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Legacy effects of elevated ozone on soil biota and plant growth

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ABSTRACT

Many studies have examined how human-induced atmospheric changes will influence ecosystems. The long-term consequences of human induced climate changes on terrestrial ecosystems may be determined to a large extend by how the belowground compartment will respond to these changes. In a free-air ozone enrichment experiment running for 5 years, we reciprocally transplanted soil cores from ambient and elevated ozone rings to test whether exposure to elevated ozone results in persistent changes in the soil biota when the plant and soil are no longer exposed to elevated ozone, and how these legacy effects of elevated ozone influenced plant growth as compared to current effects of elevated ozone. After one growing season, the current ozone treatment enhanced plant growth, but in soil with a historical legacy of elevated ozone the plant biomass in that soil was reduced compared to the cores originated from ambient rings. Current exposure to ozone increased the phospholipid fatty acids of actinomycetes and protozoa, however, it decreased dissolved organic carbon, bacterivorous and fungivorous nematodes. Interestingly, numbers of bacterivorous and fungivorous nematodes were enhanced when soils with a legacy of elevated ozone were placed under elevated ozone conditions. We conclude that exposure to elevated [O_3] results in a legacy effect in soil. This legacy effect most likely influenced plant growth and soil characteristics via responses of bacteria and fungi, and nematodes that feed upon these microbes. These soil legacies induced by changes in soil biotic community after long-term exposure of elevated ozone can alter the responses of ecosystems to current climatic changes.

1. Introduction

Tropospheric ozone [O_3] is recognized as an important damaging and widespread human-induced pollutant affecting agricultural and forest ecosystems (Nikolova et al., 2010; Galant et al., 2012), and poses a great threat to crop yields (Feng et al., 2008), forest productivity (Karnosky et al., 2007) and ecosystem carbon storage (Sitch et al., 2007). In the Northern Hemisphere, tropospheric [O_3] concentrations have increased from 10 ppb to 40 ppb currently (Biswas et al., 2008), and are predicted to further increase to 70–80 ppb by the year 2100 (Vingarzan, 2004; Zeng et al., 2008). Although the influence of elevated [O_3] has been studied widely from an aboveground perspective, relatively little attention has been paid to the effects of elevated [O_3] on the belowground subsystem (Andersen, 2003; Schrader et al., 2009; Chen et al., 2009; Li et al., 2012), whereas belowground responses might be critical in determining the long-term consequences of elevated [O_3] on terrestrial ecosystems (Andersen, 2003). Moreover, effects of enhanced [O_3] on soil conditions may result in long-lasting effects that can influence plant growth by altered resource availability and other abiotic and biotic mechanisms.

Recent work has shown that effects of climate change on soil may remain present even after climate change treatments have ceased (Nie et al., 2012; Meisner et al., 2013). These effects are called legacy effects (Baer et al., 2012). For example, Meisner et al. (2013) found that changes in soil biota induced by extreme weather events persisted in the soil after abiotic conditions had been reset and that this promoted later growing exotic plants while suppressing native ones. Similarly, Nie et al. (2012) reported that long-term elevation of CO_2 and temperature led to persistent

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changes in soil microbial communities that remained present when climate change treatments were terminated.

Legacy effects have been studied for [O_3] as well, but the focus so far has been on plants. For example, Andersen et al. (1997) showed that [O_3] effects on root growth and carbohydrate concentrations of ponderosa pine seedlings remained significant in seedlings even after the [O_3] treatment was ceased. Although soil biota play an important role in determining the responses of terrestrial ecosystems to climate change (Bardgett and van der Putten, 2014), whether exposure to elevated [O_3] also results in changes in the soil biota that remain present when the plant and soil are no longer exposed to elevated [O_3], and how such legacy effects of elevated [O_3] on soil biota may affect plant growth and its response to elevated [O_3] is still unknown.

In order to study the legacy effect of elevated [O_3] on soil biotic and abiotic characteristics, and how these effects influenced plant growth as compared to direct effects of elevated [O_3], we carried out a reciprocal transplant experiment with soil cores collected from rings with ambient and elevated ozone in a free-air ozone enrichment experiment (O_3-FACE) that has been running for 5 years. In each core, we grew wheat plants and determined microbial community structure, soil nematode community and soil physicochemical characteristics, and measured plant growth. We hypothesized that (1) effects of elevated [O_3] on soil biota and plant growth depend on the history of the soil, with more obvious treatment effects observed in the cores with a history of elevated [O_3], and (2) that the legacy of elevated [O_3] will influence the current effects of [O_3].

2. Materials and methods

2.1. Experimental site and design

The experiment was setup in an O_3-FACE experiment, located in a suburb of Jiangdu city in Jiangsu province of China (32°35′ N, 119°42′ E). The soil at the study site is a Shajiang-Aquic Cambosols (Typic Endoaquepts, FAO) with a sandy–loamy texture, with 15 g kg^{-1} total C, 1.5 g kg^{-1} total N, pH 6.8, 25.1% clay (<0.001 mm) and bulk density 1.2 g cm^{-3} at 0–15 cm depth (Zhu et al., 2011). The climate conditions are temperate with an average annual temperature and precipitation of 14.9 °C and 980 mm, respectively. An O_3-Free air enrichment (FAOE) experiment was established in 2007 in a rice–wheat rotation system. Rice was transplanted in mid-June and harvested in mid-to-late October. Winter wheat was sown in early November and harvested in late May or early June of the next year. Rice/wheat straw from the previous season was incorporated in the soil in which the rice/wheat was growing. No additional organic matter was incorporated during the wheat growing seasons.

