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Changes in microbial community composition drive the response of ecosystem multifunctionality to elevated ozone

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Abstract

Increasing tropospheric ozone poses a potential threat to both above- and belowground components of the terrestrial biosphere. However, the interaction between soil microbial communities and ecosystem functioning under elevated ozone remains poorly understood. In this study, we evaluated the responses of three crop seedlings growth (soybean, maize, and wheat) and soil microbial communities to elevated ozone (+40 ppb O_3 above ambient) in a pot experiment in the solardomes. Results showed that elevated ozone negatively affected ecosystem multifunctionality by reducing crop biomass, inhibiting soil extracellular enzyme activities, and reducing the abundance of total bacteria and functional genes involved in the soil nitrogen cycle. Ecosystem multifunctionality was associated with changes in soil microbial community composition but not with microbial alpha-diversity. In addition, elevated ozone significantly affected fungal community composition, but bacterial community composition and microbial alpha-diversity remained unaffected by ozone exposure. Crop type played a key role in determining bacterial alpha-diversity and microbial community composition. In conclusion, our findings suggest that short-term elevated ozone negatively affects the growth of crop seedlings and further influences soil microbial activity, leading to a decrease in ecosystem multifunctionality.

Keywords: Ozone exposure; Staple food crops; Microbial communities; Nutrient cycling; Ecosystem functioning

1. Introduction

As a greenhouse gas and a secondary air pollutant, tropospheric ozone can be generated by ozone precursors such as nitrogen oxides, volatile organic compounds, and carbon monoxide through complex photochemical reactions (Ainsworth et al., 2012; Ashmore, 2005). Owing to rapid industrialization and urbanization, dramatic increases in the levels of ozone precursors have occurred on a global scale, especially in the Northern Hemisphere (Cooper et al., 2014), where the tropospheric ozone emissions have increased by 0.5-2.0% per year during the past three decades (Vingarzan, 2004). The global mean tropospheric ozone concentrations have more than doubled to 35-40 ppb since the Industrial Revolution and are projected to continue to increase, particularly in rapidly developing regions (IPCC, 2013; Monks et al., 2015). Currently, this trend could threaten human health and have deleterious impacts on soil biodiversity and plant growth (Agathokleous et al., 2020; Wang et al., 2020).

High ground-level ozone concentrations have been reported in most of the major crop-growing regions of the world, including North America, Europe, and Asia (Mills et al., 2018). Several studies have proposed that ozone levels with an average of 40 ppb could reduce the yield of leading crops (i.e., rice, maize, and soybean) (Avnery et al., 2011; Feng & Kobayashi, 2009; Mills et al., 2007). Ozone exposure exerts adverse consequences on crop production by decreasing stomatal conductance and photosynthetic rates, suppressing carbon (C) sequestration, altering belowground resource allocation, and accelerating lipid oxidation and leaf senescence (Feng et al., 2008, 2016; Morgan et al., 2003). A recent modeling study suggested that elevated ozone has the potential to reduce annual global yields of soybean, maize, and wheat by 12.4, 6.1, and 7.1%, respectively (Mills et al., 2018). Based on a global chemical transport model, it is now estimated that elevated ozone will cause a

global economic loss in soybean, maize and wheat production of between $17 billion to $35 billion by 2030 (Avnery et al., 2011). Given that from crop production and food security perspectives, it is crucial to get an insight into the effects of elevated ozone on crop growth (Mills et al., 2018; Tai et al., 2014).

Elevated ozone directly affects plant properties (i.e., plant biomass, litter, and exudation), which induces shifts in a series of soil processes (Andersen, 2003; Li et al., 2012; Wu et al., 2016). For instance, elevated ozone diminishes the allocation of carbohydrates to roots, as evidenced by decreases in root biomass and exudation (Edwards, 1991; Grantz et al., 2006). These changes could cause negative effects on soil extracellular enzyme activities and thus inhibit the degradation of C and nitrogen (N) sources such as cellobiose, chitin, and peptidoglycan (Chung et al., 2006; Phillips et al., 2002). Simultaneously, ozone-induced alterations in the availability of soil substrates likely influence belowground processes driven by soil microorganisms. Using the GeoChip-based analysis, previous studies have demonstrated that microbial communities involved in soil C- and N-cycling significantly change under elevated ozone (He et al., 2014; Li et al., 2013). Recent advances have shown that soil microbial communities are crucial in maintaining ecosystem multifunctionality that is positively associated with soil nutrients and enzyme activity (Delgado-Baquerizo et al., 2020; Han et al., 2022; Zhou et al., 2020). For the development of sustainable agriculture, more empirical research concerning how elevated ozone mediates soil microbial communities, ecosystem multifunctionality, and their linkages is necessary.

There is variability in the responses to elevated ozone between different plant species. For example, deciduous species are often more sensitive to elevated ozone than evergreen species, depending on leaf mass instead of stomatal conductance (Zhang et al., 2012). Plants of the *Fabaceae*

family have a higher ozone sensitivity than plants of *Asteraceae*, *Poaceae*, and *Caryophyllaceae* families, which could be explained by the ozone-induced difference in their internal ozone/free radical detoxification system (Hayes et al., 2007). Generally, compared to ozone-tolerant cultivars, soil microbial communities in ozone-sensitive cultivars prefer to consume more complex C sources, and thus they need more soil nutrients (Bao et al., 2015). In addition, the responses of plants to ozone exposure are likely to be highly dependent on their growth stages. Previous research illustrated that wheat sensitivity varies between the vegetative and reproductive growth stages (Rai & Agrawal, 2014). Likewise, elevated ozone has a different impact on mature trees and potted seedlings (Samuelson & Michael Kelly, 2001). Even though some studies have focused on the adverse effects of elevated ozone on tree seedlings (Dai et al., 2017; Sugai et al., 2019), the available information about different crop seedlings responses to elevated ozone remains poor.

