

## ECOLOGY

# Ozone affects plant, insect, and soil microbial communities: A threat to terrestrial ecosystems and biodiversity

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Elevated tropospheric ozone concentrations induce adverse effects in plants. We reviewed how ozone affects (i) the composition and diversity of plant communities by affecting key physiological traits; (ii) foliar chemistry and the emission of volatiles, thereby affecting plant-plant competition, plant-insect interactions, and the composition of insect communities; and (iii) plant-soil-microbe interactions and the composition of soil communities by disrupting plant litterfall and altering root exudation, soil enzymatic activities, decomposition, and nutrient cycling. The community composition of soil microbes is consequently changed, and alpha diversity is often reduced. The effects depend on the environment and vary across space and time. We suggest that Atlantic islands in the Northern Hemisphere, the Mediterranean Basin, equatorial Africa, Ethiopia, the Indian coastline, the Himalayan region, southern Asia, and Japan have high endemic richness at high ozone risk by 2100.

## INTRODUCTION

Above- and belowground trophic interactions play pivotal roles in maintaining plant diversity. Plants respond to herbivores by various physiological mechanisms, affecting plant performance and plant-microbe interaction and potentially regulating ecosystem processes and community dynamics (1–3). Plant-soil feedbacks (PSFs) likewise involve interactions among plants, soil microbiota, and abiotic factors, affecting structural and functional features at different scales of biological organization. These effects allow plants to readily respond to environmental changes and mediate ecosystem processes (4). Trophic interactions depend on environmental conditions, so

changes in the environment may affect biodiversity and the functioning of terrestrial ecosystems (2, 4, 5).

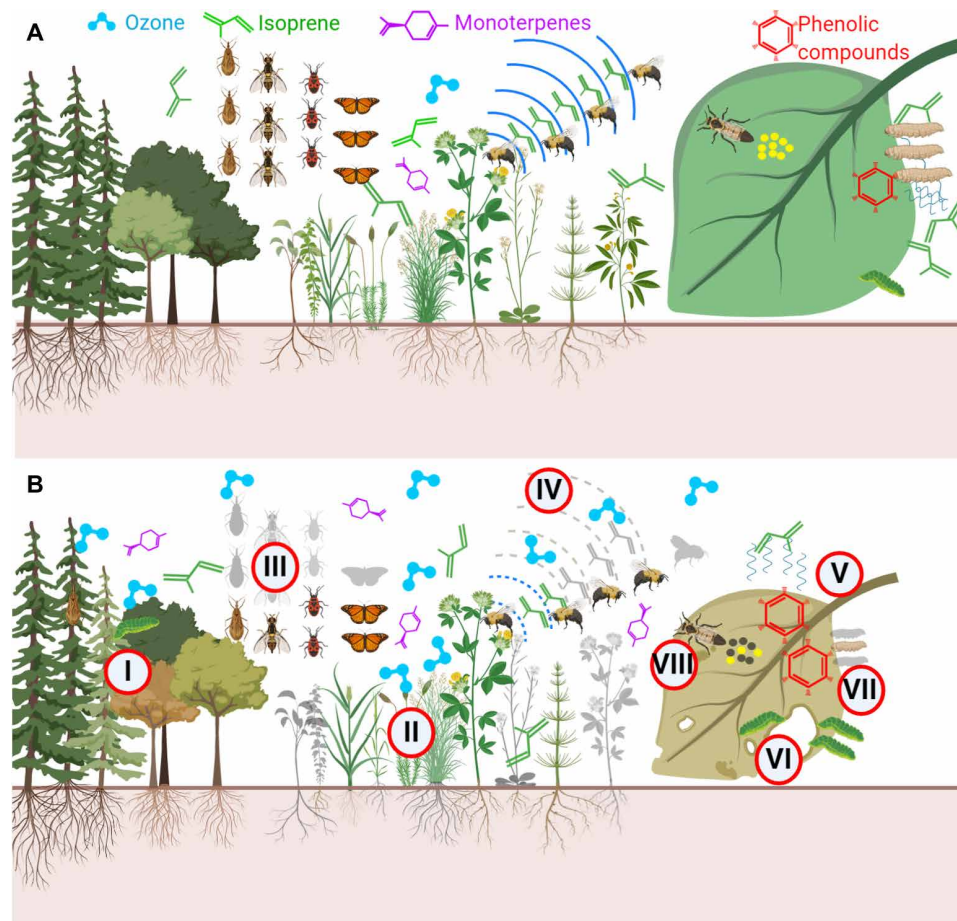
Ground-level concentrations of ozone (O<sub>3</sub>) increased considerably in the second half of the 20th century due to increased levels of NO<sub>x</sub>, volatile organic compounds (VOCs), and radical precursors responsible for its production (6–8). For example, current O<sub>3</sub> levels in rural areas of the temperate and polar zones of the Northern Hemisphere (NH) have increased by 30 to 70% compared to 1896–1975 (6, 7). The O<sub>3</sub> concentrations have remained elevated (see the Supplementary Materials) but are also projected to remain high throughout the 21st century (9), raising concerns about O<sub>3</sub> phytotoxicity, despite policies for reducing precursor emissions. Many programs have documented the incidence of visible injury from O<sub>3</sub> in numerous field-grown species of forbs, shrubs, and trees worldwide, but O<sub>3</sub> has often not been included in global assessments of threats to biodiversity (5, 10). Preliminary analyses suggest that O<sub>3</sub> will continue to pose risks to terrestrial biodiversity at various trophic levels and ecosystem processes and feedbacks in the future (10).

Despite the progress in our understanding of numerous physiological mechanisms specific to plants for responding to O<sub>3</sub> (11, 12), we still know little about the responses of communities and ecosystems to O<sub>3</sub>. Identifying plant mechanisms that may drive the structure and function of plant communities and interactions with insect and soil communities in O<sub>3</sub>-polluted atmospheres is challenging, and the effects on the functioning and biodiversity of terrestrial ecosystems are underexplored (5, 10, 13).

This paper addresses how O<sub>3</sub> affects plant communities, plant-insect interactions, and PSFs and thus plant, insect, and microbial diversity (Figs. 1 and 2). We have four aims. (i) Review the relationships between plant ecological traits and susceptibility to O<sub>3</sub>. We thus collated empirical evidence to determine whether O<sub>3</sub> affects the structure and diversity of plant communities depending on functional groups. (ii) Discuss how O<sub>3</sub> affects plant-insect interactions and whether ecosystem functioning and the structure and diversity

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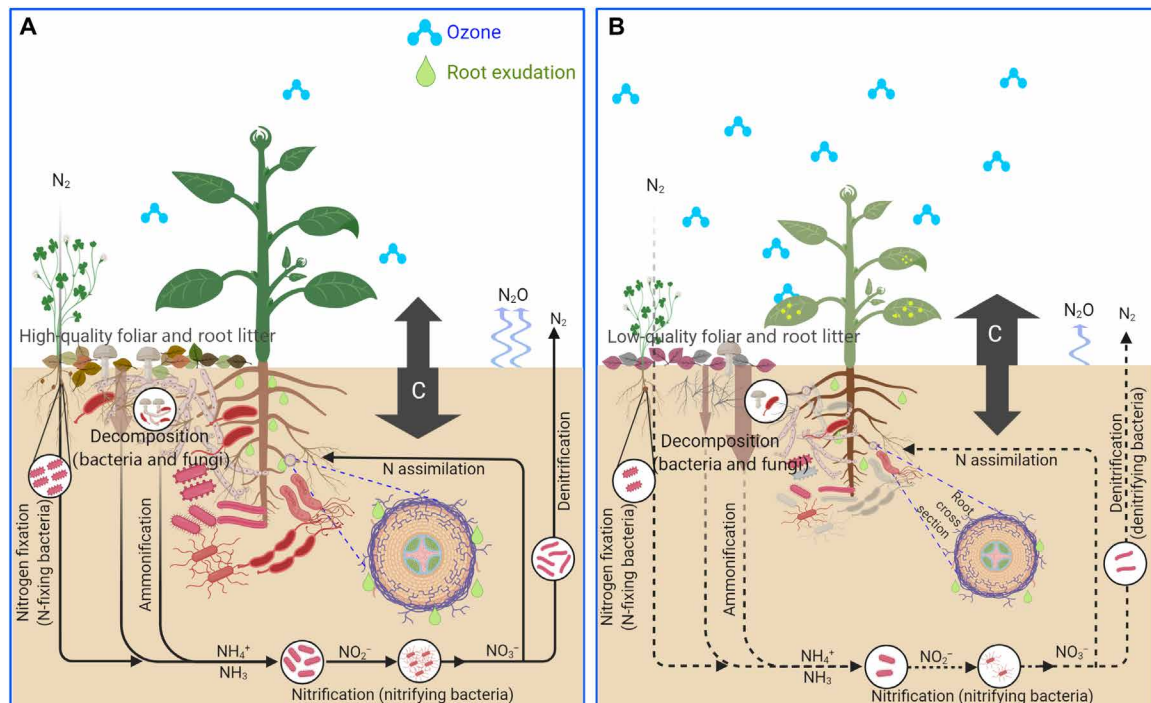
**Fig. 1. Effects of elevated ozone (O<sub>3</sub>) on aboveground ecosystem processes.** Ecological processes occurring at the ecosystem and foliar levels in a natural (not polluted) ecosystem (A) versus an ecosystem disturbed by increased levels of O<sub>3</sub> (B). Gray icons represent the loss of insect or plant diversity but not for particular species. O<sub>3</sub> reduces the growth rate and biomass of plants (including forest trees) (I). Deciduous broadleaf species are usually more susceptible than evergreen broadleaf and needle-leaf species (I). O<sub>3</sub> can also reduce plant species richness and alter community composition (II). O<sub>3</sub> reduces the abundance of insect species but not species richness in forest ecosystems (III). O<sub>3</sub> and OH degrade biogenic VOC (BVOCs), thereby impeding plant-pollinator communication (IV). O<sub>3</sub>-plant-insect interactions may be quite complex and species specific. O<sub>3</sub> inhibits isoprene emissions, increases monoterpene emissions in tolerant and evergreen species, reduces foliar size, induces foliage prematurity (V and I), and increases plant susceptibility to insects and pathogens (I and VI). In other cases, O<sub>3</sub> induces the accumulation of phenolic compounds in leaves, discouraging herbivory by insects (thus reducing insect abundance), increases insect mortality, and inhibits the growth of insect body mass (VII). O<sub>3</sub> also alters foliar phytochemistry, thereby impeding insect oviposition (VIII).

of insect communities are threatened by O<sub>3</sub>-caused changes in VOCs and foliar chemical composition. (iii) Assess how O<sub>3</sub> affects plant-soil interactions and thus soil ecosystem functioning, with special reference to plant litter, decomposition, nutrient cycling, and microbial biomass. The hypothesis that O<sub>3</sub> can affect microbial communities and diversity was verified by a series of studies demonstrating that PSF responses to O<sub>3</sub> can lead to changes in the community structure and diversity of soil microbes. (iv) Analyze global endemic richness of vascular plants versus predicted O<sub>3</sub> exposure by 2100 for assessing whether foci of the endemic richness of various biomes overlap with areas of high O<sub>3</sub> risk, with adverse effects on plant productivity. We use these discussions to seek to identify important gaps in our knowledge and to draw some conclusions about how O<sub>3</sub> can affect plant interactions with insects and microbiota and, thus, alter PSFs and community composition and diversity of plants, insects, and microbiota.