Three replicate O_3-FAOE rings, each with 14.5 m in diameter, were installed at random sites within a uniform area of 4 ha to continuously provide an elevated level of [O_3] of 60 ppb from 9:00 am to 18:00 pm (this setup is hereinafter referred to as E-O_3). A mixed gas consisting of about 5% O_3 and 95% O_2 was produced at about 50 cm above the canopy height by an O_3 generator (KCF-BT0.2, Jiangsu Koner Ozone Co. Ltd, Yangzhou, China). The O_3 concentration was released on the basis of the wind direction and wind speed to achieve the elevation of O_3 within ±15% of the set point for 90% time and was measured at the center of each plot every 20s by an O_3 analyzer (Thermo Environmental Instruments, Franklin, MA, USA). Three other rings of the same size were supplied with ambient air (about 40 ppb) (hereafter referred to as A-O_3). The rings were located at 70 m distance from each other to prevent ozone spilling from one ring to another. Each ring (plot) was split into four subplots planted with four winter wheat cultivars (*Triticum aestivum* L.): Yangfumai 2 (Y2), Yannong 19 (Y19), Yangmai 15 (Y15) and Yangmai 16 (Y16). Nitrogen was applied as urea (N = 46%) and di-ammonium phosphate at a total rate of 210 kg N ha^{-1}, and was split into a basal application at planting (60%), an application at early tillering (10%), and one at elongation stage (30%). P and K were applied as di-ammonium phosphate and potassium chloride, respectively, at a rate of 90 kg P_2O_5 ha^{-1} and 90 kg K_2O ha^{-1}, which were split-applied with 60% at planting and 40% at elongation stage, respectively (Zhu et al., 2011). The current experiment was conducted during the wheat growing season of 2013, after the treatments had been running for 5 years.

In order to determine the legacy effect of elevated [O_3] on soil biotic and abiotic characteristics and its influence on plant growth, we transplanted ambient soil cores (a) to elevated rings (E) and elevated cores (e) to ambient rings (A). Each soil core (15 cm-diameter and 15 cm-depth) was kept intact while being removed from the soil by a shovel and placed into a separate plastic tube (15 cm-diameter and 15 cm-height). In each ring, there were also soil cores from the ring itself, which were dug out, placed in a tube, and placed back in the ring of origin. In each ambient ring, for each cultivar there was a core with ambient soil from that ring (Aa), and a core with soil from an elevated ring (Ae), vice versa for the E-O_3 rings (Ea and Ee). These cores were protected from the surrounding soil by the plastic tube and embedded into the soil. We had four wheat cultivars, each being planted in soil cores filled with soil collected from areas where that cultivar was grown during the previous season. In the current season, all cultivars in tubes were placed in the subplot with that same cultivar. This resulted in 4 (wheat cultivars) × 2 (ambient core + elevated core) = 8 cores in each ring. Before planting, soil biotic and abiotic characteristics (soil moisture, soil pH, total carbon and nitrogen, microbial biomass C and N and microbial (PLFA) and nematode community) from A-O_3 and E-O_3 rings were measured as background information.

Seeds of the four wheat cultivars were obtained from Yangzhou Agricultural University. Seven seeds of a particular cultivar were sown in each core on 14th of November in 2012. At the end of February, for all cores seedlings were thinned to four per core. For one core less than four seedlings survived, additional seedlings were planted that originated from the same ring but from outside the plastic tube. Fertilizers in the cores were applied at the same rate as in the E-O_3 rings. Agricultural management in the cores was also the same. At ripening stage (8th of June in 2013), five soil samples of 2.5 cm diameter and 15 cm deep were collected from each core and combined so that a composite soil sample of each soil core was obtained. At this time the cores had been exposed to the treatment for one growing season. All above-ground biomass was harvested, and roots were rinsed. Soil samples were stored at 4 °C until further analyses.

2.2. Soil and plant analyses

The total carbon (C) and nitrogen (N) in plant biomass and soil were determined by a TruSpec CN Elemental Analyzer (Leco Corporation, USA). Dissolved organic carbon (DOC) was determined using Multi N/C 3100 analyzer (Jena Corporation, Germany). Soil inorganic N (NO_3^-–N and NH_4^+–N) was extracted with 2 M KCl, and then the filtrates were determined using a flow injection auto analyzer (FIAstar 5000 Analyzer; Foss Tecator, Hillerød, Denmark). Soil microbial biomass was determined using the fumigation–extraction method (Brookes et al., 1985; Vance et al., 1987). Both soil microbial biomass C (MBC) and N (MBN) in the filtrate were determined using a Multi C/N 3100 analyzer (Jena Corporation, Germany). Soil basal respiration was determined using static alkali absorption method. Soil pH was determined with a glass electrode in 1:2.5 soil:water solution (w/v). Wheat plants from each core were partitioned into grain and litter. Litter, grain and

root samples were dried at 65 °C until a constant weight was obtained, and then weighed.