In this study, we investigated the effects of elevated ozone on the growth of three crops (i.e., soybean, maize, and wheat) at the seedling stage and the impact of this on the diversity and composition of the soil microbial communities. To achieve this, we carried out a pot experiment under ambient and elevated ozone conditions in the solardomes. We aimed to address the following questions: (1) Does elevated ozone negatively affect the growth of crop seedlings and the activity of soil microbial communities? (2) Are changes in soil microbial communities caused by elevated ozone related to ecosystem functioning?

2. Materials and methods

2.1. Experimental design

The pot experiment was carried out in the UK CEH solardome facility at Abergwyngregyn, North Wales, UK (13 m.a.s.l., 53°15'N, 4°01'W). We collected soils from an adjacent wheat field at the Bangor University Henfaes Experimental Station, Abergwyngregyn, Gwynedd, UK. The soil is classified as a sandy clay loam textured Eutric Cambisol (FAO) or Dystric Eutrudepts (US Soil Taxonomy). The soil had a slightly acidic pH of 5.6, total soil C of 2.5%, and total N of 0.21%. Further details of the soil and site characteristics can be found in Sánchez-Rodríguez et al. (2018). The soil was sieved and homogenized (4 mm mesh size), and 1.5 kg field-moist soil was packed in the 2 L (18 cm diameter, 12 cm depth) black plastic pots.

Seedlings of maize (*Zea mays* L.), wheat (*Triticum aestivum* L.), and soybean (*Glycine max (Linn.) Merr*.) were germinated and grown in cell seedling trays for two weeks (using the same soil that was used for the main experiment), after which they were transplanted into the experimental pots. Three seedlings were arranged evenly in each pot for wheat, and one seedling was planted in each pot for maize and soybean. Pots were rotated within each solardome and irrigated with 100 mL tap water twice-weekly to avoid drought stress and keep soil water content near the field capacity throughout the experiment. The chemistry of the low nutrient tap water is detailed in Brown et al. (2021). N was added as urea at a rate of 10 g N m^{-2} after transplanting to eliminate the possible nutrient limitation.

There were two solardomes with ambient and elevated (ambient + 40 ppb) ozone treatments. The elevated ozone level was chosen to reflect baseline levels predicted to occur by 2100 and those currently occurring over short periods of time in northwest Europe (Andersson & Engardt, 2010; Kulkarni et al., 2013). Twelve pots with four replicates per crop were randomly distributed in each solardome. Crop seedlings were exposed to the ozone treatments for 40 days from 5 August to 13 September 2018. Ozone was provided to the solardomes by a G11 ozone generator (Pacific Ozone

Technology, Benicia, CA) and a work-horse eight oxygen generator (Dryden Aqua Ltd, Edinburgh, UK), with ozone added to charcoal-filtered air, and with the concentration determined by a computer-controlled ozone injection system (LabVIEW version 2012; National Instruments, Texas, US). Ozone was distributed to each solardome via Polytetrafluoroethylene tubes. The ozone concentration inside each solardome was measured for 5 min every 30 min using two ozone analyzers (ThermoScientific, Model 49i, Reading, UK) of matched calibration.

2.2. Soil properties and plant analysis

After 40 days of ozone exposure, each soil sample was collected, sieved (2-mm mesh), and then divided into two parts: one part was placed at 4°C to await chemical analysis, and the other part was stored at −80°C to await molecular analysis. Concentrations of ammonium (NH_4^+-N) and nitrate (NO_3^--N) were measured according to the colorimetric salicylate procedure of Mulvaney (1996) and the vanadate method of Miranda et al. (2001), respectively. Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted with 0.5 M K_2SO_4 and assessed using a Multi N/C 2100 TOC analyzer (Analytik Jena GmbH, Jena, Germany). Dissolved organic nitrogen (DON) was calculated as the difference between TDN and inorganic N. Following the chloroform fumigation-0.5 M K_2SO_4 extraction method of Vance et al. (1987), microbial biomass carbon (MBC) was calculated as the difference in DOC content in extracts between fumigated and non-fumigated soil samples. The potential activities of soil extracellular hydrolytic enzymes involved in the cycling of C, N, and phosphorus (P): β-glucosidase (BG), β-N-acetyl-glucosaminidase (NAG), leucine aminopeptidase (LAP), and acid phosphatase (AP) were measured following the fluorometric protocol from Saiya-Cork et al. (2002) and DeForest (2009) for MUB-linked substrates (BG, NAG, and AP) and AMC-

linked substrates (LAP). In addition, ratios of ln(BG)/ln(NAG+LAP) and ln(BG)/ln(AP) were defined as N-*acq* and P-*acq*, respectively.

The chlorophyll a, chlorophyll b, and carotenoids content in the shoots were determined according to the specific absorption coefficients by Lichtenthaler (1987). Briefly, two leaf discs per crop (diameter 0.5 cm) were extracted with 2 mL 95% ethanol for 72 h at 4°C in the dark, and then the absorbance of pigment extracts was calculated at 664, 649, and 470 nm with a Synergy 96 well plate spectrophotometer (BioTek Instruments Inc., Winooski, VT, USA). In addition, crops were separated into shoots and roots and then dried at 65 °C for 48 h to obtain their dry weight.

