflects in a significant 30 % decrease relative to the native Cd treatment with a *t*-test p-value of 0.020.

Individual climatic parameters impact the absolute abundance of various nitrogen-cycling bacteria and archaea differently in the soil with a native Cd content (Fig. 5). Relative to the ambient climate, doubled atmospheric CO_2 increased the absolute abundance of the n-cycling genes *nosZ* and *nifH* both by 10 % from $2.0 \times 10^{8} \pm 1.7 \times 10^{7}$ and $2.1 \times 10^{8} \pm 6.2 \times 10^{6}$ to $2.2 \times 10^{8} \pm 5.8 \times 10^{6}$ and $2.4 \times 10^{8} \pm 1.6 \times 10^{7}$ copies $g^{-1}_{dry\ soil}$, respectively, while the other N-related genes and the 16S rRNA gene remained unaltered. Elevated temperature significantly (*t*-test, p = 0.006) increased overall bacterial 16S rRNA gene abundance by 20 % from $1.6 \times 10^{11} \pm 4.8 \times 10^{9}$ to $1.9 \times 10^{11} \pm 8.0 \times 10^{9}$ copies $g^{-1}_{dry\ soil}$ and the abundance of the *AOA amoA* and *nirK* genes by 20 % and 30 %. Under future climatic conditions, i.e., the combination of doubled CO_2 and elevated temperature, the abundances of the *nirK* and AOA *amoA* genes increased by 30 % from $4.1 \times 10^{8} \pm 2.7 \times 10^{7}$ and $1.4 \times 10^{7} \pm 6.3 \times 10^{5}$ to $4.5 \times 10^{8} \pm 1.1 \times 10^{7}$ and $1.7 \times 10^{7} \pm 1.7 \times 10^{6}$ copies $g^{-1}_{dry\ soil}$, respectively.

For the high Cd soil, doubled atmospheric CO_2 concentration resulted in a 10 % decrease in *nirK* and a 10 % increase in the *nosZ* gene copy numbers from $4.1 \times 10^{8} \pm 3.3 \times 10^{7}$ and $2.0 \times 10^{8} \pm 2.0 \times 10^{7}$ to $3.8 \times 10^{8} \pm 1.3 \times 10^{7}$ and $2.2 \times 10^{8} \pm 5.8 \times 10^{6}$ copies $g^{-1}_{dry\ soil}$ relative to the ambient climate, respectively. Elevated temperature significantly (*t*-test, p = 0.024) increased the copy number of the AOA *amoA gene* by 50 % from $1.0 \times 10^{7} \pm 2.2 \times 10^{6}$ to $1.5 \times 10^{7} \pm 2.0 \times 10^{6}$ copies $g^{-1}_{dry\ soil}$, while the *nirK* and *nifH* gene copies increased by 10 % from $4.1 \times 10^{8} \pm 6.5 \times 10^{7}$ and $2.3 \times 10^{8} \pm 4.8 \times 10^{7}$ to $4.6 \times 10^{8} \pm 4.8 \times 10^{7}$ and $2.6 \times 10^{8} \pm 3.7 \times 10^{7}$ copies $g^{-1}_{dry\ soil}$ relative to ambient. The abundance of the *nosZ* gene increased by 10 % from $4.1 \times 10^{8} \pm 3.3 \times 10^{7}$ to $2.1 \times 10^{8} \pm 9.2 \times 10^{6}$ copies $g^{-1}_{dry\ soil}$. Combined future climatic conditions caused similar changes like elevated temperature, having an increase by 50 % and 20 % in the *amoA* and the *nirK* gene copies, from $1.0 \times 10^{7} \pm 2.2 \times 10^{6}$ and $4.1 \times 10^{8} \pm 6.5 \times 10^{7}$ to $1.6 \times 10^{7} \pm 4.0 \times 10^{6}$ and $5.0 \times 10^{8} \pm 9.4 \times 10^{7}$ copies $g^{-1}_{dry\ soil}$, respectively. While *nifH* similarly increased as under elevated temperature, the *nosZ* gene copy number increased by 20 % from $2.0 \times 10^{8} \pm 3.9 \times 10^{7}$ to $2.3 \times 10^{8} \pm 3.7 \times 10^{7}$ copies $g^{-1}_{dry\ soil}$ for the combined climatic effect (Fig. 5).