2.3. PLFA analysis

The soil microbial community was characterized using phospholipid fatty acid (PLFA) analysis as described by Bligh and Dyer (1959). Specific modifications, GC conditions and nomenclature were as described by Certini et al. (2004). Briefly, 8 g freeze-dried soil was extracted with a chloroform–methanol–citrate buffer mixture (1:2:0.8), and the phospholipids were separated from neutral lipids and glycolipids on a SPE tube (Supelco Inc., Bellefonte, PA). The phospholipids were trans-esterified to a mild alkaline methanolysis (Bossio et al., 1998) and the resulting fatty acid methyl esters were extracted in hexane and dried under N_2. Samples were then dissolved in hexane and analyzed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE).

The following biomarkers were used: gram-positive bacteria (i14:0, i15:0, a15:0, i16:0, i17:0 and a17:0), gram-negative bacteria (16:1ω7c, 16:1ω9c, 16:1ω11c, 16:1 2OH, cy17:0, 18:1ω7c, 19:1ω9c, and cy19:0) (Bach et al., 2010). The sum of gram-positive bacteria, gram-negative bacteria and non-specific bacteria (14:0, 15:0, 16:0, 16:0 N alcohol, 17:0 and 18:0) was used as measure of bacterial biomass (Dierksen et al., 2002). The fatty acid 18:2ω6c was used as indicator of fungal biomass (Bååth and Anderson, 2003) and 16:1ω5c for arbuscular mycorrhizal fungi (AMF) (Bach et al., 2010), the ratio of 18:2ω6c to total bacterial PLFAs (F:B) was used to analyze changes in the microbial community structure (Bååth and Anderson, 2003). The sum of fatty acids 20:4ω6, 9, 12, 15c were used as indicators for protozoa and methyl branched fatty acids (10Me PLFAs) were used for actinomycetes (Briar et al., 2011). The PLFAs indicated above were considered to be representative of the total PLFAs of soil microbial community. Concentration (nmol/g dry soil) and mol percentage of each PLFA were calculated for further analysis.

2.4. Nematode community analysis

Nematodes were extracted from 100 g of fresh soil by a modified cotton-wool filter method (Oostenbrink, 1960; Townshend, 1963). Nematode populations were expressed as number of nematodes per 100 g dry soil and at least 100 nematodes from each sample were identified to genus level. The nematodes were assigned to the following feeding groups (1) bacterivores; (2) fungivores; (3) omnivore–carnivores and (4) plant parasites, following Yeates et al. (1993).

2.5. Statistical analysis

Nematode abundances were ln (x + 1) transformed prior to statistical analysis. Soil characteristics not meeting assumptions of normality and homogeneity of variance were log-transformed before statistical analysis. To test the main effects and interactions of current and legacy effects of elevated [O_3] on soil properties and biota, a linear mixed effect model was used with O_3 and legacy effects as fixed factors and ring identity and cultivars as random factors using the R package 'nlme' (Pinheiro et al., 2012). Differences at $P < 0.05$ levels were considered statistically significant. Multivariate analyses were analyzed using CANOCO 4.5 (Ter Braak and Šmilauer, 2002). Soil abiotic characteristics were standardized (using the "standardize species" option) before unconstrained principal component analysis (PCA). Abundances of nematode genera were square-root transformed before PCA. The relative abundance of each PLFA was log-transformed before the unconstrained and constrained ordinations (McKinley et al., 2005). In the final PCA plots of PLFAs and nematode genera only "species" with fits higher than 20% are presented. To better highlight the links between soil/plant characteristics and soil biota, constrained redundancy analyses (RDA) were conducted to determine the current and legacy effects of elevated [O_3]. Wheat cultivar identities were entered in the model as the dummy covariables. Significance was based on permutation test using 999 permutations and using a

Table 1
Basic soil characteristics and biotic properties in the elevated [O_3] and ambient rings (values represent means ± SE) at the start of the experimental season, and *F*-values from a linear mixed effect model. * and ** indicate significant at $P < 0.05$ and $P < 0.01$ level, respectively.

	Ambient [O_3]	Elevated [O_3]	*F* values
Soil moisture (%)	0.39 ± 0.01	0.38 ± 0.01	0.20
pH	5.71 ± 0.05	5.80 ± 0.06	3.78
Total N (g/kg)	1.63 ± 0.02	1.66 ± 0.02	1.60
Total C (g/kg)	16.44 ± 0.21	16.72 ± 0.20	1.64
C/N ratio	10.06 ± 0.05	10.10 ± 0.05	1.15
MBC (mg/kg)	366.37 ± 24.84	347.90 ± 21.37	0.73
MBN (mg/kg)	24.65 ± 1.76	21.93 ± 1.41	1.94
Total PLFA (nmol/g)	196.30 ± 6.26	201.48 ± 4.56	0.58
Bacterial PLFA (nmol/g)	149.40 ± 4.74	153.92 ± 3.51	0.77
Fungal PLFA (nmol/g)	5.30 ± 0.23	5.02 ± 0.17	0.97
Fungi/Bacteria ratio	0.04 ± 0.00	0.03 ± 0.00	5.90*
Total nematodes	592.00 ± 46.00	864.00 ± 63.00	8.40**
Bacterivores	278.00 ± 27.00	413.00 ± 33.00	8.04**
Fungivores	91.00 ± 14.00	109.00 ± 17.00	0.06
Plant-parasites	103.00 ± 14.00	119.00 ± 16.00	0.38
Omnivore–predators	120.00 ± 10.00	223.00 ± 63.00	8.45**