### Plant-plant interactions: Are plant-community composition and diversity at risk from O<sub>3</sub>?

#### Indicators of plant susceptibility to O<sub>3</sub>

The main indicators of the susceptibility of plants to O<sub>3</sub> are stomatal conductance, specific leaf area, and genetically controlled defensive capacity (14, 15). Classification of plant species into tolerant and susceptible, however, is difficult because of their high intraspecific variation in tolerance (5, 16, 17). High intraspecific genetic variation has been detected in grasses and herbaceous and woody plants [e.g., *Phleum alpinum*, *Trifolium repens*, and *Plantago major* (16), as well as *Betula pendula* and *Populus* spp. (18)] and has been studied in detail in, for example, willows and silver birches (18). The genetic variation of O<sub>3</sub> tolerance in these species has been attributed to a range of factors, including foliar phenolic composition (19), effective pathways of ascorbate-glutathione detoxification, remobilization of amino acids (20), genetic plasticity, chemical composition of foliar



**Fig. 2. PSFs under elevated ozone ( $O_3$ ).** A healthy holobiont in a clean atmosphere (with natural background  $O_3$  levels), where mutually beneficial PSFs occur (A), versus a suppressed holobiont and disturbed PSFs due to  $O_3$  (B). Gray icons represent the loss of microbial biomass but not for particular species.  $O_3$  decreases root biomass, reduces the quantity, and affects the quality of foliar and root litter, potentially affecting litter-feeding soil macrofauna, decomposition, and cycling of nutrients.  $O_3$  may influence the chemical composition of roots and soluble root exudates, including reduced exudation of some extracellular enzymes (e.g.,  $\beta$ -glucosidase). The rate of decomposition can be increased or decreased species-specifically. Soil microbial biomass also decreases.  $O_3$  alters the composition and structure of soil microbial communities, with fungi being likely more susceptible to  $O_3$  than bacteria. Some N-fixing bacteria are promoted by  $O_3$ , but N fixation is reduced by  $O_3$  in other studies. Some denitrifying bacteria are likewise promoted by  $O_3$ , and the abundance of some nitrifying bacteria can be either reduced or increased by  $O_3$ . The decrease in microbial biomass disturbs the rates of N and C cycling as feedback, potentially reducing  $N_2O$  and storing less C in the rhizosphere. The changes in C and N cycling in PSFs may occur in tandem with changes in the cycling of other nutrients due to poor leaf and root litter as well as affected decomposition processes.

surface waxes (21, 22), stomatal closure, and foliar structural traits, such as apoplastic volume, thickness, and leaf dry mass per unit area (LMA) (18, 23). Model species such as *Arabidopsis thaliana* clearly indicate that  $O_3$  tolerance is due to a unique set of alterations of various defensive mechanisms, e.g., signaling pathways, regulatory genes, plant hormones, antioxidants, and physiological processes (function and regulation of stomata) (24, 25). These indicators of tolerance can be useful for individual plants or plant mixtures but are challenging in studies at evolutionary and population levels. The selection for  $O_3$  tolerance in the field is further complicated by the multiple environmental factors and stressors that usually affect plants, such as drought, salt, heavy metals, light, nutrient availability, and changeable meteorological conditions (16, 26, 27). Agrobiodiversity and the compositions of seminatural plant communities, among other factors, are often affected by management practices such as grazing, cutting, and fertilization, all of which further obscure the influence of  $O_3$ .

Plant leaves are most exposed to elevated  $O_3$  and have therefore been extensively examined for structural modifications and adaptations to elevated  $O_3$  and associated plant susceptibility (5, 15, 16). The  $O_3$  susceptibility in silver birch and trembling aspen (*Populus tremuloides*) has been associated with foliar thickness, i.e., the most susceptible genotypes had thinner leaves (28). An extensive survey of subtropical, temperate, and Mediterranean tree species found

that  $O_3$  tolerance at the foliar level was associated with LMA, and a reduction in whole-tree biomass was linked to high stomatal flux per unit foliar mass, indicating high  $O_3$  uptake per unit mass (29). High LMA and sclerophyllia can provide  $O_3$  tolerance by (i) cross-protection (e.g., resistance to several stress factors such as drought), (ii) the so-called dilution effect (lower  $O_3$  load per unit foliar mass), and (iii) a large apoplastic compartment, which is often associated with high antioxidative capacity (5, 15, 30, 31). Trichomes are foliar-surface structures that may provide protection against high levels of  $O_3$  by acting as physical barriers or by physiological detoxification (32). LMA, sclerophyllia, and trichomes are relatively easy to measure and can, therefore, be widely used for assessing the threat of  $O_3$  in the field.

#### Relationships between $O_3$ susceptibility and plant diversity

Studying how elevated  $O_3$  affects plant diversity requires focusing on functional traits associated with ecological fitness, such as reproductive fitness (seed output), plant health, and competitiveness. The responses of plant communities to  $O_3$ , however, are ultimately driven by changes in growth, physiology, biochemistry, or genetics at the level of individuals or species (10). Many indirect and well-known effects of  $O_3$  on plants may lead to changes in community composition and diversity, including impacts on growth, plant vitality (assessed as crown defoliation), photosynthesis, stomatal conductance, water balance, the trade-off between biomass production

and defensive processes, flowering, competition, and susceptibility to pests and pathogens (5, 10, 33, 34). These impacts can ultimately reduce the ability of species of a plant community to compete (inter-specific competition) and the ability of communities to compete. Specific indicators of biodiversity, however, may not represent the direct effects of O<sub>3</sub> such as reduced biodiversity within a plant community but rather variations in the competition for resources among plant species.

Visible foliar injury induced by O<sub>3</sub> is an important attribute for epidemiological assessments because it manifests susceptibility of plants to O<sub>3</sub> and is the only indicator of adverse effects of O<sub>3</sub> that can be used for routine field surveys (10, 35). The visible foliar injury appears as chlorosis, necrosis, flecks, stipples, bronzing, and/or reddening (see the Supplementary Materials for more information). While it is difficult to distinguish whether chlorosis and necrosis are induced by O<sub>3</sub> or normal senescence in the field, a characteristic of O<sub>3</sub>-induced symptoms is that they usually occur on interveinal areas of the upper leaf surface only and older leaves show the most symptoms. Species-specific visible injuries have been widely found in nature (10, 35–37), although evidence for the relationship between O<sub>3</sub>-induced visible injuries and damage is contradictory and uncertain especially for forest trees (38). Visible injuries may be associated with negative impacts on fitness traits [e.g., the reduction of carbon (C) assimilation/allocation limits growth and seed production, making the species uncompetitive] (38). Little is known, however, about the relationship between short-term phenomena such as visible foliar injury and longer-term processes affecting plant-community structure and ecosystem biodiversity (5, 10).

#### **Ozone susceptibility varies among plant functional groups**

On the basis of ecological strategies of competition and survival, some susceptible plants may be affected more than nonsusceptible plants by O<sub>3</sub>-induced stress and may thus be competitively penalized (17, 39–42). The degree of susceptibility differs widely among species but also functional groups (Table 1), although current rankings of O<sub>3</sub> susceptibility are based on the susceptibility of individual species (5, 17, 43) and the modeling of different indicators (44). For example, elevated O<sub>3</sub> may decrease the aboveground biomass of therophytes (annuals) more than non-annual plants (e.g., chamaephytes) (43), suggesting that variations in response of annual and perennial species in a community under O<sub>3</sub> are also important for long-term biodiversity effects. High oxidative stress induced by O<sub>3</sub> can also adversely affect the fitness of O<sub>3</sub>-susceptible genotypes when combined with harsh inter- and intraspecific competition within communities, ultimately altering the timing of flowering and seed development and reducing the number and biomass of flowers in some species in a community (33, 45, 46). The community composition of terrestrial ecosystems may thus exhibit long-term changes, and plant diversity may be at risk (Fig. 1), especially in areas where O<sub>3</sub> occurs at potentially phytotoxic levels (Fig. 3).

#### **Empirical evidence for O<sub>3</sub> threats to plant-community composition and diversity**

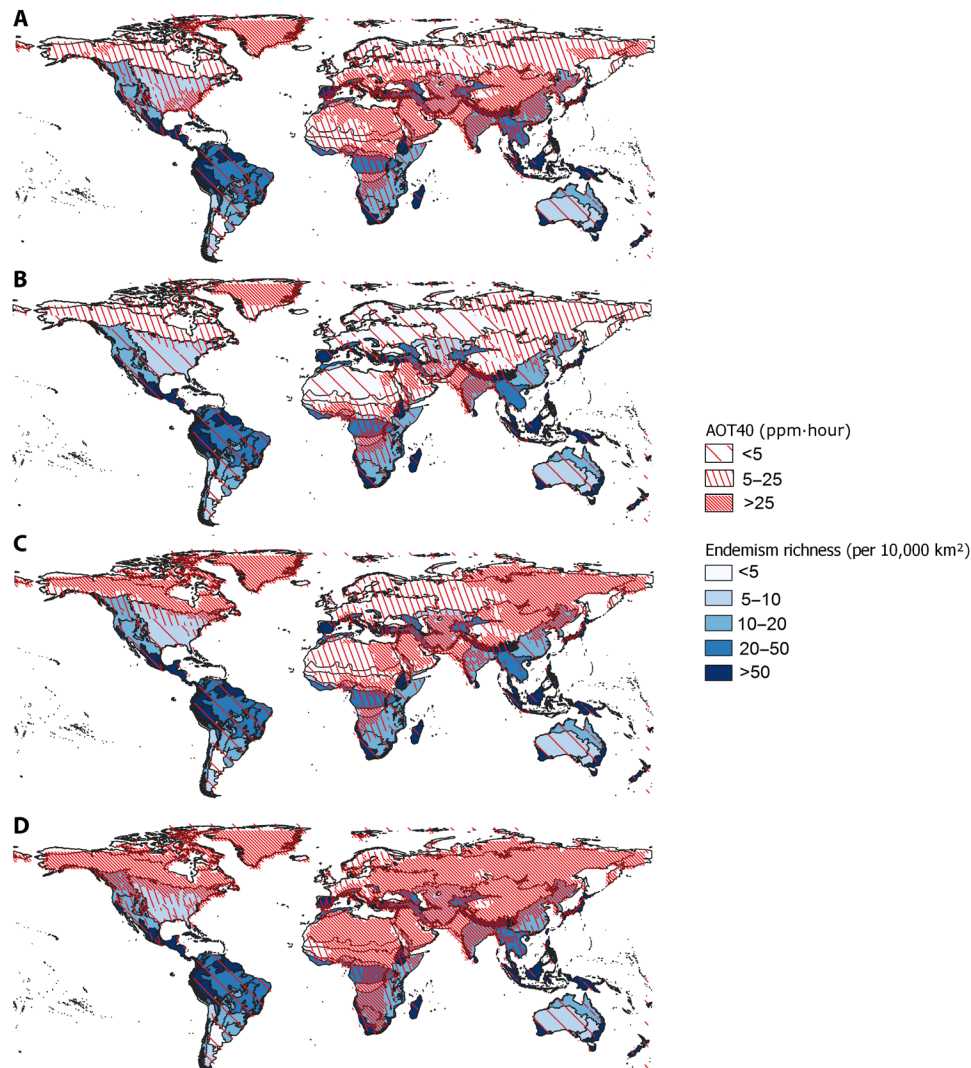
Empirical evidence from several experiments conducted in different regions of the world indicates that O<sub>3</sub> can affect the structure and diversity of plant communities (table S1). Several experiments with open-top chambers (OTCs) in pasture communities exposed to O<sub>3</sub> (commonly up to twofold above the ambient O<sub>3</sub> concentrations) for two to four growing seasons have demonstrated changes in species composition (table S1), with some species being lost over time, and in community structure (42, 47–51). For example, there is evidence

**Table 1. Evidence that the susceptibility of plants to O<sub>3</sub> is conserved at relatively high taxonomic levels.** The reader may refer to the references for further details.