4. Discussion

4.1. Future climatic conditions enhance Cd bioavailability through altered biogeochemical cycling

Mimicking future climatic conditions with doubled atmospheric CO_2 and +4 °C elevated temperature increased porewater Cd concentrations in soils with native and high Cd contents compared to soils exposed to today's climate (Table 1). This finding is in accordance with previous work (Drabesch et al., 2024), that looked into different agricultural soils and demonstrated that the combination of elevated temperature and doubled atmospheric CO_2 enhanced microbial production of metal-complexing organic acids and mobilization of adsorbed metals by competition with protons. We also detected an approximate 0.1 unit decrease in porewater pH due to temperature (Table S3). One potential source of these protons could be climate-induced microbial ammonium

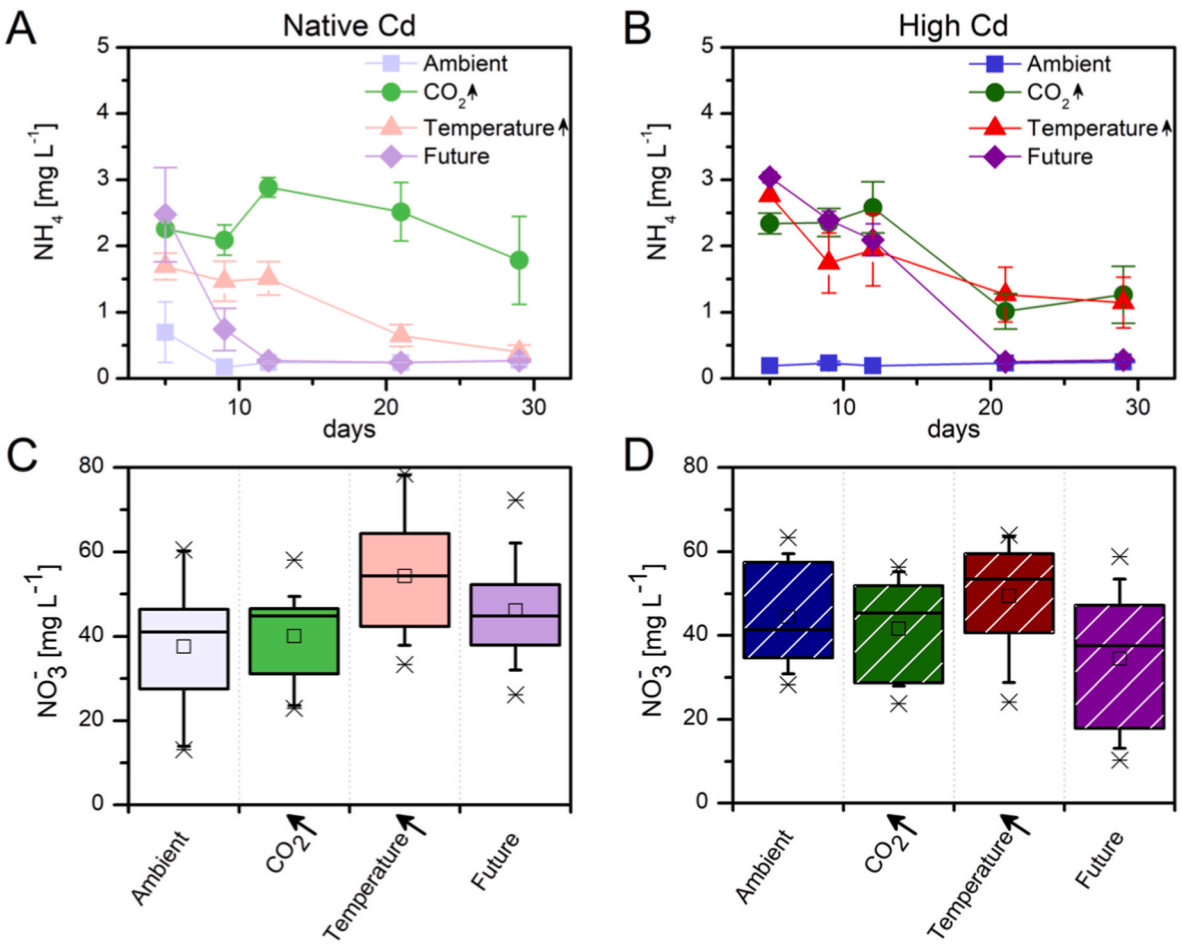


Fig. 4. Dissolved ammonium and nitrate concentrations in an agricultural soil exposed to different climate variables: Temporally resolved porewater ammonium concentrations (A, B) and incubation-averaged nitrate (C, D) concentrations for an agricultural soil with a native (0.13 mg kg^{-1} dry soil; light colors (A, B)) and high Cd (1.5 mg kg^{-1} dry soil; dark colors (C, D)) content. Soils were exposed to either ambient climatic conditions (blue), doubled atmospheric CO_2 concentrations (800 ppm_v, green), elevated temperature (+4 °C relative to ambient temperature, red), or future climatic conditions (the combination of doubled atmospheric CO_2 and elevated temperature, purple). Three/four biological replicates per time point presented as mean ± standard error for A, B. Three/four biological replicates, 5-timepoints for C, D with line inside the box indicating Q50, whiskers Q10 and Q90, and squares representing mean concentrations. Associated statistics with specific *t*-test results are presented in Table S4 and regression analysis in Table S10. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