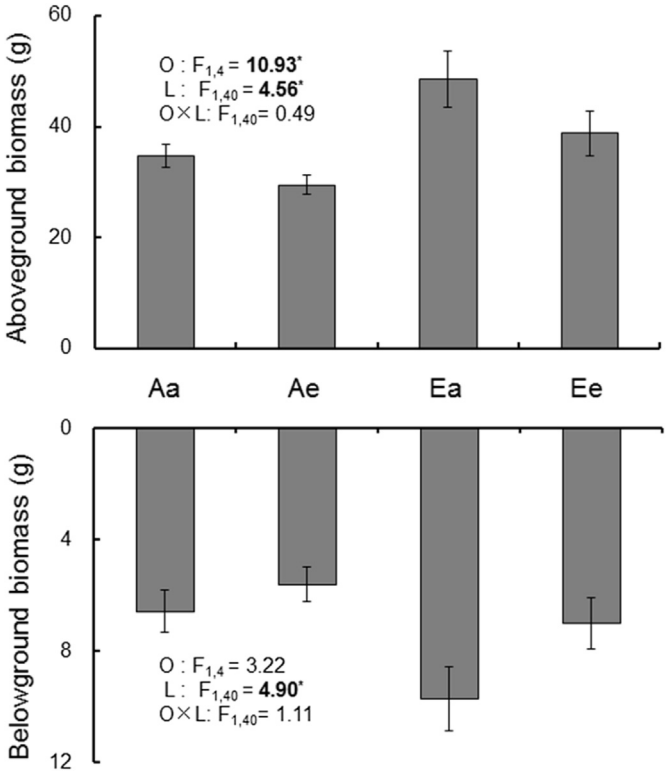

Fig. 1. Current (O) and legacy (L) effects of elevated [O_3] on above and belowground biomass. Means ± 1 standard error are shown for cores placed in rings with ambient (A) and elevated (E) [O_3] and originating from rings that had previously been exposed to ambient (a) or elevated (e) [O_3] conditions. *F* values from a linear mixed effect model are also presented; * indicates treatment effects significant at $P < 0.05$.

split-plot design. The current [O_3] treatments were treated as whole plots and the legacy [O_3] treatments as the split-plots. The number of split-plots in each whole-plot was 8. When we tested the current effect of elevated [O_3], the whole-plot was permuted 'freely exchangeable' and the split-plot was not permuted. When we tested the legacy effect of elevated [O_3], the split-plot was permuted 'freely exchangeable' and the whole-plot was not permuted. We tested the current and legacy effects of [O_3] (dummy variables), soil parameters (soil moisture, pH, DOC, NO_3^-–N, NH_4^+–N) and plant parameters (above and belowground biomass, root C and N, the C/N of root), separately. Soil and plant parameters were tested with unrestricted permutations. The current and legacy effects of elevated [O_3] were also tested with soil and/or plant characteristics included as covariables to determine whether the current and legacy effects of elevated [O_3] could be explained by changes in soil or plant characteristics (Lepš and Šmilauer, 2003).

3. Results

3.1. Initial soil conditions at the start of the experiment

Five years of exposure of elevated [O_3] did not lead to significant differences in the measured soil characteristics and total PLFAs between ambient and elevated [O_3] conditions. But the ratio of fungi to bacteria was lower in the elevated [O_3] rings than in the ambient rings ($P < 0.05$). On the contrary, the abundances of total nematodes, bacterivores and omnivores–predators were higher in