Susceptibility	Reference
Angiosperms > gymnosperms	(204)
Pioneer species > climax species	(205)
Temperate species > subtropical species	(12)
Therophytes > other Raunkiaer life forms (chamaephytes and hemicryptophytes)	(14, 43)
Deciduous trees > evergreen conifers	(5)
Deciduous broadleaf species > evergreen broadleaf and needle-leaf species	(12)
Fabaceae (legumes), Poaceae, and Asteraceae > Brassicaceae	(5, 43)
Fabaceae > Asteraceae, Caryophyllaceae, and Poaceae	(43)
Myrtaceae and Salicaceae > Boraginaceae and Brassicaceae	(5)
Legumes > other forbs and grasses	(14)
Species grown under favorable growth conditions or productive habitats > species grown in less productive habitats	(14)
Light-loving plants > Plants that normally occur in the shade	(43)
Plants grown at dry sites > plants grown in moister soils	(43)
Plants tolerant to moderate salinity > plants of no saline habitats	(43)

for reduced plant species richness (total number of species), diversity (Shannon-Weaver index), and evenness of an early successional community due to exposure to ambient and elevated O<sub>3</sub> levels over two growing seasons (49). These elevated O<sub>3</sub> concentrations occur in highly polluted areas of NH nowadays. Solardome experiments have also suggested potential changes in the structure of grassland communities (33, 45). An O<sub>3</sub> treatment accounted for ≈40% of the variation in species composition (41). Reduced species richness (total number of species), diversity (Shannon-Weaver index), and evenness due to exposure to O<sub>3</sub> were particularly important over two growing seasons in an early successional community (49). The species richness of communities with different histories of O<sub>3</sub> exposure was similar, but the relative abundance of the dominant species differed among the communities (50). Changes in community structure due to past exposure to O<sub>3</sub> suggest that O<sub>3</sub> might have already contributed to changes in natural communities.

The use of free-air O<sub>3</sub> concentration enrichment (FACE) experiments can generate more realistic estimates of the impacts of O<sub>3</sub> on vegetation because the plants fully interact with the surrounding natural environment. A few FACE studies have assessed the impacts of O<sub>3</sub> (commonly up to 1.6-fold higher than the ambient O<sub>3</sub> concentrations) in subalpine and other types of grasslands after two to



**Fig. 3. Ozone exposure levels and global plant endemic richness.** Surface mean AOT40 [parts per million (ppm)-hour] for 2000–2003 (A) and for RCP2.6 (B), RCP4.5 (C), and RCP8.5 (D) by 2100, overlapping the global patterns of the endemic richness of vascular plants (number of species of vascular plants per 10,000 km<sup>2</sup>) across biogeographic regions worldwide (except Antarctica). RCP represents a representative concentration pathway, and AOT40 represents accumulated ozone exposure above a threshold of 40 parts per billion (ppb). Data sources: (9) and (197). The ozone maps are from (9).

seven growing seasons (table S1). Some experiments found O<sub>3</sub>-induced shifts in the fractions of plant functional groups, changes in species composition depending on the functional component, and increased abundance of some species such as *Nardus stricta* (52). Empirical evidence from long-term FACE experiments, therefore, supports the suggestions from OTC experiments that O<sub>3</sub> induces alterations to the structure and species composition of grassland communities.

Forbs are generally more susceptible to O<sub>3</sub> than grasses, but the relative abundance of a grass species may decrease, depending on potential interacting environmental factors (table S1). Note that some species may increase in abundance and others may decrease but without necessarily a large loss of species richness or a shift in their distributional evenness (53). These changes in community composition may also lead to changes in the nutritional value of vegetation used for animal consumption. For instance, reductions

in the legume fraction, but not the grass fraction, of harvested biomass as a result of elevated O<sub>3</sub> exposure occurred in intact and managed pasture (54, 55), *T. repens*–*Lolium perenne* mixtures established under field conditions from seed (56), and *T. repens*–*L. perenne* established in mesocosms (57). Similarly, reductions in the clover/grass ratio due to elevated O<sub>3</sub> appeared in *T. repens* and *L. perenne* (58) and for *T. repens* and *Festuca arundinacea* (59). Comparable reductions can also occur if the aboveground biomass of the grass component increases due to reduced competition from the legume. Altered pasture quality from samples analyzed from seven component studies also occurred (60). This was the result of species composition alterations and the nutritional quality change of individual species within the community.

The hypothesis of impacts on community composition associated with O<sub>3</sub> was also supported by an assessment of the impacts on community composition at 64 field sites selected from the U.K. National

Vegetation Classification of U4 “*Festuca ovinae*–*Agrostis capillaris*–*Galium saxatile* grasslands,” which represent calcifugous grasslands, widely distributed in the British Isles (table S1) (53). The use of genetic markers and DNA fingerprinting in wild populations also demonstrated that genetic diversity is declining in areas with high levels of O<sub>3</sub> [reviewed in (5)]. These studies suggest potentially changing diversity in the real world. Recent technological developments are providing powerful tools for monitoring the loss of biodiversity in vegetation with various plant functional groups, e.g., remote sensing and hyperspectral imaging techniques combined with drones (61); drone techniques are particularly useful in wetland ecosystems. These techniques can potentially more robustly assess the global threat of O<sub>3</sub> to biodiversity in the future.

Some studies have reported negligible or no significant effects on species composition, community structure, or diversity. Several factors, which can affect the estimated impacts of O<sub>3</sub>, such as OTC environments, can affect the species composition of pastures (55). Within-family competition can also be a major driver of the general response of a community (42), and competition between species can be affected by both O<sub>3</sub> and the mixing ratio of the individuals within a community (62). The compositions of species with low abundances may also not greatly change, even if the species are strongly affected by O<sub>3</sub> (52, 63). The outcome of inter- or intraspecific competition also depends on other environmental factors such as soil-water content (62), soil nitrogen (N) content (42, 52), and spatial heterogeneity (64). A mixture of contaminants may also occur in the environment, and multiple co-occurring environmental contaminants may have larger effects than the effects of single contaminants (53). A new generation of studies accounting for various co-occurring factors is therefore needed to provide a basis for assessing real-world risks.

### Plant-insect interactions: Is insect community composition and diversity at risk from O<sub>3</sub>?

#### Foliar quality drives plant-insect interactions

Ozone can affect both the foliar content of N, a major nutrient driving insect dynamics (65), and secondary metabolites. For example, several studies show that elevated O<sub>3</sub> enhanced the concentration of lignin, a key secondary metabolite determining the palatability of biomass to insects (66). Secondary metabolites play important roles in the defense of plants against herbivores by deterring feeding and reducing digestibility by being toxic at high concentrations (2, 67–71) but may also attract herbivores at lower concentrations (70, 72). Elevated O<sub>3</sub> doses that exceed the toxicological threshold inhibit photosynthesis and thus the biosynthesis of secondary metabolites, whereas low O<sub>3</sub> doses that are below the toxicological threshold stimulate defensive signaling pathways and induce the biosynthesis of secondary metabolites (21, 73). Insect herbivores are expected to damage plants more if O<sub>3</sub> decreases the rate of C assimilation so that less C is available for C-based defensive chemicals (74). Numerous studies have demonstrated that O<sub>3</sub> affects phenolics and terpenes, especially in angiosperms (75), although different groups of phenolics and terpenes may respond differently to O<sub>3</sub> (19, 76, 77). Despite the recent advancements in the understanding of O<sub>3</sub> effects on foliar quality, O<sub>3</sub> effects on latex and other constituents of saps exuded from damaged tissues remain completely unknown, although plant latex plays an important role in defense against herbivores (78).

Plant defense modulates insect growth and development directly via toxic secondary metabolites or indirectly by recruiting the natural

enemies of insect pests via herbivore-induced plant volatiles and extra floral nectar (67, 79, 80). For example, the mortality of gypsy moths increased when fed with leaves treated with O<sub>3</sub> (81). A recent study also found that the increase in body mass in *Samia ricini* larvae was inhibited when the larvae were fed with cauliflower (*Brassica oleracea*) leaves treated with O<sub>3</sub> (65), similarly to *Pieris brassicae* reared on *Brassica nigra* (82). This inhibition was due to the effect of O<sub>3</sub> on foliar quality and could, thus, affect herbivory levels by overcompensating for the poor nutritional quality of the tissues (65, 83). These effects suggest potential shifts between generalist species, which can adapt easier to plant defensive compounds and thrive in a wide variety of environmental conditions, and specialist species, which have a limited diet and can thrive only within a narrow range of environmental conditions (84).

Shifts in secondary metabolites under elevated O<sub>3</sub> may lead to changes in relative insect performances, including feeding and ovipositional preferences, longevity, and the ability to reproduce, potentially influencing the population density and community composition of the insects (73, 81, 85). Recent studies of a community of trees grown in a FACE system for 5 years, however, found that populations of different species of insects, with different host plants, decreased significantly in elevated O<sub>3</sub> (74, 86). These studies suggest that these decreases were not due to foliar palatability, also confirmed by laboratory assays (74, 86, 87), or to direct negative effects of O<sub>3</sub> on insects (88), leading to the suggestion that VOCs played an important role in plant-herbivore interactions under O<sub>3</sub> (89). These observations agree with those from other studies of different plant-insect systems (85).

#### Impacts of O<sub>3</sub>-induced changes in VOC emissions on insect dynamics

Biogenic VOCs (BVOCs), i.e., VOCs emitted by plants, play important roles in a range of conspecific and heterospecific interactions and plant survival (90–92). They are emitted by most plant components, both above- and belowground (roots), and provide pivotal ecological cues detectable over a range of distances (93–96). Typical BVOCs include isoprene, terpenes, green leaf volatiles, carbonyls, organic acids, halides, sulfurous compounds, and benzenoids (97). Ozone can induce, reduce, or have no effect on BVOC emissions, depending on the species and conditions of exposure (98–100). Isoprene emissions are typically inhibited under elevated O<sub>3</sub>, but monoterpene emissions of O<sub>3</sub>-tolerant and evergreen species are stimulated (101).