oxidation (Drabesch et al., 2024), which may facilitate Cd release from mineral surfaces through cation exchange(Jin et al., 2018). While N cycling is a plausible explanation, other mechanisms, such as abiotic pH changes or microbial community shifts affecting other soil biogeochemical processes cannot be ruled out. This manuscript looks at impacts of overall soil biogeochemical processes with a stronger focuses on N cycling due to its well-established role in soil acidification and relevance under changing climatic conditions (Philippot et al., 2024).

The soil's microbiome was fully able to resist and actively adapt to the stress exerted by Cd (Bravo et al., 2018; Pal et al., 2022), as indicated by a recovered total bacterial abundance and diversity 14 weeks after Cd inputs (Table S10). This resulted in a fully functional, Cd-adapted microbial community at the start of the experiment. Future climatic conditions then amplified microbial carbon mineralization and nitrogen cycling in native and high Cd soils (Rustad et al., 2001; Conant et al., 2011; Toosi et al., 2014; Dacal et al., 2022), evidenced by heightened DOC (Fig. 3) and elevated soil respiration (Fig. 2A). In Cd-spiked soils, increased mobilization of Cd due to elevated temperature, most likely induced a small degree of microbial death and cell lysis(Landi et al., 2000; Wang et al., 2004), which is not resolved in cell count data (Table S10) but is indicated by higher available and degradable DOC (Fig. 3), which could be cell-derived sugars, proteins, and membrane lipids.