2.3. DNA extraction, quantification, and MiSeq sequencing

The DNA was extracted from soil samples using the DNeasy PowerSoil DNA Isolation Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions and stored at −80°C until required. The abundances of total bacteria and functional genes involved in nitrification, denitrification, ammonification, and N fixation were quantified by quantitative real-time PCR using a Quant Studio™ 6 Flex system (Applied Biosystems, Life Technologies, USA). Details of primer sequences for the target genes and amplification conditions are shown in Table S1. Standard curves were obtained from serial dilutions of a known amount of linearized plasmids containing specific gene fragments. PCR products were detected by electrophoresis on 2% agarose gels before being purified with Qiagen Gel Extraction Kit (QIAGEN, Germany), pooled in equimolar concentrations, and then paired-end sequenced on a MiSeq system (Illumina Inc., San Diego, CA). Paired-end reads were assigned to samples based on their unique barcode, truncated by cutting off the barcode and primer sequence, and merged using FLASH (v 1.2.7) (Magoč & Salzberg, 2011). Quality filtering on the resulting reads was performed under specific filtering conditions to obtain the high-quality

sequences according to the QIIME (v 1.7.0) (Caporaso et al., 2010). Subsequently, these were compared with the reference database (Gold database, http://drive5.com/uchime/uchime_download.html), and the chimera sequences were removed from the datasets using UCHIME (Edgar et al., 2011). The operational taxonomic units (OTUs) were generated at 97% sequence similarity after discarding the low-quality and chimeric sequences. All raw sequences were deposited in the NCBI SRA with accession number PRJNA777642.

2.4. Statistical analyses

All data were checked using Shapiro-Wilk's and Levene's tests to test for normality and equality of variance, respectively. The averaging approach was used to estimate multifunctionality using the "*multifunc*" package (Byrnes et al., 2014). This approach determines the average levels of multiple functions (i.e., photosynthetic pigment content, crop biomass, soil chemical properties, soil MBC, and extracellular enzyme activities) by standardizing each function to a common scale and averaging standardized values into a single index. To evaluate the effects of ozone, crop type, and their interaction on leaf photosynthetic pigments, crop biomass, soil chemical properties, microbial biomass, extracellular enzyme activities, gene abundances, the alpha-diversity of the bacterial and fungal communities, as well as ecosystem multifunctionality, a two-way analysis of variance (ANOVA) was conducted with post-hoc Tukey HSD tests. For the OTU-based analysis, soil microbial alpha-diversity was compared using normalized Observed OTUs, Shannon, ACE, and Chao1 indices. The non-metric multidimensional scaling (NMDS) was used to depict soil microbial beta-diversity with the permutational multivariate analysis of variance (PERMANOVA). Soil microbial alpha-diversity and beta-diversity analyses were performed using the "*vegan*" package (Oksanen et al.,

2017). We considered $P < 0.05$ as statistically significant, $0.05 < P < 0.1$ as marginally significant. All statistical analyses were performed by R software version 3.2.2.

3. Results

3.1. Crop growth, soil chemical and biological properties

Both ozone and crop type and their interaction significantly affected crop physiological and growth parameters ($P < 0.01$; Table S2). Overall, the negative effects of elevated ozone on chlorophyll a, chlorophyll b, and carotenoids content were greater in soybean than in wheat and maize. Elevated ozone significantly reduced photosynthetic pigment content in soybean and wheat by 62.8–69.0% and 16.9–31.8%, respectively. Photosynthetic pigment content in maize was decreased by 13.2–24.8% under elevated ozone, although this effect was not significant statistically (Fig. 1a). In addition, shoot and root biomass in wheat were reduced to a greater extent than in soybean and maize under elevated ozone, with 45.9 and 70.6% decreases for wheat, 32.8 and 44.2% decreases for soybean, as well as 25.3 and 22.9% decreases for maize. The ratio of root and shoot biomass in wheat and soybean were lower under elevated ozone (Fig. 1a).

There were no significant changes in soil chemical properties under elevated ozone ($P > 0.05$; Table S3). A significant interaction between ozone and crop type was detected in soil NO_3^-, DON, and TDN content as indicated by the declines in maize (47.7–57.2%), but the increases in wheat (60.3–228.3%) and soybean (8.3–30.5%) under elevated ozone ($P < 0.05$; Table S3, Fig. 1b). Although elevated ozone did not significantly influence soil MBC, it led to declines in soil MBC in wheat and soybean ($P > 0.05$; Table S4). Besides, both ozone and crop type significantly affected soil extracellular enzyme activities, with NAG and AP activities impacted by the interaction between

ozone and crop type ($P < 0.05$; Table S4). In general, elevated ozone corresponded with lower soil extracellular enzyme activities. Significantly reduced BG, NAG, LAP, and AP activities in maize (9.0–28.1%) and soybean (13.5–23.5%) were detected under elevated ozone. In addition, there were no pronounced increases or decreases in N-*acq* and P-*acq* within each crop type under elevated ozone (Fig. 1c).

3.2. N-cycling processes

Ozone and crop type had significant or marginal impacts on the abundances of total bacteria and functional genes involved in soil N-cycling (except for Comammox) ($P < 0.001$ and $P < 0.1$; Table S5). No significant interaction between ozone and crop type was found for gene abundances ($P > 0.05$; Table S5). Compared to ambient ozone, the abundances of total bacteria and functional genes involved in soil N-cycling (except for Comammox) were significantly reduced in wheat and maize under elevated ozone, with a generally larger decrease in wheat (51.3–70.6%) than in maize (40.3–77.3%; Fig. 2a). Elevated ozone did not show a pronounced influence on the abundances of total bacteria and functional genes involved in soil N-cycling in soybean, except for a significant decrease in AOB *amoA* (41.0%). Meanwhile, there were no significant changes in the ratios of AOA/Bacteria, AOA/AOB, and (*nirK*+*nirS*)/*nosZ* across each crop type under elevated ozone (Fig. 2a). Across the three crops, the abundances of total bacteria and functional genes involved in soil N-cycling were reduced by 50.5% and 27.5–54.3% on average under elevated ozone, respectively. Concomitantly, on average, soil NH_4^+ content was reduced by 6.0%, and soil NO_3^- content was increased by 63.0% under elevated ozone (Fig. 2b).