Interactions involving O<sub>3</sub>, BVOCs, and insects are highly complex. Ozone can react with a multitude of VOCs in the atmosphere, breaking them down into mostly unknown reaction products, which may impair communication between plants and insects mediated by volatiles and may compromise pollination (82, 93, 102–105). For example, a laboratory study assessing the effects of O<sub>3</sub> on the orientation of a beetle (*Acalymma vittatum*) to flowers reported that O<sub>3</sub> levels <80 parts per billion (ppb) did not affect orientation, whereas levels >80 ppb disrupted orientation toward floral volatiles (106). Similar results were found for the attraction of other beetles (*Agelastica coerulea*) to leaves (89). Both elevated O<sub>3</sub> (107) and diesel exhaust (108), which comprises some precursors of O<sub>3</sub>, have recently been reported to rapidly degrade floral volatiles. The distance over which floral scents can be detected by pollinators thus decreased, negatively affecting the orientation of the pollinators toward floral food sources (107).

Behavioral tests run at elevated O<sub>3</sub> concentrations indicated that elevated O<sub>3</sub> could also considerably alter the composition of volatile

blends induced by herbivory and consequently alter tritrophic interactions by influencing the behavior of the natural enemies of the herbivores (109–111). The results from these studies, however, were inconsistent. For example, O<sub>3</sub> affected plant volatile bouquets induced by herbivory, but the changes did not affect the orientation of parasitoids, indicating a minimal role of oxidation products in signal perception for this system (109). A combination of elevated O<sub>3</sub> and *P. brassicae* larval feeding on *B. nigra*, however, induced substantially larger volatile emissions than either stress alone, which decreased the attractiveness of the plants to the parasitoid *Cotesia glomerata* relative to plants exposed to herbivores alone (111). Exposure to elevated O<sub>3</sub> may also typically reduce insect oviposition on host plants (86, 112), which may be influenced by multiple mechanisms such as the degradation by O<sub>3</sub> of specific VOCs that stimulate oviposition (105, 113).

Understanding the knock-on effects on insect diversity and the impact of O<sub>3</sub>-induced VOC emissions on insect diversity is needed, despite the growing number of studies addressing the effects of O<sub>3</sub> on the composition of BVOC emissions and specific ecological interactions. The impact of O<sub>3</sub>-induced VOC emissions on insect diversity should also be studied further.

#### **Empirical evidence for O<sub>3</sub> risks of insect community composition and diversity**

Numerous studies have addressed how O<sub>3</sub> alters insect performance by modifying secondary metabolism and VOC emissions, but the impacts of O<sub>3</sub> on insect diversity remain relatively understudied. The fecundity of individual insects does not predict the responses of populations or communities to O<sub>3</sub> (114), but three studies of the effects of O<sub>3</sub> on insect diversity suggest that O<sub>3</sub> can alter species abundance and community structure, thereby threatening diversity (50, 115, 116). For example, an examination of >47,000 insects from four orders and 83 families sampled in a FACE system at regular intervals over four growing seasons found that O<sub>3</sub> tended to increase the abundance of phloem-feeding herbivores and decreased the abundance of chewing herbivores and parasitoids in aspen (115). This study also found that elevated O<sub>3</sub> reduced the total abundance of insects (17%) compared to ambient O<sub>3</sub>, with prominent effects on parasitoids such as Braconidae (–33%), Chalcidoidea (–26%), Figitidae (–59%), and Ichneumonidae (–41%) (115). Arthropod communities also changed in two experiments when seeds from a community of natural plants previously exposed to filtered air, 90-ppb O<sub>3</sub> or 120-ppb O<sub>3</sub>, with episodic patterns of varying daily peak concentrations over four growing seasons, were used to re-establish the plant community in a new environment with low-level O<sub>3</sub> pollution (50). The carnivore/herbivore ratio particularly increased (increased abundance of carnivores) with increasing historical levels of O<sub>3</sub> in the 2 years following historical exposures (50). The effects on insect abundance and species richness are specific to plant species and vary temporally (116). More studies are needed, but these results suggest that O<sub>3</sub> may influence biotic communities and pose a threat to biodiversity even years after exposure.

#### **Interactions between plant and soil microbiota: Are microbial community composition and diversity at risk from O<sub>3</sub>?**

##### **Plant-microbe coevolutionary relationship**

Diverse communities of bacteria and fungi live near and on the surfaces of plant roots (rhizosphere) and leaves (phyllosphere) and in internal plant tissues (endosphere). Beneficial microbes help plants

to acquire water and nutrients, defend against pathogenic microbes, tolerate abiotic stress (including drought and O<sub>3</sub> and other air pollutants), adapt to environmental changes, establish mycorrhizae, and regulate plant growth (117–121). Microbes can regulate plant growth directly by releasing phytohormones or organic growth promoters, contributing to ecosystem processes, such as N fixation, ammonia oxidation, and phosphate solubilization, or indirectly by producing biocontrol compounds, such as antibiotics, siderophores, and enzymes, or signaling compounds (121–123). Microbes, in return, benefit from a stable niche, and the supply of primary and secondary metabolites and C from root exudates (123, 124). Microorganisms are evolutionarily older than land plants, and our current understanding is that plants are coevolving species assemblages—holobionts (Fig. 2), consisting of plants and their microbiota (125). The long coevolution of plants and their associated microbiota has likely led to phylogenetically and functionally divergent microbiomes in different environments and climatic zones. Our current knowledge of the impacts of O<sub>3</sub> on plant microbiota and biodiversity is mainly restricted to rhizospheric processes.

##### **Plant-soil feedbacks**

An extensive body of literature addresses the interactions between plants and biotic and abiotic soil properties, known as PSFs (4). The effect of increasing atmospheric concentrations of O<sub>3</sub> on these interactions, however, has rarely been studied (10, 13). A few studies have investigated the effects of manipulated O<sub>3</sub> levels on community structure and composition and the function of soil microbial communities, but the results have been inconclusive (126–131), perhaps due to varying durations of the experiments or other experimental factors such as the facilities used for exposing plants to O<sub>3</sub>, ecosystem type, and type of management (132). Many of these new studies, however, suggest potentially important impacts of elevated O<sub>3</sub> on PSFs, including soil microbial diversity and decoupled PSF interactions (Fig. 2).

##### **Soil ecosystem functioning**

Elevated O<sub>3</sub> can modify resource allocation between above- and belowground parts of the plant, an important response in all plants under O<sub>3</sub> stress (13, 133). A meta-analysis showed that O<sub>3</sub> generally inhibits the allocation to roots, relatively to shoots, albeit a variable phenomenon (133). A more recent analysis of 239 data entries of dry root mass of woody plants revealed that 40% of the entries reflected a statistically significant decrease and only 3% of the entries reflected a statistically significant increase (13). Likewise, an analysis of 104 data entries of root/shoot biomass ratio showed that 27% of the entries reflected a statistically significant decrease and only 5% of the entries reflected a statistically significant increase (13). Hence, it appears that elevated O<sub>3</sub> generally reduces the allocation of resources to roots more than to shoots, suggesting potential feedbacks to the soil ecosystem.

Rhizospheric microbial communities are highly diverse and have a fundamental role in nutrient acquisition, water economy, growth, and disease tolerance (118). Elevated O<sub>3</sub> reduces the allocation of C derived from the soil, which reduces the amount of resources for heterotrophic microbes and thereby affects belowground processes driven by microbes (132, 134, 135). O<sub>3</sub> can thus modify decomposition rates, activities of soil enzymes, root turnover, rhizodeposition (all material lost from plant roots and deposited into the soil) and belowground cycling of C and N, although the magnitude and direction of the influence are plant specific and depend on various environmental conditions other than O<sub>3</sub> (136–139). The role of

rhizodeposition is particularly important in northern biomes, where productivity is often strongly limited by the uptake and cycling of N (140). Exudation of other molecules such as chelators or organic acids is also crucial for the acquisition of phosphorus or microelements, but they lack experimentation regarding their role in O<sub>3</sub> effects.

Many studies have reported that O<sub>3</sub> accelerates foliar senescence, thereby changing the timing of litter deposition, and reduces the amount of leaf litter due to diminished foliar area (13, 141, 142). O<sub>3</sub> can also affect litter quality (12, 13, 143). A series of experiments on the effects of O<sub>3</sub> on nutrient translocation from senescing to younger leaves identified species-, soil-, and study-specific changes in the levels of micro- and macronutrients in the foliar litter, which appeared early during stress, altering ecological stoichiometry (144, 145). More studies are needed to draw general conclusions about these highly complex phenomena. These changes in the amount and quality of litter could affect soil macrofauna that feeds on litter, including detritivores [e.g., (143)].

Ozone reduces rhizodeposition because ecosystems exposed to elevated O<sub>3</sub> support a lower net primary productivity, similar to leaves. The rate of turnover of fine roots may also be higher, which may also affect decomposition (13). Many experiments have found that O<sub>3</sub> affects the chemical composition of fine roots, such as decreasing or increasing the contents of monosaccharides, total soluble carbohydrates, and total sugars, depending on the severity of stress (13). The levels of fatty acids, starches, and nutrients were also affected but not consistently in direction or magnitude, which are expected to vary with time and stress level (13). Ozone influences the chemical composition of both roots and soluble root exudates (146, 147). All these alterations can lead to species-specific changes in the rates of decomposition (138, 148).

Recent advances show that elevated O<sub>3</sub> alters the expression of microbial genes involved in C cycling, which, in turn, likely affects C cycling regulated by microbes (149). For instance, in two Chinese wheat cultivars exposed to elevated O<sub>3</sub> in a FACE system, the abundance of C cycling genes was generally decreased in both cultivars (including *fts* genes involved in the reductive acetogenesis pathway), although the abundance of a few genes increased in both cultivars (*mcrA* and mannanase and xylanase genes) or one of the cultivars (*amyX*, *nplT*, and *lip* genes in one cultivar; *pcc*, *aceA/B*, bacterial *ara*, and carbon monoxide dehydrogenase and phenol oxidase genes in the other cultivar) (149). Ozone can also reduce the ability of microbes to use C sources, especially in the rhizosphere, as indicated by the reduced signal intensity of some C degradation genes (e.g., related to hemicellulose, aromatics, and chitin) detected in a soil depth of 0 to 5 cm (129). The excretion of some extracellular enzymes involved in C metabolism (e.g.,  $\beta$ -glucosidase) can be similarly suppressed by O<sub>3</sub> (150, 151), reducing the availability of C sources, which can also decrease the methanogenic activity of microbes in paddy fields (152). Ozone generally decreases C cycling by decreasing C-based exudation caused by a reduced translocation of photosynthates to the roots (13).