Since up to 90 % of soil nitrogen is associated with soil organic matter (Chen et al., 2014a; Enggrob et al., 2020), increases in accessible DOC are expected to affect nitrogen dynamics. This has been discussed separately in relation to soil Cd (Zhao et al., 2021) and under climate change conditions, but not in their combination (Bai et al., 2023; Wang et al., 2023a). Oxidized organic matter becomes a major NH_4^+ source (Mullen, 2011; Mooshammer et al., 2014) as observed by higher porewater ammonium concentrations in the future treatment (Fig. 4A and B). An increased *nifH*-gene-carrying microbial sub-community confirms elevated nitrogen fixation under combined future conditions (Fig. 5), also contributing to higher porewater ammonium levels (Berthrong et al., 2014). Subsequently, future climatic conditions activated ammonium-oxidizing and nitrifying archaea carrying the *amoA* gene, such as *Nitrosomonadaceae and Nitrososphaeraceae* (Fig. 5A, Fig. S6), converting ammonium to nitrite and nitrate. However, this did not increase porewater nitrate, as combined future conditions raised the *nirK*-gene as well (Fig. 5B), leading to a 7.2-fold increase in N_2O emissions compared to ambient conditions. Since Cd is known to interfere with *nirK* and other nitrogen-cycling genes (Xu et al., 2016; Duan et al., 2019), climate-induced increases in porewater Cd impacted *nirK* strongly. Climate-mobilized Cd also increased the *nosZ*-gene abundance, converting N_2O to N_2. Yet, the stimulation of the denitrifying community with *nirK* outweighed the stimulation of the *nosZ* community,