3.3. Soil microbial communities

We found that only bacterial alpha-diversity was significantly affected by crop type ($P < 0.05$; Table 1). No significant differences in bacterial and fungal alpha-diversity were found in response to ozone exposure and its interaction with crop type ($P > 0.05$; Table 1). The sequences obtained from each sample were classified into different phyla. The relative abundance at the phylum level of bacterial communities greater than 0.1% is shown in Fig. 3a. The most abundant phyla across all samples were *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Acidobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*, and *Bacteroidetes*, accounting for 94.6–96.1% of the total bacterial OTUs. Results of six fungal phyla are given in Fig. 3b, and the dominant fungal phyla were *Ascomycota*, *Zygomycota*, and *Chytridiomycota*, accounting for 97.9–98.6% of the total fungal OTUs.

Under elevated ozone treatments, we found that the relative abundance of four bacterial genera was significantly changed ($P < 0.05$). *Paenibacillus* and *unidentified_Elusimicrobia* in wheat were significantly enriched. In contrast, *Alkanindiges* in soybean and *Perlucidibaca* in wheat were significantly depleted. No significant differences in bacterial genera in maize were observed under elevated ozone (Fig. 4). Significant alterations in the fungal families were primarily detected in soybean under elevated ozone ($P < 0.05$). Furthermore, the relative abundance of two fungal families (*unassigned_Eurotiomycetes* and *Davidiellaceae*) in maize was significantly increased, while the relative abundance of four fungal families (*Clavicipitaceae*, *unassigned_Ascomycota*, *Ascobolaceae*, and *Leptosphaeriaceae*) in maize was significantly decreased under elevated ozone ($P < 0.05$; Fig. 4). There were no significant differences in fungal families in wheat under elevated ozone ($P > 0.05$).

Shifts in microbial community composition of the three crops in response to elevated ozone were observed in the NMDS analysis, with the stress value less than 0.2 (Fig. 3c and d). The PERMANOVA analysis revealed that both bacterial and fungal community composition were

significantly influenced by crop type ($P < 0.001$; Table S6) and that ozone exposure and its interaction with crop type also significantly affected fungal community composition ($P < 0.01$; Table S6). In the NMDS for bacterial communities, all the samples were divided into three parts according to crop type (Fig. 3c). In the NMDS for fungal communities, there was a separation between soybean samples and maize and wheat samples (Fig. 3d).

3.4. The relationship between ecosystem multifunctionality and soil microbial communities

Ecosystem multifunctionality was calculated using five metrics, including photosynthetic pigment content, crop biomass, soil chemical properties, soil MBC, and soil extracellular enzyme activities. We found that elevated ozone had a negative impact on ecosystem multifunctionality ($P < 0.001$; Table 1), which was positively correlated with the NMDS2 coordinate of bacterial communities ($r = 0.59$, $P = 0.003$; Fig. 5), but negatively correlated with the NMDS1 coordinate of fungal communities ($r = -0.38$, $P = 0.079$; Fig. 5). In addition, there was no significant relationship between microbial alpha-diversity and ecosystem multifunctionality.

4. Discussion

4.1. Effects of elevated ozone on crop physiology and growth

Photosynthetic pigments act as good indicators of foliar stress under ozone exposure as they influence the capacity of plants to absorb light and further affect photosynthetic rates, crop biomass, and crop yield (Morgan et al., 2003; Rai et al., 2011). To date, ozone-induced adverse effects on photosynthetic pigments have been reported for many crop types, including soybean, maize, and wheat (Feng et al., 2016; Leitao et al., 2007; Morgan et al., 2003), which is consistent with our findings. In the present study, after 40 days of elevated ozone, chlorophyll a, chlorophyll b, and carotenoid contents were

274 significantly decreased in soybean and wheat (Fig. 1a). Consistent with our findings, legumes are
275 reported to be more sensitive to ozone than other families (Hayes et al., 2007), and soybean has a
276 more pronounced crop yield sensitivity to ozone than wheat, maize, and rice (Mills et al., 2018).
277 Correspondingly, the reduced content and rates of photosynthetic pigments in leaves may result in less
278 available C for crop growth (Morgan et al., 2003). Further, we found that elevated ozone decreased
279 photosynthetic pigment content in maize, albeit not significant statistically, suggesting that maize as a
280 moderately sensitive crop is less sensitive to ozone than soybean and wheat (Mills et al., 2007).

281 On the other hand, the current study agrees with several meta-analyses demonstrating that
282 elevated ozone reduces crop biomass (Morgan et al., 2003; Pleijel et al., 2018). Our findings indicated
283 that wheat was the crop with the largest reduction in biomass (Fig. 1a), which was not in line with the
284 extent of declines in photosynthetic pigment content. We speculate that this phenomenon may be
285 related to the leaf thickness and tissue density of wheat. Previous studies have shown that leaf mass
286 per area is the main characteristic determining plant sensitivity to ozone and that ozone-induced
287 decreases in plant biomass are associated with stomatal flux per unit leaf mass (Dai et al., 2017; Feng
288 et al., 2018; Hoshika et al., 2022). Besides, we observed that elevated ozone reduced the ratio of root
289 and shoot biomass in wheat and soybean (Fig. 1a), a common notion of ozone damage to plants by
290 reducing carbohydrate allocation to roots (Andersen, 2003).