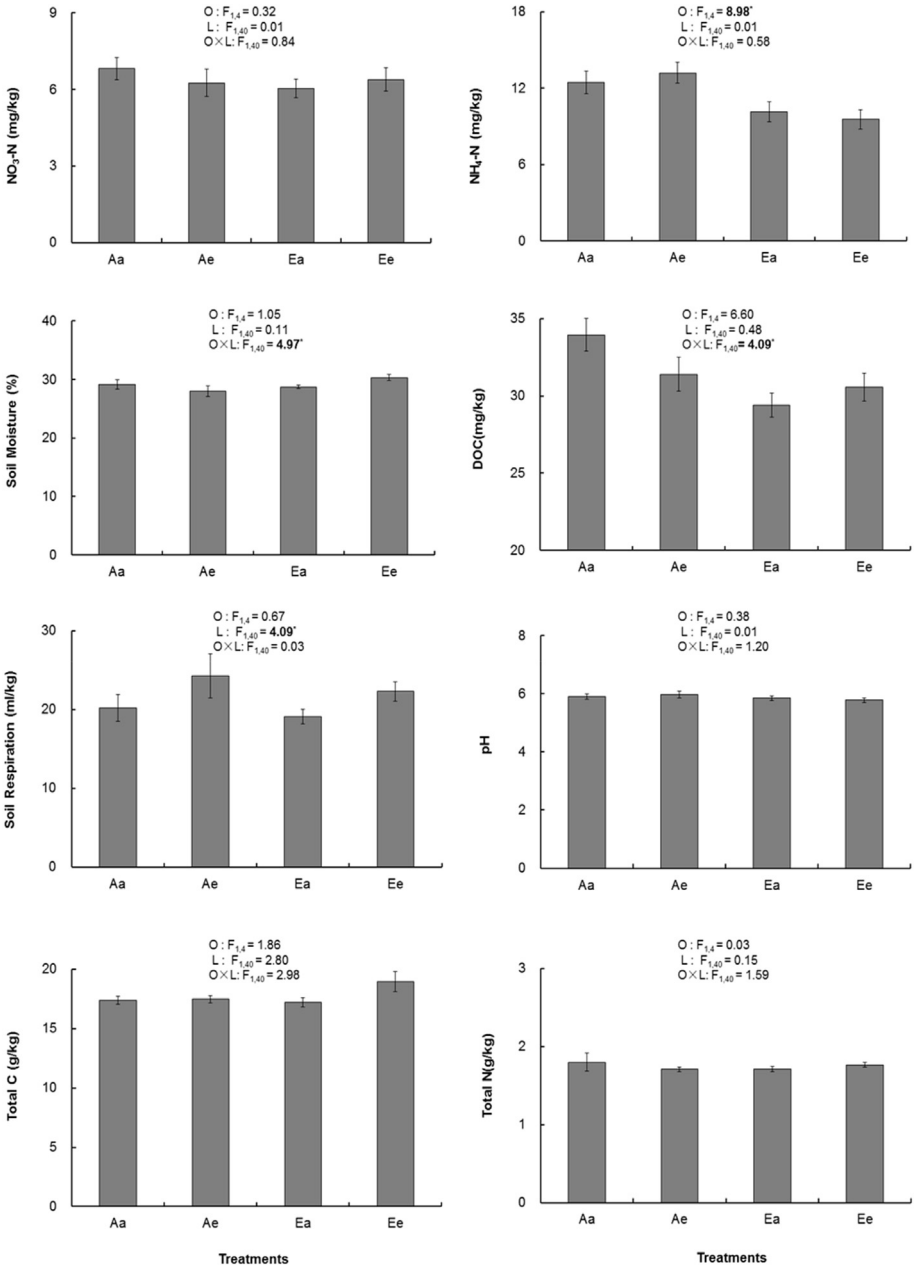

Fig. 2. Current (O) and legacy (L) effects of elevated [O_3] on soil characteristics. Means ± 1 standard error are shown for cores placed in rings with ambient (A) and elevated (E) [O_3] and originating from rings that had previously been exposed to ambient (a) or elevated (e) [O_3] conditions. *F* values from a linear mixed effect model are also presented; * indicates treatment effects significant at $P < 0.05$.

the elevated [O_3] rings than in the ambient rings ($P < 0.01$) (Table 1).

3.2. Effects on plant growth

After one growing season, elevated [O_3] enhanced aboveground crop biomass (Fig. 1). However, in cores originating from elevated [O_3] rings, above and belowground biomass were lower than in cores originating from ambient [O_3] rings (ozone legacy effect; $P < 0.05$); this was the case both for plants growing at current conditions of ambient and of elevated [O_3] (Fig. 1). No significant effects of current [O_3] treatment or legacy effects of previous [O_3] treatment were observed on the C and N content of shoot or root tissues (Supplement Fig. S1).

3.3. Effects on soil characteristics

There was a significant interaction between current [O_3] treatment and the legacy effect of previous [O_3] treatment for both soil moisture and DOC. In rings with ambient [O_3], soil moisture and DOC content were lower in cores originating from elevated [O_3] rings than in cores originating from ambient rings, while an opposite effect was observed in rings with elevated [O_3]. Soil respiration was significantly higher ($P < 0.05$) in cores originating from elevated [O_3] rings than in cores originating from ambient rings (Fig. 2). There was less NH_4^+–N in rings with elevated [O_3] than in rings with ambient conditions ($P < 0.05$). A PCA of all soil characteristics also indicated that the legacy effects of previous [O_3] treatment differed under current ambient and elevated [O_3] conditions. In the PCA the current [O_3] treatments clearly separated, while the position of the two legacy [O_3] treatments was reversed in current ambient and elevated [O_3] conditions (Fig. 3a).

3.4. Effects on soil biota

There was a trend of more PLFA subgroups under current elevated [O_3] conditions, and this was significant for PLFAs of protozoa and actinomycetes (Table 2). A significant legacy effect of [O_3] was observed for G^+ bacteria ($F_{1,40} = 4.56$, $P = 0.04$), with higher amounts of G^+ bacteria observed in cores originating from elevated [O_3] rings than in cores originating from ambient rings under both current ambient and elevated [O_3] conditions. In a PCA of all PLFAs combined the first axis discriminated between current ambient and elevated [O_3] conditions, while the soil legacy treatments were separated on the second axis (Fig. 3b).

The abundances of total nematodes (Fig. S2), as well as of bacterivorous and fungivorous nematodes, were influenced by an interaction between the current [O_3] treatment and the legacy effects of previous [O_3] treatment. Under ambient conditions, the abundance of bacterivorous and fungivorous nematodes was lower in cores originating from elevated [O_3] rings than in cores originating from ambient [O_3] rings. However, under current conditions of elevated [O_3] the abundance was higher in soil originating from elevated [O_3] rings (Fig. 4). The abundance of plant-parasitic nematodes tended to be reduced by current conditions of elevated [O_3], but this was not significant, which might be due to low number degrees of freedom (3 rings per treatment). For omnivorous and predatory nematodes, no obvious treatment effects were observed. A PCA analysis of the entire nematode community also clearly separated the four [O_3] treatments (Fig. 3c).