Ozone can also impair N cycling in soil driven by microbial activity (139, 150, 153–157). For example, N fixation by legumes can decrease in response to increasing O<sub>3</sub> concentrations (158). Several studies show that N-fixing plants (e.g., legumes) grown within multispecies plant communities can be more sensitive to O<sub>3</sub> than their co-occurring species (table S1), although it remains elusive how O<sub>3</sub> affects their N-fixing capacity in these multispecies communities. Ozone can also negatively affect N<sub>2</sub>O emissions from meadows and

soybean fields (159, 160) and rice and wheat fields (161–163), suggesting that reduced denitrification due to decreases in plant-derived C inputs induced by O<sub>3</sub> may play a dominant role. The direction of the effects of O<sub>3</sub> on N<sub>2</sub>O emissions is also driven by the system of cultivation (163). Recent advances suggest that O<sub>3</sub> can reduce the expression of microbial genes involved in N fixation, denitrification, and N mineralization associated with legumes (129), including decreased abundances of *nirK*, *nirS*, and *nosZ* (which are widely used to describe denitrifier communities) and *amoA* (an indicator of nitrifier communities) (160). Reduced availability of soil N and/or labile C for nitrifiers or denitrifiers may account for these observations in the abundances of genes associated with N cycling. The effects of O<sub>3</sub> on the cycling of soil N, however, clearly vary among plant genotypes or cultivars depending on their susceptibility to O<sub>3</sub> (139, 149, 163). These findings demonstrate the decoupling of PSF interactions by O<sub>3</sub>.

Several studies suggest that O<sub>3</sub> usually decreases soil microbial biomass (127, 150, 152, 164–166). The negative response of microbial biomass to O<sub>3</sub> is most likely due to a reduction in root biomass and substrate availability (13). O<sub>3</sub> can reduce both fungal biomass and the ratio of fungi to bacteria, suggesting that fungi may be more susceptible than bacteria to O<sub>3</sub> (149, 165, 166). Elevated O<sub>3</sub> can significantly decrease microbial biomass in microaggregates (inhabited predominately by bacteria) relative to macroaggregates (132), suggesting that bacteria in microaggregates may be more susceptible to O<sub>3</sub>. Ozone, however, increased microbial biomass in a community dominated by sedges (167), perhaps due to an increase in substrate availability, because O<sub>3</sub> slightly increased the total number of sedge leaves toward the end of the experiment, and/or to a faster turnover of fine roots caused by O<sub>3</sub> (168). A decrease in soil microbial biomass would also contribute to a suppressed holobiont and disturbed PSFs (Fig. 2).

#### **Empirical evidence for O<sub>3</sub> threats to microbial community composition and diversity**

Elevated O<sub>3</sub> can alter the composition and structure of soil microbial communities (127, 129, 130, 165, 166, 169–171). For example, 11 years of exposure to elevated O<sub>3</sub> doubled the ratio of Basidiomycota to Ascomycota in soil microbial communities (128). The relative abundances of bacteria at the order level in the phylum Actinobacteria (which may promote the degradation of recalcitrant substances) increased under O<sub>3</sub> (130). O<sub>3</sub> also reduced the relative abundance of bacterial groups belonging to the family Rhodospirillaceae and the order Clostridiales in rice systems (169). Further studies have recently reported the effects of O<sub>3</sub> on bacteria that facilitate the oxidation of ammonia, the rate-limiting step of nitrification. Elevated O<sub>3</sub> decreased the relative abundances of some nitrifiers (e.g., Proteobacteria and *Nitrospira*) but increased those of some denitrifiers (e.g., *Acremonium* and *Bacillus*) in soils growing *Machilus ichangensis* and *Taxus chinensis* (127). O<sub>3</sub> likewise increased the relative abundances of some nitrifying bacteria (e.g., Nitrososphaeraceae, Nitrospiraceae, Nocardiodaceae, and 0319-6A21) and N-fixing bacteria (e.g., Sphingomonadaceae, Rhizobiaceae, Termomonosporaceae, Micromonosporaceae, Streptomycetaceae, and Bradyrhizobiaceae) in the soil microbial community of a maize field (131). The abundance of microbes is an important indicator of N mineralization (172), and these results suggest that the effects of O<sub>3</sub> on microbial structural diversity can affect N mineralization.

The structure of microbial communities may be affected by the soil environment and plant functions. Decreases in root exudation



caused by elevated O<sub>3</sub> represent a plausible mechanism by which plants could modulate their interaction with microbes. Root exudates have an important role in plant-microbe interactions and help to determine the composition of the rhizospheric microbiome (173–175). Plants generally exude up to 20% of the fixed C and 15% of the N, which includes an array of simple molecules such as sugars, organic acids, and secondary metabolites and complex polymers such as mucilage (174, 176). The amount and composition of root exudates vary among plant genotypes. Root exudation is modulated by various abiotic stresses (177, 178). For example, microbial composition differed between maize genotypes, a phenomenon linked to differences in the amount of root exudation induced by O<sub>3</sub> (131). Few studies, however, have focused on the effect of O<sub>3</sub> on the relationships between root exudates and soil bacterial communities, so our understanding of the response of soil microorganisms to O<sub>3</sub> is incomplete.

The alpha diversity of soil fungi (especially ectomycorrhizae), bacteria, and archaea is often reduced by O<sub>3</sub> as an indirect consequence of changes in plant (and rhizospheric) structure and function caused by elevated O<sub>3</sub> (130, 179–182). Ozone in other cases, however, has increased microbial alpha diversity (130, 183, 184), highlighting the degrees to which the literature remains inconclusive and additional studies are needed for a comprehensive mechanistic understanding.

### Ozone risks by 2100 and global biodiversity: Model predictions

#### Rationale

The previous sections documented that the effects of O<sub>3</sub> on insects and microbiota were mediated by plants and that the effects of O<sub>3</sub> on plants could affect the composition and diversity of plant, insect, and microbial communities (Figs. 1 and 2). The impacts of O<sub>3</sub> on plant diversity is not clear, but the evidence of indirect impacts on the diversity of plant microbiota is much clearer, suggesting that microbial diversity may be at a higher O<sub>3</sub> risk than plant diversity. Plant biomass, a trait critical to fitness that also indicates permanent adverse effects of O<sub>3</sub> (17), can be used as an indicator of the threat of O<sub>3</sub> to the health of the plant, insect, and microbial communities. The productivity of plant communities, such as in predominant global forests, is also positively correlated with species richness (185). We identified areas with overlapping O<sub>3</sub> risk and high terrestrial endemic richness for vascular plants under scenarios of representative concentration pathways (RCPs).

#### Methodology

Projected changes in O<sub>3</sub> vary considerably among models (186) and scenarios of emission of O<sub>3</sub> precursors (9). The latest emission scenarios, the RCPs, were developed for the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (187). The RCP scenarios include various assumptions on climate, policies of energy access, and changes in land cover and use (188). Different RCP scenarios lead to different concentrations and emissions, e.g., CH<sub>4</sub>, O<sub>3</sub> (even stratospheric O<sub>3</sub> inputs), and emissions of NO<sub>x</sub> from lightning, associated with climate change, all of which affect O<sub>3</sub> levels. The RCPs have been described elsewhere (9, 186, 189).

Sixteen global or regional chemical models within the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP) were validated and used to evaluate projected changes in air quality under various scenarios of emission and climate (186, 189). On the basis of (9), we selected the global three-dimensional (3D) chemistry transport model MOCAGE (Modèle de Chimie

Atmosphérique à Grande Echelle) among the 16 ACCMIP models to assess the worldwide threats of O<sub>3</sub> to plant endemic richness.

MOCAGE is a global 3D chemistry transport model with a high grid resolution (0.5° by 0.5°), which provides numerical simulations of the interactions between dynamical, physical, and chemical processes in the troposphere and lower stratosphere (190). It uses a semi-Lagrangian advection scheme to transport the chemical species (190). MOCAGE reproduces well the spatial pattern of O<sub>3</sub> exposure at the global scale, e.g., in high-elevation areas or areas downwind of O<sub>3</sub> precursor sources (9). The MOCAGE historical runs cover the period 2000–2003, while the time slice of RCPs is centered around 2100. MOCAGE can simulate 110 gas species including VOC species as well as soil and lightning NO<sub>x</sub>, with a horizontal resolution of 2° by 2°, and includes 47 vertical pressure levels from the surface up to 5 hPa. MOCAGE simulates biogenic emissions of hydrocarbons from vegetation (isoprene, monoterpenes, and other VOCs) and also parameterizes dry deposition of hundreds of compounds including O<sub>3</sub> (190). A shortfall of the MOCAGE model is that it overestimates O<sub>3</sub>, especially near sea surfaces, essentially in NH (190, 191). This phenomenon was due to limitations of the thermodynamic equilibrium hypothesis in a marine atmosphere and an overestimation of NO<sub>x</sub> emissions in the lowest part of the troposphere, especially in the winter months (190, 191). It may result from a positive bias in OH and CO emissions at the south of the Equator, which are mainly from a biomass burning origin, and are too strong (191).

The O<sub>3</sub> exposure-based index AOT40 [parts per million (ppm)·hour] is a metric used to assess the potential O<sub>3</sub> risk to vegetation from local to global scales and adopted by European regulatory agencies (192–194). AOT40 is less biologically relevant than flux-based metrics because, among other reasons, it does not incorporate species-specific O<sub>3</sub> influx (11). However, O<sub>3</sub> flux information can be obtained for only few ecotypes of (semi)natural plants, thus not permitting reliable O<sub>3</sub> flux estimates across large regions of the globe. Global flux data for (semi)natural vegetation, which is the focus of this study, are not available. Furthermore, mapping global-scale O<sub>3</sub> flux at high spatial resolution cannot be practically performed because of physical scarcity of data, such as hourly meteorological data, hourly soil moisture, dominant tree species per pixel, and stomatal conductance per dominant species. Moreover, world-leading agencies base regulatory ecological risk assessment upon estimated environmental concentrations (exposures) and not upon dose intake by organisms (influx), including the Deterministic Approach and the Probabilistic Approach [e.g., U.S. Environmental Protection Agency, Scientific Advisory Panel of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Ecological Committee on FIFRA Risk Assessment Methods; www.epa.gov]. AOT40 is computed as the sum of the hourly exceedances above 40 ppb, for daylight hours (radiation, >50 W m<sup>-2</sup>) over vegetation- or species-specific growing seasons (195), namely, April to September for temperate climates (e.g., Europe) and all year round for Mediterranean, subtropical, and tropical-type climates (196). On the basis of (193), we calculated AOT40 for a model grid (8:00 to 20:00, local time) for all days of the year. A critical level of 5 ppm·hour calculated over the growing season has been recommended for the protection of trees (5% reduction, total biomass) and (semi)natural vegetation dominated by perennials (10% reduction, above- or belowground biomass and/or cover of individual species) (195). We selected this level as a stringent reference point for both annual and perennial (semi)natural vegetation; a level of 3 ppm·hour (195) would produce exceedances of critical

level throughout NH. Details of the O<sub>3</sub> data used and the procedure followed are provided by (9).

Kier *et al.* (197) analyzed the “endemism richness” of vascular plants (i.e., ferns, gymnosperms, and angiosperms) in large datasets and maps across 90 biogeographic regions worldwide (excluding Antarctica) using a standard area of 10,000 km<sup>2</sup>. This index (endemism richness) combines both endemic and species richness and is considered superior to species richness or species endemism, and it can indicate the specific contribution of an area to global biodiversity (197). The dataset contains the full spectrum of abiotic conditions and includes all major biomes. We used the dataset provided by Kier *et al.* (197) to map the endemism richness of <5, 5 to 10, 10 to 20, 20 to 50, and >50 species of terrestrial vascular plants per 10,000 km<sup>2</sup>. Mean richness for mainland areas was 18.2 species per 10,000 km<sup>2</sup>, so we created these arbitrary categories and considered an endemic richness of ≥20 species per 10,000 km<sup>2</sup> to be high, i.e., exceeding the average endemic richness (see the Supplementary Materials).