291 **4.2. Effects of elevated ozone on soil microbial activity, functional genes, and community**
292 **composition**

293 As mentioned above, the lower soil MBC in wheat and soybean under elevated ozone could be
294 ascribed to the lower ozone-induced C inputs to the underground parts of the plant (Fig. 1c), implying
295 that elevated ozone reduces nutrition supply for microbial metabolism and thereby influences the

296 microbial abundance and activity (Edwards, 1991; Grantz et al., 2006; Islam et al., 2000). As
297 expected, we found that soil extracellular enzyme activities associated with C-, N-, and P-cycles (i.e.,
298 BG, NAG, LAP, and AP) generally declined in response to elevated ozone (Fig. 1c). Previous studies
299 have examined the effects of elevated ozone on soil extracellular enzyme activities in forest and
300 grassland ecosystems; however, findings in the literature are inconsistent, showing either significant
301 changes or no significant effect of elevated ozone on soil extracellular enzyme activities (Chung et al.,
302 2006; Edwards & Zak, 2011; Phillips et al., 2002; Wang et al., 2019a). These contradictory results
303 may be due to differences in experimental duration, ecosystem type, and other factors. In addition, it
304 is well documented that ratios of some extracellular enzyme activities could reflect the acquisition of
305 C, N, and P via microbes (Xu et al., 2017). The values of N-*acq* and P-*acq* in this study indicated that
306 nutrient cycling dominated by soil microbes was not C, N, and P-limited (Fig. 1c).

307 Soil microorganisms can regulate soil nutrients and greenhouse gas emissions through a myriad
308 of biogeochemical processes (Bhatia et al., 2011; Wu et al., 2016). We found that elevated ozone
309 generally decreased the abundance of functional genes involved in soil N-cycling associated in all
310 crops (Fig. 2a). The lower *ureC* abundance may contribute to lower soil NH_4^+ through
311 ammonification, while lower *nifH* abundance could inhibit N fixation and thereby influence soil NH_4^+.
312 The positive response of soil NO_3^- to elevated ozone may be related to the lower abundance of *narG*
313 associated with the first step of denitrification (He et al., 2014), which was not tested in this study. In
314 addition, the effects of elevated ozone on soil N-cycling processes varied among different plant
315 cultivars and soil conditions. For example, after three years of elevated ozone, the abundance of *nifH*
316 related to N fixation in the rhizosphere soil decreased in the relatively ozone-sensitive wheat but
317 increased in the ozone-sensitive wheat (Li et al., 2013). A survey using intact soils showed that after

five years of ozone fumigation reductions in nitrification and ammonification occurred only in ozone-sensitive wheat cultivars and not in ozone-tolerant wheat cultivars (Wu et al., 2016). Contrary to our study, elevated ozone was reported to enhance denitrification by increasing the abundance of *nosZ* in the rhizosphere and bulk soils due to higher soil organic C supply for denitrifying microbes (Pujol Pereira et al., 2011).

Fungi are more susceptible to elevated ozone than bacteria (Li et al., 2012; Phillips et al., 2002). In the current study, elevated ozone significantly altered the composition of the fungal community. Short-term ozone exposure may be a major explanatory factor for the insignificant responses of bacterial community composition to elevated ozone (Li et al., 2022). Crop type had a pronounced impact on bacterial and fungal community composition (Table S6). This phenomenon may be ascribed to the fact that different plant types could influence soil bacteria and fungi via various root exudation (Andersen, 2003; Li et al., 2012). As the primary energy sources for soil microbes, root exudates contain many dissolved organic compounds, e.g., amino acids, sugars, and organic acids (Dennis et al., 2010). A laboratory experiment using wheat, maize, rape, and barrel clover confirmed that the rhizosphere bacterial community structure is likely shaped by the root exudates of plant species (Haichar et al., 2008). Similarly, the NMDS analysis in our study showed that bacterial communities were divided into three parts according to crop type, with the microbial community of soybean being distantly separated from that of maize and wheat (Fig. 3c and d).

4.3. Linkages between ecosystem multifunctionality and soil microbial communities

Elevated ozone could threaten ecosystem multifunctionality by influencing crop growth, soil conditions, as well as microbial biomass and activity (Table 1). Crop production plays a key role in sustaining human welfare and regulating belowground ecosystem processes. Soil NH_4^+ and NO_3^- are

340 considered to be the main N sources for plants and soil microorganisms (Delgado-Baquerizo et al.,
341 2016). Soil extracellular enzymes have been widely used to evaluate soil fertility and ecosystem
342 functioning because they are involved in C metabolism and are associated with soil substrate
343 availability (Bhatia et al., 2011; DeForest, 2009; Edwards, 1991). Various ecosystem processes
344 ultimately determine ecosystem multifunctionality and exhibit strong effects on soil microbial
345 diversity and community composition. A common notion is that ecosystem functioning is associated
346 with microbial diversity (Delgado-Baquerizo et al., 2016). However, among abiotic factors, the
347 changed soil pH is the dominant factor controlling microbial alpha-diversity on a global scale
348 (Delgado-Baquerizo et al., 2017; Zhou et al., 2020). Hence, global change factors may not
349 consistently reduce microbial alpha-diversity, as supported by our findings showing that microbial
350 alpha-diversity was unaffected under elevated ozone (Table 1). A recent meta-analysis showed that
351 shifts in microbial community structure rather than microbial alpha-diversity regulate the effects of
352 global change on ecosystem functioning (Zhou et al., 2020). Consistent with this, we found a link
353 between changes in microbial community composition and ecosystem multifunctionality (Fig. 5), with
354 changes in the relative abundance of some bacteria and fungi associated with crop growth, nutrient
355 cycling, and decomposition (Cesarano et al., 2017; Jin et al., 2020; Wang et al., 2019b).

356 As main decomposers, *Ascomycota* and *Basidiomycota* play a key role in the decomposition of
357 crop residues (Ma et al., 2013). Several studies have shown that organic inputs enrich the families
358 from the phylum *Ascomycota*, such as *Ascobolaceae* as the main groups of saprophytic fungi in plant
359 residues and *Lasiosphaeriaceae* that is positively correlated with the phenol oxidase activity (Bei et
360 al., 2018; Dang et al., 2021; Wang et al., 2019b). The current study found that some fungal groups
361 belonging to the phyla *Ascomycota* and *Basidiomycota* (*Ascobolaceae* and *Leptosphaeriaceae* in

maize as well as *Lasiosphaeriaceae*, *Pleosporaceae*, *Hygrophoraceae*, and *Schizoporaceae* in soybean) were significantly depleted under elevated ozone (Fig. 4), which is supported by the view that elevated ozone could reduce the release of dissolved organic compounds into the soil through diminishing root biomass and exudates (Andersen, 2003). In addition, *Davidiellaceae* effectively assimilates root exudates C and the increase in this family in maize is consistent with the ozone-induced increase in the ratio of root and shoot biomass in maize (Wang et al., 2019c) (Fig. 1a). Another explanation could be that maize releases more root exudates because of its larger fibrous root system (Gunina & Kuzyakov, 2015).