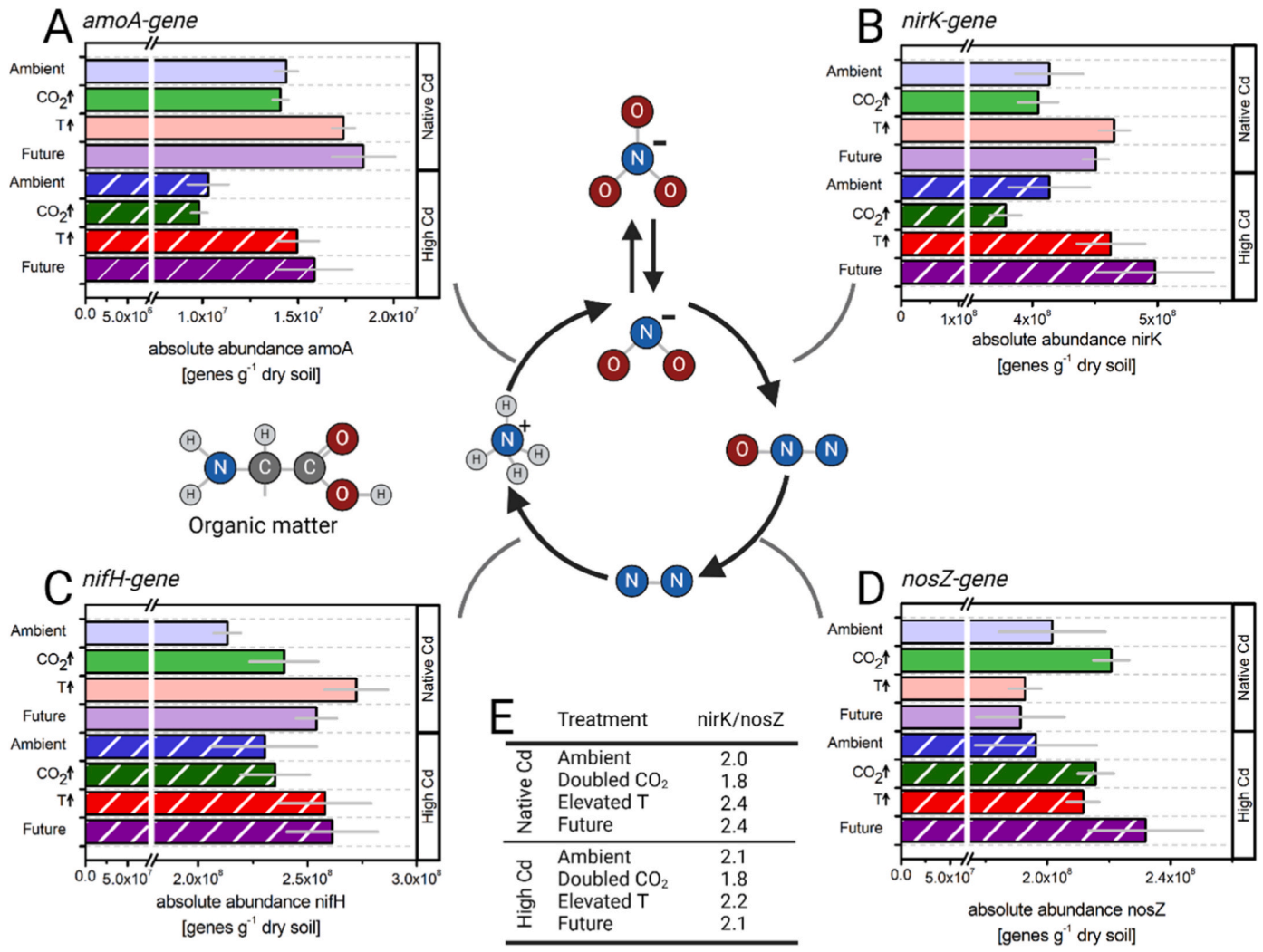


Fig. 5. Microbial nitrogen-cycling genes in an agricultural soil exposed to different climate variables: Absolute gene copy numbers after 31 days of the ammonia monooxygenase; amoA (A), nitrite reductase; nirK (B), nitrogenase; nifH (C), nitrous oxide reductase; nosZ (D) gene, quantified via qPCR from an agricultural soil with a native (0.13 mg kg^{-1} dry soil; light colors) and high Cd content (1.5 mg kg^{-1} dry soil; dark colors, striped). Soils were exposed to either ambient climatic conditions (blue), doubled atmospheric CO_2 concentrations (800 ppm_v, green), elevated temperature (+4 °C relative to ambient temperature, red), or futsure climatic conditions (the combination of doubled CO_2 and elevated temperature, purple). The relative ratio of nirK to nosZ gene abundance is given in the table (E). Average values are based on three/four biological replicates presented as mean ± standard error at day 29. Data for nitrogen-cycling gene transcripts were below the detection limit, thus data for genes is presented. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

leading to overall higher N_2O emissions. Thus, a subsequent N_2O contribution of 45 % to the warming potential from this soil incubation under high Cd and combined future conditions was noted (Fig. 2C). This emphasizes the pivotal role of managing N_2O emissions from agricultural soils burdened with high Cd under combined future climatic scenarios and underscores the resilience and adaptability of specific, and especially nitrogen-cycling microbial populations to altered environmental conditions(Hoffmann and Sgrò, 2011).

Our findings clearly showed that elevated temperature increased porewater Cd mobility substantially, which aligns with previous work demonstrating enhanced Cd bioavailability under warming due to increased mineral dissolution and microbial activity (Morse and Arvidson, 2002; Cornu et al., 2016). In contrast, the absence of significant impacts from elevated atmospheric CO_2 on Cd mobility differs from some studies suggesting indirect effects via microbial community alterations under higher CO_2 conditions (Kelly et al., 2013; Qiu et al., 2019). The observed antagonistic interaction under combined elevated temperature and CO_2 conditions, which resulted in a smaller increase in Cd mobility compared to elevated temperature alone, highlights a novel outcome that previous single-factor studies were unable to reveal (Rillig et al., 2019). Furthermore, our results showed substantially higher N_2O emissions under elevated temperature due to stimulated nitrogen cycling, consistent with findings from (Kammann et al., 2008). However, this differs from results obtained by (Sun et al., 2018), who reported reduced N_2O emissions due to moisture constraints under warming conditions. Such contrasting outcomes underscore the importance of explicitly studying combined climatic factors, as interactions among factors can lead to unexpected ecological responses. Whether increases in temperature and atmospheric CO_2 equally contributed to the observed impacts of future climatic conditions on microbial dynamics and, specifically, the nitrogen-cycling sub-community is discussed in the following.