## RESULTS AND DISCUSSION

AOT40 averaged 31.8 ppm-hour in NH and 3.5 ppm-hour in the Southern Hemisphere (SH) in the 2000s (Fig. 3A). In the early 2000s, AOT40 below the critical level of 5 ppm-hour occurred mainly in the SH: South America, Pacific islands, the Cape region of South Africa, Madagascar, Polynesia-Micronesia, Melanesia, Indonesia, the Philippines, Australia, Tasmania, New Caledonia, and New Zealand (Fig. 3A). Higher O<sub>3</sub> burdens (AOT40, >25 ppm-hour) have been simulated for areas at high elevations, e.g., the Rocky and Appalachian Mountains and the Tibetan Plateau, and for Greenland, northeastern Siberia, the Mediterranean Basin, and areas downwind of precursor sources, e.g., southern Asia (Fig. 3A).

Changes in AOT40 compared to the early 2000s were –60% (NH) and +69% (SH) for RCP2.6 (most optimistic), –28% (NH) and +203% (SH) for RCP4.5, and +67% (NH) and +449% (SH) for RCP8.5 (most pessimistic). Surface AOT40 for RCP2.6 would decrease worldwide, except in equatorial Africa, where AOT40 was higher (Fig. 3B). Mean surface O<sub>3</sub> concentrations decreased most where historical O<sub>3</sub> concentrations were high. The O<sub>3</sub> foci (AOT40, >25 ppm-hour) for RCP2.6 were in Greenland, India, the Near East, and equatorial Africa (Fig. 3B), but the vegetation in Greenland was very limited. AOT40 for RCP4.5 was slightly lower than historical runs, except over Canada and eastern Asia (particularly Siberia)

where a high increase was observed (Fig. 3C). The surface O<sub>3</sub> levels and AOT40 were higher for RCP8.5 than historical runs and increased the most in northwestern United States, Greenland, the Mediterranean Basin, the Near East, and eastern Asia (Fig. 3D). AOT40 greatly exceeded 25 ppm-hour worldwide, except in Central and South America and Australia (Fig. 3D).

AOT40 for the three RCPs did not change significantly in SH. MOCAGE indicated that the critical levels for the protection of perennial (semi)natural vegetation for RCP8.5 based on AOT40 (5 ppm-hour) would be exceeded over many areas of NH by as much as 10-fold, despite improvements for RCP2.6 and RCP4.5.

AOT40 is expected to be high (>5 ppm-hour) by 2100 for 23 to 51% of the land areas with an endemic richness of 20 to 50 or >50 species per 10,000 km<sup>2</sup>, depending on the RCP (Table 2). Only 4% of the land areas with an endemic richness of >200 species per 10,000 km<sup>2</sup>, however, is projected to be exposed to high AOT40 values (>5 ppm-hour) and only for the most pessimistic scenario, RCP8.5 (Table 2).

All NH areas with very high endemic richness (>50 species per 10,000 km<sup>2</sup>) in the 2000s overlapped with high O<sub>3</sub> exposures (>5 ppm-hour), except Central America, northern South America, and the Philippines. Plant endemic richness was high in regions with a Mediterranean climate, and regions in NH where endemic richness was high (e.g., California, the Mediterranean Basin, and Ethiopia) had high O<sub>3</sub> levels in summer (198, 199).

A comparison of the global distributions of the endemic richness of vascular plants (>20 plant species per 10,000 km<sup>2</sup>) where AOT40 was >5 ppm-hour for the three RCPs indicated that both endemic richness and O<sub>3</sub> risk were high for NH Atlantic islands in the latitude band 15° to 45°N (e.g., the Canary Islands, Azores, and the Caribbean), the Mediterranean Basin, equatorial Africa, Ethiopia, the Indian coastline, the Himalayan region, southern Asia, and Japan.

## Unresolved questions

A gap of knowledge exists in O<sub>3</sub> effects on natural and seminatural communities in tropical and subtropical environments; thus, these studies are encouraged. The long-term impacts of O<sub>3</sub> on biodiversity remain completely unknown, especially in terms of global biodiversity. A new generation of long-term “real-world” experiments designed to study the effects of O<sub>3</sub> on biodiversity are greatly needed. The complex structure, physiognomy, and high biodiversity of southern biomes are obstacles to conducting field or laboratory studies of this subject.

**Table 2. Land areas for three ranges of the endemic richness of vascular plants exposed to high O<sub>3</sub> exposures (AOT40, >5 ppm-hour) by 2100 estimated using three RCPs.**

	Land area exposed to AOT40 > 5 ppm-hour (%)			
	Historical	RCP2.6	RCP4.5	RCP8.5
Area with 20–50 species per 10,000 km <sup>2</sup> (15.1% of total land area)	41.7	23.9	29.9	41.7
Area with >50 species per 10,000 km <sup>2</sup> (11.4% of total land area)	37.7	23.2	23.2	51.3
Area with >200 species per 10,000 km <sup>2</sup> (1.8% of total land area)	0.0	0.0	0.0	4.4

Can elevated O<sub>3</sub> alter the ecological plasticity of wild and semi-wild plant species? Potential selection pressure due to plant-plant competition under O<sub>3</sub> is likely weak and will be difficult to demonstrate. Previous studies have reported controversial results (16, 200) due to the high intraspecific variation of many species (5). Little evidence has been found for the impact of temporal changes in O<sub>3</sub> concentrations on diversity. Understanding and improving the tolerance to O<sub>3</sub> are more advanced for crop plants than wild and semi-wild species, but the tolerance of genetically improved plants to O<sub>3</sub> has rarely been demonstrated in the field (201). The use of genetic markers and DNA fingerprinting in wild populations has demonstrated that genetic diversity is decreasing in areas with high O<sub>3</sub> levels [reviewed in (5)], which may lead to reduced ecological plasticity in changing environments. Population differentiation studies at high-risk areas under future scenarios may provide further insights and are thus encouraged. Limited information is yet available for the impacts of O<sub>3</sub> on mosses, ferns, lichens, algae, and fungi, although current data suggest high tolerance to O<sub>3</sub> for many of these organisms (5). More research is needed to understand the role of belowground processes and phyllospheric microbiota for assessing biodiversity.

Can O<sub>3</sub> affect insect diversity? The effects of elevated O<sub>3</sub> on insects could be indirect and would depend on the magnitude of change in the quality of the host plant (bottom-up factors) and/or the impact on natural enemies (top-down factors) (83). Elevated O<sub>3</sub> may affect populations of natural enemies by shifts in the diversity, abundance, and quality of prey or changes in behavior that may affect finding hosts (85, 115). Information about the temporary and long-term impacts of elevated O<sub>3</sub> on insect diversity remains elusive.

Can elevated O<sub>3</sub> alter the diversity of phyllospheric microbiota? The differences in the composition of microbiota from different plant tissues represent an adaptation to different plant niches and the specific function of the tissue, such as C assimilation in leaves and the uptake of water and nutrients by roots. The atmospheric environment has direct contact with the phyllosphere, which forms the largest area of bioactive surfaces on Earth, at the interface between the plant and atmosphere, mediating plant responses to a changing environment (202). The phyllosphere is a habitat for a large variety of microbes (phyllobiome), with microbial densities of up to 10<sup>7</sup> cells cm<sup>-2</sup> (203), but the impacts of elevated O<sub>3</sub> or other air pollutants on the phyllobiome are poorly known (169). Rhizospheric and phyllospheric microbiotas are interconnected and overlap with endophytic microbiota through regulatory traits, particularly for the promotion of plant growth (hormone biosynthesis) and catalytic pathways (degradation of C-based and defensive compounds) (120). A preliminary study did not detect significant effects of O<sub>3</sub> on the phyllosphere microbiome; however, it was conducted in an artificial environment, outside the natural habitat of rice, where plants were grown in pots inside a greenhouse (169). Hence, new research is needed for understanding the impacts of elevated O<sub>3</sub> on the phyllobiome.

## CONCLUSIONS

Despite several uncertainties, some key conclusions of our review are the following:

The composition of a plant community may exhibit long-term changes, and diversity may be at risk, due to a genotype-specific susceptibility to O<sub>3</sub>, especially in areas where O<sub>3</sub> is at potentially phytotoxic levels. The consensus is that elevated O<sub>3</sub> affects plant-

community composition but if and to what extent O<sub>3</sub> may contribute to changes in biodiversity in terrestrial ecosystems remain unclear. Climatic scenarios for 2100 suggest that regions with high endemic richness, e.g., NH Atlantic islands in the latitude band 15° to 45°N, the Mediterranean Basin, equatorial Africa, Ethiopia, the Indian coastline, the Himalayan region, southern Asia, and Japan, are most threatened by high levels of O<sub>3</sub>.

Ozone affects the foliar chemical composition and the composition of BVOC emissions, altering plant-insect interactions and thus threatening key ecosystem functions (e.g., plant-insect communication). O<sub>3</sub> can indirectly affect species abundance and the structure of insect communities.

Ozone also alters plant properties and soil processes that define plant-soil-microbe interactions and PSFs, such as the input of plant litter, plant exudation, root turnover, nutrient cycling, activities of soil enzymes, and decomposition, threatening the functioning of the soil ecosystem. Soil microbial communities can be indirectly affected, including decreasing microbial biomass, altering the composition and structure of communities and usually reducing alpha diversity.

The responses of plant-insect interactions and PSFs to O<sub>3</sub> are species specific and affected by several factors, such as the spatial variation of O<sub>3</sub>, temperature, relative humidity, degree of urbanization, and the quality of control services in urban, rural, and forested areas. Ozone has, nonetheless, been demonstrated to decouple plant-insect interactions and PSFs, which should be considered when predicting the impacts of climate change.

Potential threats of elevated O<sub>3</sub> to biodiversity and ecosystem services should be considered when adopting the post-2020 global biodiversity initiative “Roadmap for EU Biodiversity Strategy to 2030” at the UN Biodiversity Conference in Kunming, China (October 2020), for curtailing biodiversity loss and preserving and restoring its ecosystems.