3.5. Relationship between soil biota and soil/plant characteristics

RDA analyses showed that the explained variation in PLFA composition by [O_3] legacy effects on soil remained significant when soil and/or plant parameters were included as covariables (Table 3). For nematode communities after removing variation explained by plant characteristics, the legacy effect of previous [O_3] exposure explained a small but significant amount of the variation

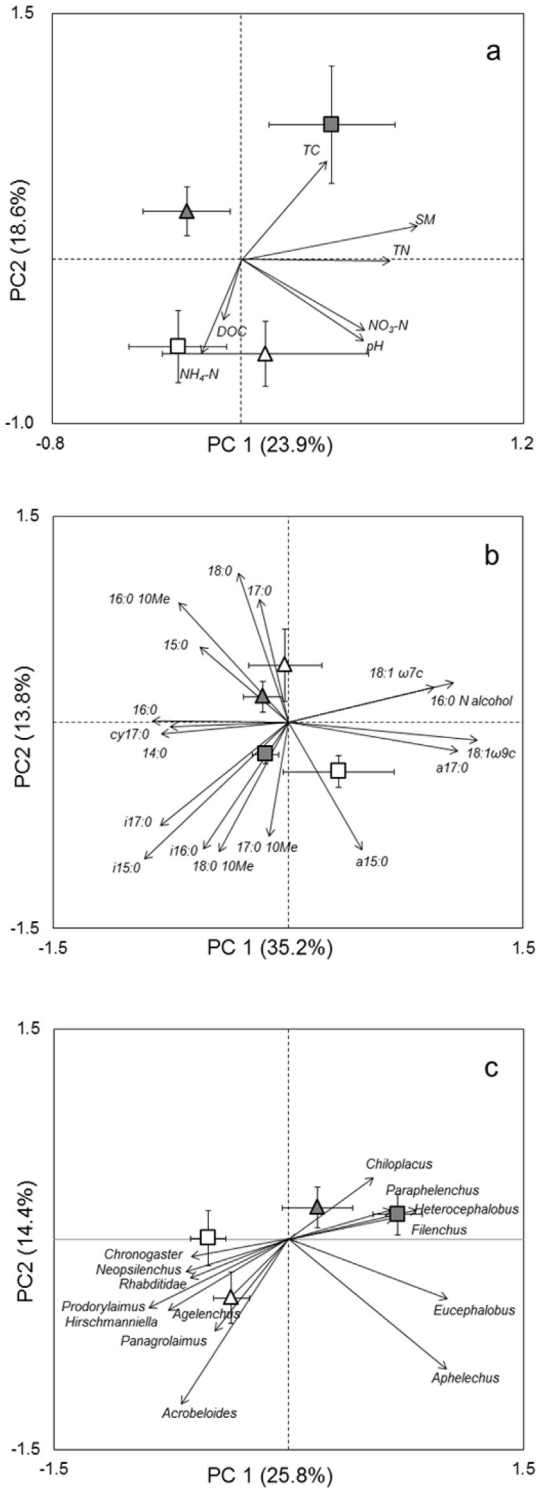

Fig. 3. PCA analysis of soil characteristics (**a**), microbial community (**b**), and nematode community composition (**c**) for cores placed in rings with current ambient (white symbols) and elevated (gray symbols) [O_3] conditions (direct [O_3] effect) and originating from rings that had been previously exposed to ambient (triangles) or elevated (squares) [O_3] conditions ([O_3] legacy effect). Means ± 1 standard error are shown (n = 3 rings).

Table 2
Current (O) and legacy (L) effects of elevated [O_3] on soil microbial community composition. Means (±SE) are shown for cores in ambient (A) and elevated (E) [O_3] and originating from rings that had been exposed to ambient (a) or elevated (e) [O_3] conditions. *F*-values from a linear mixed effect model are also presented. * indicates significant at $P < 0.05$ level.

PLFA (nmol/g)	Aa	Ae	Ea	Ee	*F*-values		
					O	L	O × L
Total PLFA	208.20 ± 11.85	221.25 ± 15.07	248.54 ± 7.82	260.83 ± 14.68	5.15	0.99	0.01
Fungal	6.41 ± 0.55	6.78 ± 0.75	7.39 ± 0.27	7.69 ± 0.44	1.30	0.47	0.01
AMF	6.23 ± 0.34	6.68 ± 0.51	7.52 ± 0.40	7.58 ± 0.49	1.95	0.42	0.24
Protozoa	2.97 ± 0.31	2.51 ± 0.31	3.58 ± 0.16	3.72 ± 0.17	8.58*	0.41	1.57
Actinomycetes	21.76 ± 1.35	22.59 ± 1.19	26.20 ± 0.83	27.34 ± 1.58	11.32*	0.60	0.02
Bacterial G^+	47.61 ± 2.71	54.99 ± 3.36	57.00 ± 2.12	62.59 ± 3.85	5.90	4.56*	0.09
Bacterial G^-	60.38 ± 3.86	62.09 ± 4.51	73.79 ± 2.97	74.80 ± 4.37	4.89	0.13	0.01
Bacterial	156.38 ± 7.98	166.25 ± 10.82	185.88 ± 6.08	195.47 ± 11.04	5.85	1.20	0.01

(Table 3). The current [O_3] effect explained about 10% of the variation in nematode community composition and this was significant when soil and/or plant parameters were included as covariables. The soil and plant parameters explained 20.5% and 15.4% of the variation in microbial community composition, and 13.0% and 11.4% of nematode composition, respectively.