4.2. Elevated temperature overestimates future Cd bioavailability and its impact on respiration and N-cycling

Elevated atmospheric temperature as a singular climate change parameter seems to overestimate the impact of future climatic

Table 1
Summary of individual and combined climatic factor effects on the biogeochemistry of native and high Cd soils. Individual climatic parameters either increased (change >1.05, light green), decreased (change <0.95, red) or had no effect (grey) on the quantified soil parameters. Furthermore, the combination of individual climatic parameters may result in a synergistic effect, whereby the effects are amplified beyond the additive effect (dark green), or an antagonistic effect, whereby the effects are partially cancelled out (purple).

Parameter	Cd level	Only CO_2*	Only temperature*	Combination* #
Cadmium	Native	0.99	1.35	1.16
	High	1.01	1.28	1.09
DOC	Native	0.95	1.09	1.14
	High	1.00	1.06	1.07
CO_2 production	Native	1.19	1.66	1.50
	High	1.26	1.73	1.74
Ammonium	Native	10.46	4.82	1.87
	High	8.27	6.92	5.86
Nitrate	Native	1.14	1.60	1.21
	High	1.10	1.08	0.89
N_2O production	Native	1.35	5.86	3.15
	High	3.38	5.12	10.13

No effect | *Increase* | *Decrease* | Synergistic effect | Antagonistic effect

* *X-fold change relative to the ambient climate*
\# *Combination > Only temperature + only CO_2 = Synergistic effect*
Combination < Only temperature + only CO_2 = Antagonistic effect

conditions on Cd mobility by 15–20 % (Fig. 1, Table 1). The combined elevated temperature and CO_2 treatment in our study showed an attenuated Cd mobility response compared to elevated temperature alone. This observation aligns with findings from hydroponic rice experiments, where elevated temperature increased Cd uptake, but elevated CO_2 moderated this effect (Feng et al., 2023). This is mainly due to an overall increase microbial activity, which affects various biogeochemical processes in the soil. Regardless of the total soil Cd content, elevated temperature stimulated the microbial community, increasing porewater organic carbon concentrations to similar extent as combined future climatic conditions. However, the native soil Cd respirational output under elevated temperature was 10 % higher compared to combined future climatic conditions (Fig. 2A), leading to the perception that more organic carbon was mobilized and degraded compared to combined future climatic conditions. Microbial communities closer to their temperature optimum exhibit enhanced oxidation of soil organic matter (Conant et al., 2011; Wiesmeier et al., 2019). *Vice versa*, microbial communities experiencing a temperature increase beyond their optimal range, cell death or inactivation of parts of the community can occur. Interestingly, both scenarios can lead to increased porewater organic carbon (Lu et al., 2013b). This increase happens either through enhanced turnover of more recalcitrant organic matter due to increased microbial activity or through the contribution of lysed cells to the porewater organic carbon pool. Either way, increased available DOC facilitates microbial activity and augments respiration (Fig. 2, Table 1) (Ren et al., 2018). The observed adaptation of microbiome to elevated temperatures and soil Cd levels provides insight into their capacity to cope with the combined effects of Cd contamination and changing climatic conditions.

In the high Cd soil, the optimum temperature for Cd-tolerant bacteria was likely favored under elevated temperature allowing them to sustain their metabolic activity more than Cd-non-tolerant bacteria and mitigating Cd toxicity compared to ambient climatic conditions (Conant et al., 2011; Wiesmeier et al., 2019). Therefore, combining increased soil Cd and climate change did not have compounded effects on the microbial community. This finding aligns with previous research, suggesting that elevated temperatures can cause Cd to exhibit a hormetic effect (Zhang et al., 2023a) on microbial respiration, depending on the doses (Khan et al., 2010; Zhao et al., 2021; Zhang et al., 2023a). This hormetic response may arise from increased microbial (autotrophic) respiration or cell lysis, both of which can enhance nutrient turnover and microbial activity(Chen et al., 2014b), further underscoring the complex interplay between temperature, microbial response, and nutrient dynamics in Cd-contaminated environments.