## SUPPLEMENTARY MATERIALS

Supplementary material for this article is available at <http://advances.sciencemag.org/cgi/content/full/6/33/eabc1176/DC1>

## REFERENCES AND NOTES

1. A. Biere, A. E. Bennett, Three-way interactions between plants, microbes and insects. *Funct. Ecol.* **27**, 567–573 (2013).
2. J. K. Holopainen, J. D. Blande, Where do herbivore-induced plant volatiles go? *Front. Plant Sci.* **4**, 185 (2013).
3. M. M. Howard, J. Kao-Kniffin, A. Kessler, Shifts in plant-microbe interactions over community succession and their effects on plant resistance to herbivores. *New Phytol.* **226**, 1144–1157 (2020).
4. F. I. Pugnaire, J. A. Morillo, J. Peñuelas, P. B. Reich, R. D. Bardgett, A. Gaxiola, D. A. Wardle, W. H. van der Putten, Climate change effects on plant-soil feedbacks and consequences for biodiversity and functioning of terrestrial ecosystems. *Sci. Adv.* **5**, eaaz1834 (2019).
5. E. Bergmann, J. Bender, H.-J. Weigel, Impact of tropospheric ozone on terrestrial biodiversity: A literature analysis to identify ozone sensitive taxa. *J. Appl. Bot. Food Qual.* **90**, 83–105 (2017).
6. D. Tarasick, I. E. Galbally, O. R. Cooper, M. G. Schultz, G. Ancellet, T. Leblanc, T. J. Wallington, J. Ziemke, X. Liu, M. Steinbacher, J. Staehelin, C. Vigouroux, J. W. Hannigan, O. Garcia, G. Foret, P. Zanis, E. Weatherhead, I. Petropavlovskikh, H. Worden, M. Osman, J. Liu, K.-L. Chang, A. Gaudel, M. Lin, M. Granados-Muñoz, A. M. Thompson, S. J. Oltmans, J. Cuesta, G. Dufour, V. Thouret, B. Hassler, T. Trickl, J. L. Neu, Tropospheric Ozone Assessment Report: Tropospheric ozone from 1877 to 2016, observed levels, trends and uncertainties. *Elem. Sci. Anthr.* **7**, 39 (2019).
7. A. S. Lefohn, C. S. Malley, L. Smith, B. Wells, M. Hazucha, H. Simon, V. Naik, G. Mills, M. G. Schultz, E. Paoletti, A. De Marco, X. Xu, L. Zhang, T. Wang, H. S. Neufeld, R. C. Musselman, D. Tarasick, M. Brauer, Z. Feng, H. Tang, K. Kobayashi, P. Sicard, S. Solberg, G. Gerosa, Tropospheric ozone assessment report: Global ozone metrics for climate change, human health, and crop/ecosystem research. *Elem. Sci. Anthr.* **6**, 28 (2018).

8. L. Y. Yeung, L. T. Murray, P. Martinerie, E. Witrant, H. Hu, A. Banerjee, A. Orsi, J. Chappellaz, Isotopic constraint on the twentieth-century increase in tropospheric ozone. *Nature* **570**, 224–227 (2019).
9. P. Sicard, A. Anav, A. De Marco, E. Paoletti, Projected global tropospheric ozone impacts on vegetation under different emission and climate scenarios. *Atmos. Chem. Phys. Discuss.* **17**, 12177–12196 (2017).
10. J. Fuhrer, M. Val Martin, G. Mills, C. L. Heald, H. Harmens, F. Hayes, K. Sharps, J. Bender, M. R. Ashmore, Current and future ozone risks to global terrestrial biodiversity and ecosystem processes. *Ecol. Evol.* **6**, 8785–8799 (2016).
11. E. Agathokleous, R. G. Belz, V. Calatayud, A. De Marco, Y. Hoshika, M. Kitao, C. J. Saitanis, P. Sicard, E. Paoletti, E. J. Calabrese, Predicting the effect of ozone on vegetation via linear non-threshold (LNT), threshold and hormetic dose-response models. *Sci. Total Environ.* **649**, 61–74 (2019).
12. P. Li, Z. Feng, V. Calatayud, X. Yuan, Y. Xu, E. Paoletti, A meta-analysis on growth, physiological, and biochemical responses of woody species to ground-level ozone highlights the role of plant functional types. *Plant Cell Environ.* **40**, 2369–2380 (2017).
13. E. Agathokleous, C. J. Saitanis, X. Wang, M. Watanabe, T. Koike, A review study on past 40 years of research on effects of tropospheric O<sub>3</sub> on belowground structure, functioning, and processes of trees: A linkage with potential ecological implications. *Wat. Air Soil Pollut.* **227**, 33 (2016).
14. S. Bassin, M. Volk, J. Fuhrer, Factors affecting the ozone sensitivity of temperate European grasslands: An overview. *Environ. Pollut.* **146**, 678–691 (2007).
15. P. Li, V. Calatayud, F. Gao, Y. Uddling, Z. Feng, Differences in ozone sensitivity among woody species are related to leaf morphology and antioxidant levels. *Tree Physiol.* **36**, 1105–1116 (2016).
16. A. W. Davison, J. D. Barnes, Effects of ozone on wild plants. *New Phytol.* **139**, 135–151 (1998).
17. E. Agathokleous, C. J. Saitanis, Plant susceptibility to ozone: A tower of babel? *Sci. Total Environ.* **703**, 134962 (2020).
18. E. Oksanen, S. Manninen, E. Vapaavuori, T. Holopainen, Near-ambient ozone concentrations reduce the vigor of *Betula* and *Populus* species in Finland. *Ambio* **38**, 413–417 (2009).
19. E. Häikiö, V. Freiwald, R. Julkunen-Tiitto, E. Beuker, T. Holopainen, E. Oksanen, Differences in leaf characteristics between ozone-sensitive and ozone-tolerant hybrid aspen (*Populus tremula* × *Populus tremuloides*) clones. *Tree Physiol.* **29**, 53–66 (2009).
20. J. Dumont, S. Keski-Saari, M. Keinänen, D. Cohen, N. Ningre, S. Kontunen-Soppela, P. Baldet, Y. Gibon, P. Dizengremel, M.-N. Vaultier, Y. Jolivet, E. Oksanen, D. Le Thiec, Ozone affects ascorbate and glutathione biosynthesis as well as amino acid contents in three Euramerican poplar genotypes. *Tree Physiol.* **34**, 253–266 (2014).
21. S. Kontunen-Soppela, V. Ossipov, S. Ossipova, E. Oksanen, Shift in birch leaf metabolome and carbon allocation during long-term open-field ozone exposure. *Glob. Chang. Biol.* **13**, 1053–1067 (2007).
22. S. Kontunen-Soppela, J. Riikonen, H. Ruhanen, M. Brosché, P. Somervuo, P. Peltonen, J. Kangasjärvi, P. Auvinen, L. Paulin, M. Keinänen, E. Oksanen, E. Vapaavuori, Differential gene expression in senescing leaves of two silver birch genotypes in response to elevated CO<sub>2</sub> and tropospheric ozone. *Plant Cell Environ.* **33**, 1016–1028 (2010).
23. J. Riikonen, M. Mäenpää, M. Alavillamo, T. Silfver, E. Oksanen, Interactive effect of elevated temperature and O<sub>3</sub> on antioxidant capacity and gas exchange in *Betula pendula* saplings. *Planta* **230**, 419–427 (2009).
24. M. Brosché, E. Merilo, F. Mayer, P. Pechter, I. Puzörjova, G. Brader, J. Kangasjärvi, H. Kollist, Natural variation in ozone sensitivity among *Arabidopsis thaliana* accessions and its relation to stomatal conductance. *Plant Cell Environ.* **33**, 914–925 (2010).
25. B. Grimmig, M. N. Gonzalez-Perez, G. Leubner-Metzger, R. Vögeli-Lange, F. Meins Jr., R. Hain, J. Penuelas, B. Heidenreich, C. Langebartels, D. Ernst, H. Sandermann Jr., Ozone-induced gene expression occurs via ethylene-dependent and -independent signalling. *Plant Mol. Biol.* **51**, 599–607 (2003).
26. N. Suzuki, R. M. Rivero, V. Shulaev, E. Blumwald, R. Mittler, Abiotic and biotic stress combinations. *New Phytol.* **203**, 32–43 (2014).
27. Z. Feng, B. Shang, Z. Li, V. Calatayud, E. Agathokleous, Ozone will remain a threat for plants independently of nitrogen load. *Funct. Ecol.* **33**, 1854–1870 (2019).
28. E. Oksanen, J. Sober, D. F. Karnosky, Impacts of elevated CO<sub>2</sub> and/or O<sub>3</sub> on leaf ultrastructure of aspen (*Populus tremuloides*) and birch (*Betula papyrifera*) in the aspen FACE experiment. *Environ. Pollut.* **115**, 437–446 (2001).
29. Z. Feng, P. Büker, H. Pleijel, L. Emberson, P. E. Karlsson, J. Uddling, A unifying explanation for variation in ozone sensitivity among woody plants. *Glob. Chang. Biol.* **24**, 78–84 (2018).
30. P. E. Karlsson, S. Braun, M. Broadmeadow, S. Elvira, L. Emberson, B. S. Gimeno, D. Le Thiec, K. Novak, E. Oksanen, M. Schaub, J. Uddling, M. Wilkinson, Risk assessments for forest trees: The performance of the ozone flux versus the AOT concepts. *Environ. Pollut.* **146**, 608–616 (2007).
31. F. Bussotti, Functional leaf traits, plant communities and acclimation processes in relation to oxidative stress in trees: A critical overview. *Glob. Chang. Biol.* **14**, 2727–2739 (2008).
32. G. Karabourniotis, G. Liakopoulos, D. Nikolopoulos, P. Bresta, Protective and defensive roles of non-glandular trichomes against multiple stresses: Structure-function coordination. *J. For. Res.* **31**, 1–12 (2019).
33. F. Hayes, G. Mills, H. Harmens, K. Wyness, Within season and carry-over effects following exposure of grassland species mixtures to increasing background ozone. *Environ. Pollut.* **159**, 2420–2426 (2011).
34. A. De Marco, M. Vitale, I. Popa, A. Anav, O. Badea, D. Silaghi, S. Leca, A. Screpanti, E. Paoletti, Ozone exposure affects tree defoliation in a continental climate. *Sci. Total Environ.* **596–597**, 396–404 (2017).
35. P. Sicard, A. De Marco, L. Dalstein-Richier, F. Tagliaferro, C. Renou, E. Paoletti, An epidemiological assessment of stomatal ozone flux-based critical levels for visible ozone injury in Southern European forests. *Sci. Total Environ.* **541**, 729–741 (2016).
36. Z. Feng, J. Sun, W. Wan, E. Hu, V. Calatayud, Evidence of widespread ozone-induced visible injury on plants in Beijing, China. *Environ. Pollut.* **193**, 296–301 (2014).
37. E. Paoletti, A. Alivernini, A. Anav, O. Badea, E. Carrari, S. Chivulescu, A. Conte, M. L. Ciriani, L. Dalstein-Richier, A. De Marco, S. Fares, G. Fasano, A. Giovannelli, M. Lazzara, S. Leca, A. Materassi, V. Moretti, D. Pitar, I. Popa, F. Sabatini, L. Salvati, P. Sicard, T. Sorgi, Y. Hoshika, Toward stomatal flux-based forest protection against ozone: The MOTTLES approach. *Sci. Total Environ.* **691**, 516–527 (2019).
38. R. Marzuoli, G. Gerosa, F. Bussotti, M. Pollastrini, Assessing the impact of ozone on forest trees in an integrative perspective: Are foliar visible symptoms suitable predictors for growth reduction? A critical review. *Forests* **10**, 1144 (2019).
39. C. J. Saitanis, E. Agathokleous, Stress response and population dynamics: Is Allee effect hormesis? *Sci. Total Environ.* **682**, 623–628 (2019).
40. F. Scebba, F. Canaccini, A. Castagna, J. Bender, H.-J. Weigel, A. Ranieri, Physiological and biochemical stress responses in grassland species are influenced by both early-season ozone exposure and interspecific competition. *Environ. Pollut.* **142**, 540–548 (2006).
41. K. V. Wedlich, N. Rintoul, S. Peacock, J. N. Cape, M. Coyle, S. Toet, J. Barnes, M. Ashmore, Effects of ozone on species composition in an upland grassland. *Oecologia* **168**, 1137–1146 (2012).
42. H. Calvete-Sogo, I. González-Fernández, J. Sanz, S. Elvira, R. Alonso, H. García-Gómez, M. A. Ibáñez-Ruiz, V. Bermejo-Bermejo, Heterogeneous responses to ozone and nitrogen alter the species composition of Mediterranean annual pastures. *Oecologia* **181**, 1055–1067 (2016).
43. F. Hayes, M. L. M. Jones, G. Mills, M. Ashmore, Meta-analysis of the relative sensitivity of semi-natural vegetation species to ozone. *Environ. Pollut.* **146**, 754–762 (2007).
44. M. L. M. Jones, F. Hayes, G. Mills, T. H. Sparks, J. Fuhrer, Predicting community sensitivity to ozone, using Ellenberg Indicator values. *Environ. Pollut.* **146**, 744–753 (2007).
45. F. Hayes, J. Williamson, G. Mills, Ozone pollution affects flower numbers and timing in a simulated BAP priority calcareous grassland community. *Environ. Pollut.* **163**, 40–47 (2012).
46. F. Hayes, G. Mills, P. Williams, H. Harmens, P. Büker, Impacts of summer ozone exposure on the growth and overwintering of UK upland vegetation. *Atmos. Environ.* **40**, 4088–4097 (2006).
47. M. R. Ashmore, R. H. Thwaites, N. Ainsworth, D. A. Cousins, S. A. Power, A. J. Morton, Effects of ozone on calcareous grassland communities. *Wat. Air Soil Pollut.* **85**, 1527–1532 (1995).
48. M. R. Ashmore, N. Ainsworth, The Effects of ozone and cutting on the species composition of artificial grassland communities. *Funct. Ecol.* **9**, 708–712 (1995).
49. D. N. Barbo, A. H. Chappelka, G. L. Somers, M. S. Miller-Goodman, K. Stolte, Diversity of an early successional plant community as influenced by ozone. *New Phytol.* **138**, 653–662 (1998).
50. M. A. Martínez-Ghersa, A. I. Menéndez, P. E. Gundel, A. M. Folia, A. M. Romero, J. B. Landesmann, L. Ventura, C. M. Ghersa, Legacy of historic ozone exposure on plant community and food web structure. *PLOS ONE* **12**, e0182796 (2017).
51. R. H. Thwaites, M. R. Ashmore, A. J. Morton, R. J. Pakeman, The effects of tropospheric ozone on the species dynamics of calcareous grassland. *Environ. Pollut.* **144**, 500–509 (2006).
52. S. Bassin, M. Volk, J. Fuhrer, Species composition of subalpine grassland is sensitive to nitrogen deposition, but not to ozone, after seven years of treatment. *Ecosystems* **16**, 1105–1117 (2013).
53. R. J. Payne, C. J. Stevens, N. B. Dise, D. J. Gowing, M. G. Pilkington, G. K. Phoenix, B. A. Emmett, M. R. Ashmore, Impacts of atmospheric pollution on the plant communities of British acid grasslands. *Environ. Pollut.* **159**, 2602–2608 (2011).
54. J. Fuhrer, Effects of ozone on managed pasture: I. Effects of open-top chambers on microclimate, ozone flux, and plant growth. *Environ. Pollut.* **86**, 297–305 (1994).
55. J. Fuhrer, H. Shariat-Madari, R. Perler, W. Tschannen, A. Grub, Effects of ozone on managed pasture: II. Yield, species composition, canopy structure, and forage quality. *Environ. Pollut.* **86**, 307–314 (1994).