In the current study, the shoot and root biomass of the three crops showed a declining trend in the presence of elevated ozone (Fig. 1a). Concomitantly, elevated ozone reduced *Clavicipitaceae* in maize, *Trichocomaceae* in soybean, and *Perlucidibaca* in wheat, which were positively correlated with crop biomass and yield (Cesarano et al., 2017; Jin et al., 2020). On the contrary, ozone-induced higher *unassigned_Sebacinales* and *Mortierellaceae* in soybean may also be beneficial for plant production (Telagathoti et al., 2021; Weiß et al., 2011). These inconsistent findings reflect the complex interactions between soil microorganisms and plant growth, with these members presumably shown to be associated with plant growth stages (Chen et al., 2021). Besides, we found that *Perlucidibaca* in wheat responded negatively to elevate ozone and that this genus could promote soil N-cycling (Jin et al., 2020), which is supported by the ozone-induced reduction in the abundance of functional genes involved in soil N-cycling. Moreover, changes in the relative abundance of some members under elevated ozone could induce a wide range of feedback to plant health. For instance, elevated ozone resulted in a significant reduction of *Alkanindiges* in soybean, which is commonly used as an indicator of healthy plants (Santos & Olivares, 2021). Accordingly, *Thanatephorus*, a

genus of phytopathogenic fungi of the family *Ceratobasidiaceae* in soybean, was significantly improved under elevated ozone (Zhang et al., 2020). Overall, our study provides empirical evidence for the link between soil microbial communities and multiple ecosystem functioning under elevated ozone, with implications for tailoring strategies to maintain ecosystem multifunctionality.

5. Conclusions

In summary, this study showed that elevated ozone negatively affected the growth of crop seedlings (i.e., soybean, maize, and wheat) and the activity of soil microbial communities, although the responses varied among the different crops. Meanwhile, short-term ozone exposure led to a decrease in ecosystem multifunctionality. In addition, significant correlations between ecosystem multifunctionality and changes in microbial community composition were found. Elevated ozone significantly affected fungal community composition, while crop type was the primary driving factor of changes in microbial community composition. Field-based research is now needed to better assess the long-term effects of elevated ozone over successive cropping cycles and its impact on soil microbial communities and ecosystem functioning.

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Figure captions

Figure 1. Percent changes of **(a)** leaf photosynthetic pigments and biomass of wheat, maize, and soybean, **(b)** soil chemical properties, and **(c)** soil microbial biomass and extracellular enzyme activities in an agricultural soil under elevated ozone. Values are means ± 95% confidence intervals (*n* = 4). R/S ratio, the ratio of root and shoot biomass; DOC, dissolved organic carbon; DON, dissolved organic nitrogen; TDN, total dissolved nitrogen; MBC, microbial biomass carbon; BG, β-glucosidase; NAG, β-N-acetyl-glucosaminidase; LAP, leucine aminopeptidase; AP, acid phosphatase; N-*acq* and P-*acq* indicate ratios of ln(BG)/ln(NAG + LAP) and ln(BG)/ln(AP), respectively.

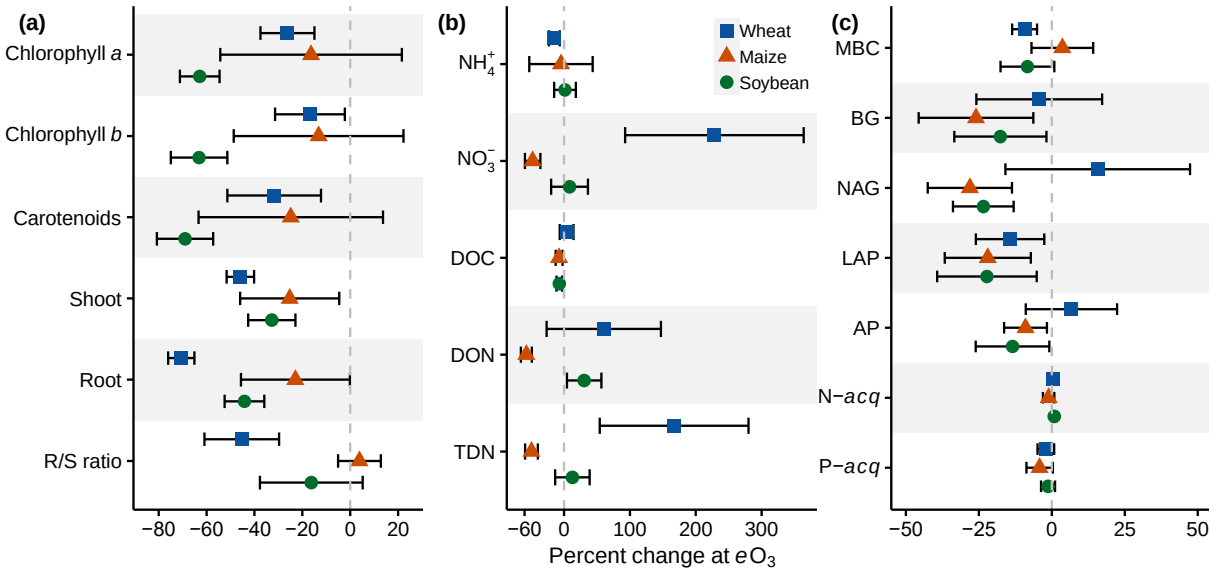

Figure 2. Percent changes of **(a)** the abundances of total bacteria, functional genes involved in N-cycling, and their ratios, and **(b)** the averaged responses of these across three crops in an agricultural soil under elevated ozone. Values are means ± 95% confidence intervals (n = 4).