Elevated temperature stimulated the degradation of organic matter and showed a high N_2-fixation via the *nifH* gene (Fig. 5C). This increased and prolonged the supply of ammonium for microbial ammonium oxidation compared to combined future climatic conditions in both native and high Cd soils. As a result, porewater nitrate increased (Fig. 4, Table 1). This led to maximum N_2O emissions compared to all other treatments in the native Cd soil due to an increased abundance of the *nirK gene* and simultaneous reduction in *nosZ gene* abundance. Our observed increase in N_2O emissions agrees with Kammann et al. (2008), but contrasts with findings from Sun et al. (2018), potentially due to differences in soil moisture and experimental setups. Thus, under elevated temperature protons were released (Table S3), displacing Cd from mineral surfaces in the native Cd soil. Additionally, small molecular weight organic carbon molecules as products of soil organic matter degradation (Li et al., 2009) may also scavenge Cd from mineral surfaces via complexation, contributing to an overall higher porewater Cd load.

In the presence of high soil Cd, the N_2-fixing, denitrifying and N_2O-reducing microbial community were less impacted by increased atmospheric temperature, leading to lower N_2O emissions compared to combined future climatic conditions. Intermittent nitrate was still highest under combined Cd and elevated temperature but not as distinctly high as in the low Cd treatment indicating a subtle interactive toxic effect of amended Cd on the nitrogen cycling community, which was further supported by less stimulated nitrogen cycling genes compared to combined future climatic conditions. This suggests that overall temperature-induced microbial activity is partially offset by high soil Cd toxicity intricately affecting the nitrogen cycling community in soils.

4.3. Doubled atmospheric CO_2 selectively increases nitrogen fixation

Elevated atmospheric CO_2 as a singular climate parameter does not impact the mobility of Cd compared to ambient CO_2 conditions (Fig. 1). Nonetheless, since the mobility of Cd under combined future climatic conditions was on average 14 % lower as under singular elevated temperature conditions, there must be an inhibitory effect of atmospheric

CO_2 on Cd mobility (Table 1). However, because of the high microbial respiration in soils, the partial pressure of CO_2 is in the lower percent range in soil pores space (Oh et al., 2005). Therefore, contrasting to a paddy soil (Wang et al., 2023b), doubled atmospheric CO_2 likely does not affect soil geochemistry on a pH scale. Still, it may influence the carbonate buffer system, potentially leading to more precipitated carbonate formations (Ferdush et al., 2023) or changes microbial dynamics as elevated atmospheric CO_2 can increase the rates of organic matter mineralization (Carney et al., 2007). Despite a 20 % increase in CO_2 emissions (Fig. 2A), the total DOC remained unchanged compared to ambient conditions (Fig. 3A), though changes in the composition of organic carbon may have occurred. Nonetheless, ammonium production was prominently affected by increased atmospheric CO_2, which is in accordance with (He et al., 2010); and in our study indicated by an increased abundance of the nitrogen-fixing *nifH*-gene (Fig. 5C). The resulting higher porewater ammonium (Fig. 4A) was not consumed via microbial ammonium oxidation as increased atmospheric CO_2 did not additionally stimulate *amoA* abundance (Fig. 5A). Thus, no additional protons were produced that could have mobilized Cd and N_2O emissions remained low (Figs. 2 and 5B).