4. Discussion

Our results show that there are legacy (or carry-over) effects of previous [O_3] exposure on soil biotic and abiotic characteristics, which influence subsequent plant growth. Importantly, the current [O_3] treatment and legacy effects of previous [O_3] exposure had opposite effects on plant growth. Moreover, the direction of the effects of the current [O_3] treatment on soil nematodes and soil abiotic characteristics depended on whether the soil had previously been exposed to elevated [O_3] or not. The potential importance of climate change-induced soil legacy effects has recently been identified in microcosm experiments (Nie et al., 2012; Meisner et al., 2013). Our study, which was carried out in the field and examined for the first time legacy effects of elevated [O_3], now shows that effects of previous exposure to climate change that remain in the soil, can alter the responses of ecosystems to current climatic changes.

In the present study our results demonstrate that exposure to elevated [O_3] can cause a soil legacy effect that led to a decrease in above- and belowground biomasses of plants growing in that soil. These legacy effects on plant growth can be either due to [O_3] induced changes in the abiotic or in the biotic characteristics in the soil. However, at the start of the experimental season, significant changes were only found in soil biotic characteristics. Therefore our results suggest that these legacy effects were induced by changes of soil biota. Previous findings have observed that soil biotic legacy effects can influence plant invasiveness (Meisner et al., 2013) and plant growth by modifying aboveground multitrophic interactions (Kostenko et al., 2012). Other studies in the same experimental field suggested that elevated ozone can influence functional genes of the soil microbial community (Li et al., 2013) and the soil nematode community (Li et al., 2012). Our current study now shows that these responses of the soil biotic community after long-term exposure to

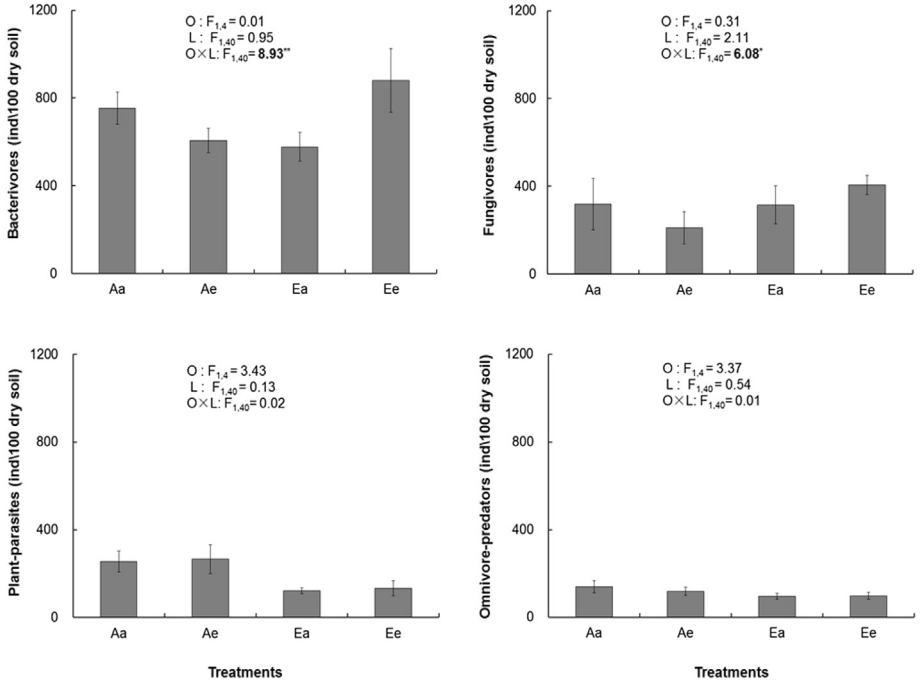

Fig. 4. Current (O) and legacy (L) effects of elevated [O_3] on soil nematode trophic groups. Means ± 1 standard error are shown for cores placed in rings with ambient (A) and elevated (E) [O_3] conditions and originating from rings that had previously been exposed to ambient (a) or elevated (e) [O_3] conditions. *F* values from a linear mixed effect model are also presented; *,** indicate treatment effects significant at $P < 0.05$ and $P < 0.01$.

Table 3
Results of redundancy analysis (RDA) of soil and plant characteristics and the current and legacy effects of elevated O_3 on microbial (PLFA) and nematode community composition. The current and legacy effects of elevated O_3 are also tested with soil and/or plant parameters included as covariables (indicated by /). Explained variance is based on the sum of all canonical eigen values. *P*-values are based on a Monte-Carlo permutation test with 999 permutations, and restricted for split-plot design. Signifcant differences were labelled with bold.