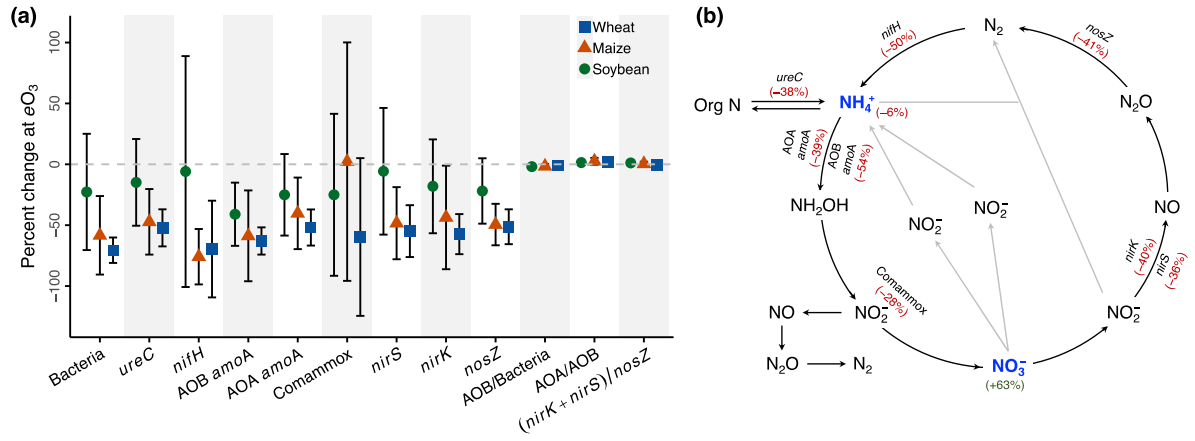

Figure 3. The relative abundance at the phylum level (top panel) and non-metric dimensional scaling (NMDS) ordination (low panel) of soil bacterial **(a, c)** and fungal communities **(b, d)** under three crops in an agricultural soil under ambient (aO_3) and elevated (eO_3) ozone. Bacteria phyla with a relative abundance lower than 0.1% were summarized with 'others.' Sample type presented the major driver of community variation. The percentage of variation on each axis refers to the explained fraction of total variation in the community.

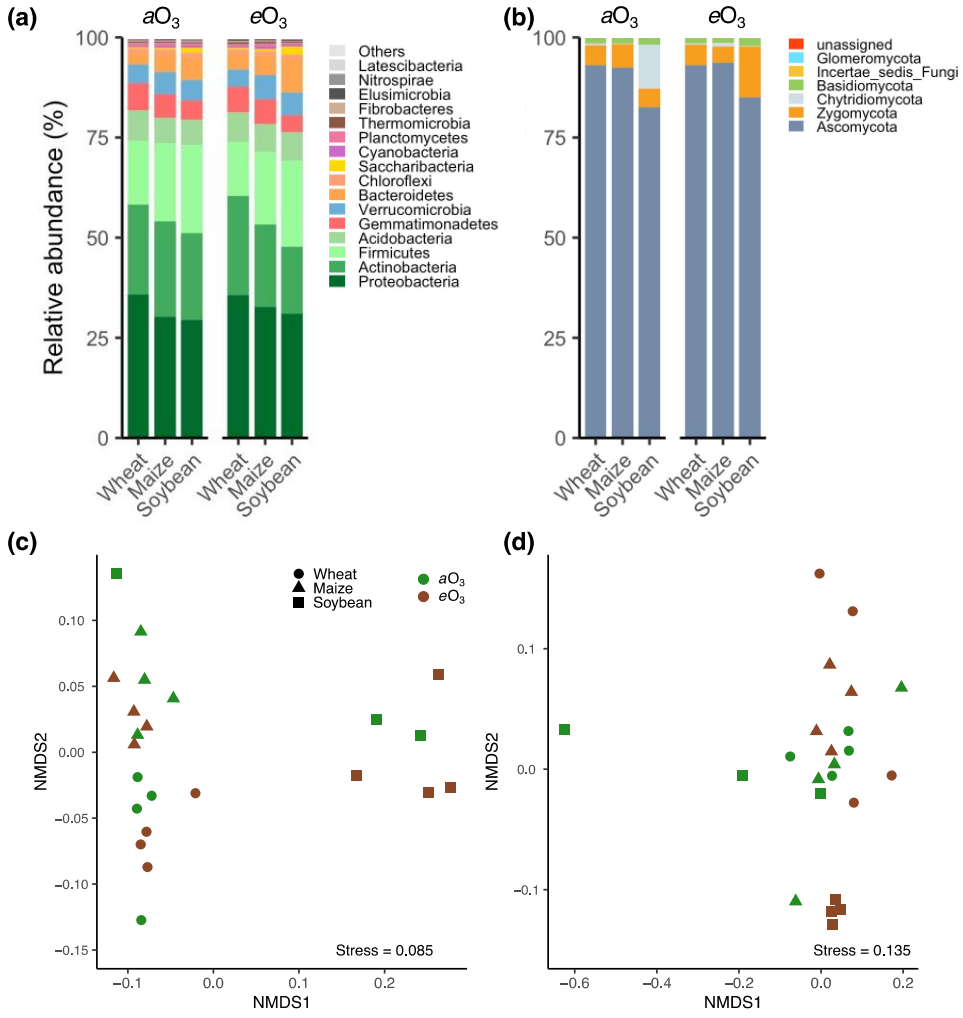

Figure 4. Enrichment and depletion of the OTUs from bacterial and fungal communities under elevated ozone in an agricultural soil under wheat, maize, and soybean crops. The size of the dots corresponds to the value of $\log_{10}$ averaged abundance (as counts per million, CPM).