In soil with a high Cd content, ammonium concentrations were similarly high under doubled atmospheric CO_2 and under higher temperatures plus elevated CO_2, i.e. future conditions, but not as high as in the native Cd soil. Therefore, the observed CO_2-stimulated ammonium production through enhanced *nifH* activity leading to N_2-fixation in the native Cd soil seemed to be inhibited by high Cd. However, under doubled atmospheric CO_2 and high soil Cd, the reduced abundance of the denitrifying *nirK*-gene did not decrease N_2O emissions. Instead, N_2O emissions were 340 % higher after Cd addition compared to the ambient climate setup without Cd addition ($p = 0.035$) but 70 % lower than at higher temperature and doubled CO_2 (Fig. 2B). A higher potential activity of *Nitrospiraceae (*Fig. S6C*)* suggests increased ammonium oxidation over time under doubled atmospheric CO_2 (Fig. 4B). This, in turn, could have augmented nitrate production and made denitrification processes more efficient despite not increasing in the abundance of genes as it was observed under combined future conditions. This indicates an essential selective coupling under doubled atmospheric CO_2 for individual parts of the nitrogen cycle, especially when combined with abiotic Cd stress. Moreover, our findings highlight the importance of considering temperature as a universal stressor affecting the entire microbiome, while doubled atmospheric CO_2 is a more selective stressor specific to parts of the nitrogen cycle.

4.4. Conclusion and environmental relevance

Our study demonstrates that combined future climatic conditions (elevated temperature and CO_2) strongly influence soil Cd bioavailability, microbial nitrogen cycling, and greenhouse gas emissions. These interactions are not predictable from individual-factor studies alone. The novelty of this study is to compare individual versus combined climate effects. Elevated temperature alone increased porewater Cd concentrations by up to 50 % (Fig. 1, Table 1). Elevated CO_2 alone showed minimal impacts on Cd mobility. Under combined future conditions, Cd increases were attenuated to approximately 30 % (Fig. 1, Table 1). Elevated temperature substantially increased microbial nitrogen cycling and N_2O emissions approximately 7-fold compared to ambient conditions (Fig. 2). This aligns with findings by Kammann et al. (2008) but contrasts studies showing moisture-limited reductions (Sun et al., 2018). These contrasting outcomes support recent frameworks classifying interactions as antagonistic, synergistic, or additive (Krishna et al., 2025). Antagonistic interactions observed here show combined climate effects are not simply additive. While climate-controlled chamber experiments provide precise control over temperature and CO_2, they may not fully capture the complexity of natural field conditions, where additional variables such as fluctuating moisture, plant interactions, and microbial community dynamics could influence outcomes. Future studies should validate these laboratory-derived findings under field conditions to enhance applicability. Further research across various soil types and metal contaminants would be insightful to broadly validate these findings and enhance their applicability, especially considering that the current results are based on a single soil type where the impact of climate on Cd mobility may differ from that in soils with contrasting properties. A comprehensive understanding of these interactions is vital for developing targeted mitigation strategies against environmental challenges posed by climate change, particularly as increased Cd mobility under combined climate drivers may elevate plant uptake and food safety risks, as shown by (Wang et al., 2020a).

CRediT authorship contribution statement

S. Drabesch: Writing – review & editing, Writing – original draft, Visualization, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **S. Mueller:** Writing – review & editing, Methodology, Investigation, Formal analysis. **J.M. León Ninin:** Writing – review & editing, Methodology, Formal analysis. **B. Planer-Friedrich:** Writing – review & editing, Methodology, Formal analysis. **A. Kappler:** Writing – review & editing, Supervision, Conceptualization. **E.M. Muehe:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Data availability statement

Raw sequencing data be deposited into the Sequence Read Archive (SRA) at NCBI. The dataset supporting the findings of this study will be made available on Zenodo prior to publishing.

The data that support the findings of this study are included in a compressed Source Data file at Zenodo (https://doi.org/10.5281/zenodo.17913011) and raw sequencing data were deposited at the Sequence Reads Archive (SRA, BioProject: PRJNA1380388, (http://www.ncbi.nlm.nih.gov/bioproject/1380388).

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.envpol.2025.127508.

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