Explanatory variables	Microbial community			Nematode community		
	F-value	*P*	% Explained	*F*-value	*P*	% Explained
Soil characteristics	**2.13**	**<0.01**	**20.5**	1.26	0.08	13.0
Plant characteristics	1.51	0.07	15.4	1.09	0.28	11.4
Current O_3 effect	2.13	0.48	4.5	5.12	0.10	9.9
O_3/soil	2.11	0.05	3.9	**4.57**	**0.01**	**8.6**
O_3/plant	1.24	0.36	2.5	**3.82**	**<0.01**	**7.5**
O_3/(soil + plant)	1.20	0.11	2.2	**4.03**	**0.01**	**7.6**
Legacy O_3 effect	**2.55**	**0.01**	**5.3**	1.10	0.18	2.3
Legacy/soil	**2.96**	**0.01**	**5.4**	1.09	0.24	2.2
Legacy/plant	**1.86**	**0.04**	**3.7**	**1.51**	**0.04**	**3.1**
Legacy/(soil + plant)	**2.28**	**0.04**	**4.1**	**1.64**	**0.02**	**3.3**

elevated [O_3] can remain present and feedback to plant growth during the next season. We do not currently know what the mechanisms behind these soil legacies via soil biotic changes are. However, we speculate that they are mediated by competition between plants and the soil microbial community for nutrients. Several studies have argued that depending on the current availability of nutrients in the soil, an increase in microbial activity indicated in our study by higher soil respiration can influence plant growth negatively via increases in the nutrient demand of the soil microbial community (Dunn et al., 2006; Van der Heijden et al., 2008).

Many studies have shown that exposure to elevated [O_3] negatively affects plant growth (Ainsworth et al., 2012). In contrast, in our study exposure to [O_3] of 60 ppb for one growth season increased aboveground biomass of wheat and PLFAs of microbial biomass compared to a control level of 40 ppb. The increase in plant growth and microorganisms may result in a decrease in soil NH_4^+–N through higher N uptake under elevated [O_3]. Similar stimulatory effects of elevated [O_3] on plant growth and the soil microbial community have been reported in a few other studies (Phillips et al., 2002; Grantz et al., 2006; Pregitzer et al., 2008). Nikolova et al. (2010), for example, reported that the fine root production of beech was stimulated by elevated [O_3] in a relatively wet year. We speculate that under continuous elevated [O_3], the soil subsystem will adapt. When the soil subsystem has evolved several years under elevated [O_3], these changed soil conditions (through biotic and/or abiotic characteristics) will be less supportive for plant growth. Therefore these short-term positive effects of elevated [O_3] on wheat biomass in our study were counteracted in soils that had been exposed to elevated [O_3] for a longer time. Further studies on elevated [O_3] should consider that the effects of elevated [O_3] may only become apparent after the legacy of elevated [O_3] have become operational. This has important implications for the duration of climate change experiments.

The legacy effects induced by previous exposure to elevated [O_3] changed the direction of the effect of the current [O_3] treatment on the abundance of soil microbivorous nematodes, which was consistent with our hypothesis that the legacy effects of elevated [O_3] influence the current effects of elevated [O_3]. These legacy effects of elevated ozone on soil nematode communities may subsequently influence the effects of current climatic conditions on soil abiotic and biotic properties. One possible explanation for these contrasting effects of the current [O_3] and legacy treatments is that there were historical differences between the soil treatments that remained in the soil until the next growth season. At the beginning of the experiment, nematodes trophic groups, such as bacterivores and omnivores–predators were more abundant in cores originating from elevated [O_3] rings than in those originating from the ambient rings. Thus, it may take some time for the soil subsystem to adapt to the new conditions under current ambient or elevated [O_3] conditions. This may lead to differences in responses between soil cores originating from the ambient rings and from the elevated [O_3] rings. For the cores originating from the ambient rings, the lower levels of DOC (in Ea) may negatively affect the microbial community and then lead to the decrease of the abundance of microbivorous nematodes (Neher et al., 2004). Beyond the context of our study this would suggest that legacy effects of the climatic changes can be memorized by the soil biota, and that this can influence the responses of soil ecosystems to current climatic changes. These legacy effects of ozone on soil biotic and abiotic conditions need to be taken into consideration explicitly when making predictions of long-term effects of ozone on soil biodiversity and functioning from short-term ozone exposure experiments.

At the start of the current experiment, significant differences were found in bacterivores and omnivores–predators between ambient and elevated [O_3] conditions. After the growth season, we did not observe current or legacy effects of elevated [O_3] on the omnivore–predators. The treatment effects only operated at the lower trophic levels of food web, such as microbes and microbivorous nematodes. It may take more time before the treatment effects are expressed at the higher levels of the soil food web. A similar finding was reported by Scherber et al. (2010) who observed that plant diversity affected the lower trophic levels of the soil food web more than the higher trophic levels. Further studies are needed to reveal how bottom-up and top-down effects influence these responses and how changes in soil biota may interact with changes in abiotic soil properties, such as altered DOC levels.

5. Conclusion

In conclusion, we observed that the current effect of elevated [O_3] on plant growth depended on whether the soil originated from an environment that had been exposed to elevated [O_3] for a longer time or not. Our results show that long-term exposure to elevated [O_3] results in changes in soil biotic community, which have a feedback effect on plant responses to current atmospheric [O_3]. Our findings are important as they emphasize that responses of terrestrial ecosystems to elevated [O_3] may operate via complex interplays of plants and abiotic, as well as biotic soil components. These soil biotic changes can be remembered and alter the responses of ecosystems to current or future climatic changes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2015.08.029.

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