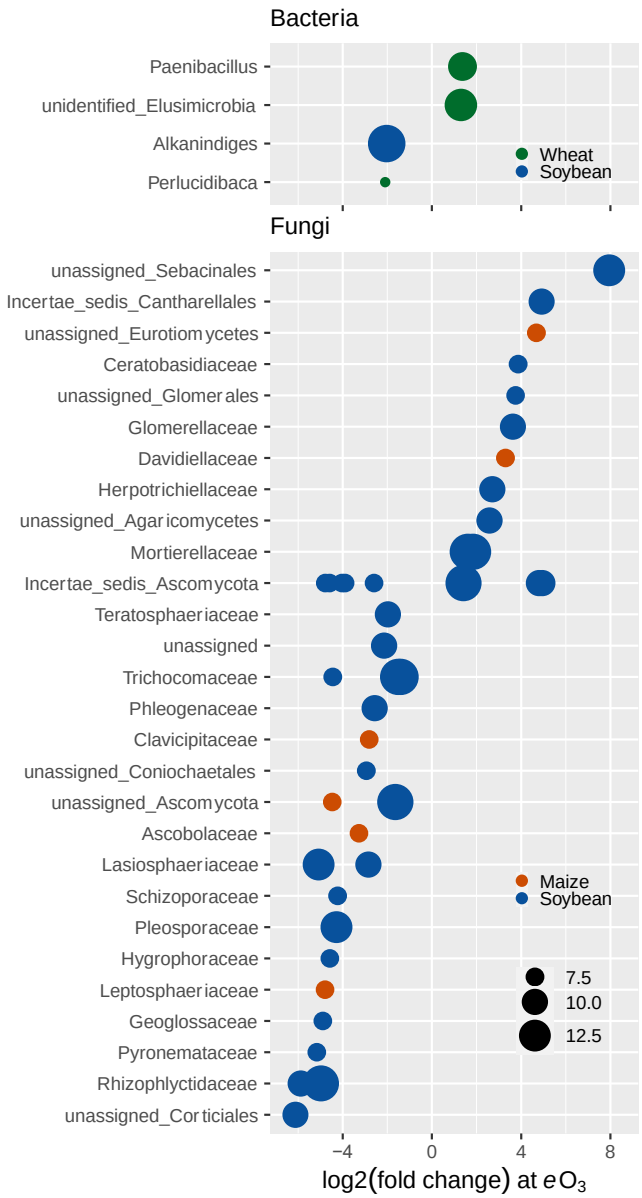

Figure 5. The relationship between ecosystem multifunctionality and non-metric multidimensional scaling (NMDS) coordinates of soil bacterial (top) and fungal communities (low) in the three crops.

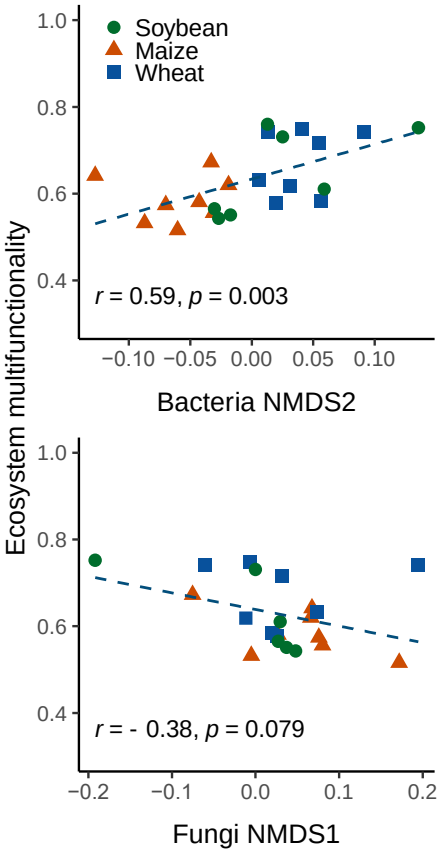

Table 1 Ecosystem multifunctionality and the alpha-diversity of the bacterial and fungal communities of wheat, maize, and soybean grown under either ambient or elevated ozone. Values represent means ± SEM (n = 4). *P*-values significant at the 0.05 level are marked in bold.

		Bacteria				Fungi				Ecosystem
		Observed				Observed				multifunctionality
Crop	Ozone	OTUs	Shannon	ACE	Chao1	OTUs	Shannon	ACE	Chao1	
Wheat	Ambient	3108±117	6.01±0.05	4345±174	4327±171	687±30	4.19±0.10	894±38	882±41	0.74±0.01
	Elevated	2913±42	5.95±0.04	4096±126	4045±108	678±16	4.22±0.06	856±25	859±26	0.60±0.01
Maize	Ambient	3115±61	6.05±0.02	4404±116	4414±110	711±22	4.31±0.04	937±21	921±31	0.63±0.02
	Elevated	3167±105	6.18±0.03	4555±223	4510±187	614±88	4.11±0.09	795±134	782±128	0.54±0.01
Soybean	Ambient	3494±246	6.16±0.18	4097±373	4914±350	723±41	4.10±0.24	940±45	933±45	0.75±0.01
	Elevated	3356±57	6.23±0.04	4641±129	4586±89	760±15	4.40±0.03	961±35	979±35	0.57±0.02
ANOVA										
Ozone		0.317	0.430	0.473	0.258	0.478	0.750	0.301	0.423	**<0.001**
Crop		**0.005**	**0.013**	**0.039**	**0.018**	0.183	0.782	0.353	0.221	**<0.001**
Interaction		0.494	0.338	0.474	0.409	0.330	0.077	0.465	0.369	**0.011**