56. S. Wilbourn, A. W. Davison, J. H. Ollerenshaw, The use of an unenclosed field fumigation system to determine the effects of elevated ozone on a grass–clover mixture. *New Phytol.* **129**, 23–32 (1995).
57. F. Hayes, G. Mills, M. Ashmore, Effects of ozone on inter- and intra-species competition and photosynthesis in mesocosms of *Lolium perenne* and *Trifolium repens*. *Environ. Pollut.* **157**, 208–214 (2009).
58. S. Nussbaum, M. Geissmann, J. Fuhrer, Ozone exposure-response relationships for mixtures of perennial ryegrass and white clover depend on ozone exposure patterns. *Atmos. Environ.* **29**, 989–995 (1995).
59. A. S. Heagle, J. Rebbeck, S. R. Shafer, U. Blum, W. W. Heck, Effects of long-term ozone exposure and soil moisture deficit on growth of a ladino clover-tall fescue pasture. *Phytopathology* **79**, 128–136 (1989).
60. F. Hayes, G. Mills, L. Jones, J. Abbott, M. Ashmore, J. Barnes, J. Neil Cape, M. Coyle, S. Peacock, N. Rintoul, S. Toet, K. Wedlich, K. Wyness, Consistent ozone-induced decreases in pasture forage quality across several grassland types and consequences for UK lamb production. *Sci. Total Environ.* **543**, 336–346 (2016).
61. L. Granlund, S. Keski-Saari, T. Kumpula, E. Oksanen, M. Keinänen, Imaging lichen water content with visible to mid-wave infrared (400–5500 nm) spectroscopy. *Remote Sens. Environ.* **216**, 301–310 (2018).
62. S. Nussbaum, P. Bungener, M. Geissmann, J. Fuhrer, Plant-plant interactions and soil moisture might be important in determining ozone impacts on grasslands. *New Phytol.* **147**, 327–335 (2000).
63. S. Bassin, M. Volk, M. Suter, N. Buchmann, J. Fuhrer, Nitrogen deposition but not ozone affects productivity and community composition of subalpine grassland after 3 yr of treatment. *New Phytol.* **175**, 523–534 (2007).
64. A. Stampfli, J. Fuhrer, Spatial heterogeneity confounded ozone-exposure experiment in semi-natural grassland. *Oecologia* **162**, 515–522 (2010).
65. E. Agathokleous, Y. WaiLi, G. Ntatsi, K. Konno, C. J. Saitanis, M. Kitao, T. Koike, Effects of ozone and ammonium sulfate on cauliflower: Emphasis on the interaction between plants and insect herbivores. *Sci. Total Environ.* **659**, 995–1007 (2019).
66. M. Frei, Lignin: Characterization of a multifaceted crop component. *ScientificWorldJournal* **2013**, 436517 (2013).
67. R. Karban, The ecology and evolution of induced resistance against herbivores. *Funct. Ecol.* **25**, 339–347 (2011).
68. G. K. Taggar, R. S. Gill, A. K. Gupta, J. S. Sandhu, Fluctuations in peroxidase and catalase activities of resistant and susceptible black gram (*Vigna mungo* (L.) Hepper) genotypes elicited by *Bemisia tabaci* (Gennadius) feeding. *Plant Signal. Behav.* **7**, 1321–1329 (2012).
69. A. R. War, M. G. Paulraj, B. Hussain, A. A. Buhroo, S. Ignacimuthu, H. C. Sharma, Effect of plant secondary metabolites on *Helicoverpa armigera*. *J. Pest Sci.* **86**, 399–408 (2013).
70. A. M. Trowbridge, in *Ecology and the Environment*, R. K. Monson, Ed. (Springer, 2015), pp. 1–28.
71. R. Kaur, A. K. Gupta, G. K. Taggar, Induced resistance by oxidative shifts in pigeonpea (*Cajanus cajan* L.) following *Helicoverpa armigera* (Hübner) herbivory. *Pest Manag. Sci.* **71**, 770–782 (2015).
72. M. Wink, Plant secondary metabolites modulate insect behavior-steps toward addiction? *Front. Physiol.* **9**, 364 (2018).
73. R. L. Lindroth, Impacts of elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> on forests: Phytochemistry, trophic interactions, and ecosystem dynamics. *J. Chem. Ecol.* **36**, 2–21 (2010).
74. E. Agathokleous, T. Sakikawa, S. A. Abu ElEla, T. Mochizuki, M. Nakamura, M. Watanabe, K. Kawamura, T. Koike, Ozone alters the feeding behavior of the leaf beetle *Agelastica coerulea* (Coleoptera: Chrysomelidae) into leaves of Japanese white birch (*Betula platyphylla* var. *japonica*). *Environ. Sci. Pollut. Res.* **24**, 17577–17583 (2017).
75. E. Valkama, J. Koricheva, E. Oksanen, Effects of elevated O<sub>3</sub>, alone and in combination with elevated CO<sub>2</sub>, on tree leaf chemistry and insect herbivore performance: A meta-analysis. *Glob. Chang. Biol.* **13**, 184–201 (2007).
76. R. L. Lindroth, B. J. Kopper, W. F. Parsons, J. G. Bockheim, D. F. Karnosky, G. R. Hendrey, K. S. Pregitzer, J. G. Isebrands, J. Sober, Consequences of elevated carbons dioxide and ozone for foliar chemical composition and dynamics in trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *Environ. Pollut.* **115**, 395–404 (2001).
77. J. J. Couture, T. D. Meehan, K. F. Rubert-Nason, R. L. Lindroth, Effects of elevated atmospheric carbon dioxide and tropospheric ozone on phytochemical composition of trembling aspen (*Populus tremuloides*) and paper birch (*Betula papyrifera*). *J. Chem. Ecol.* **43**, 26–38 (2017).
78. K. Konno, Plant latex and other exudates as plant defense systems: Roles of various defense chemicals and proteins contained therein. *Phytochemistry* **72**, 1510–1530 (2011).
79. G.-I. Arimura, K. Matsui, J. Takabayashi, Chemical and molecular ecology of herbivore-induced plant volatiles: Proximate factors and their ultimate functions. *Plant Cell Physiol.* **50**, 911–923 (2009).
80. A. R. War, M. G. Paulraj, T. Ahmad, A. A. Buhroo, B. Hussain, S. Ignacimuthu, H. C. Sharma, Mechanisms of plant defense against insect herbivores. *Plant Signal. Behav.* **7**, 1306–1320 (2012).
81. J. J. Couture, R. L. Lindroth, Atmospheric change alters performance of an invasive forest insect. *Glob. Chang. Biol.* **18**, 3543–3557 (2012).
82. E. Khaling, S. Papazian, E. H. Poelman, J. K. Holopainen, B. R. Albrechtsen, J. D. Blande, Ozone affects growth and development of *Pieris brassicae* on the wild host plant *Brassica nigra*. *Environ. Pollut.* **199**, 119–129 (2015).
83. T. Cornelissen, Climate change and its effects on terrestrial insects and herbivory patterns. *Neotrop. Entomol.* **40**, 155–163 (2011).
84. J. G. Ali, A. A. Agrawal, Specialist versus generalist insect herbivores and plant defense. *Trends Plant Sci.* **17**, 293–302 (2012).
85. H. Cui, J. Su, J. Wei, Y. Hu, F. Ge, Elevated O<sub>3</sub> enhances the attraction of whitefly-infested tomato plants to *Encarsia formosa*. *Sci. Rep.* **4**, 5350 (2014).
86. T. Sugai, S. Okamoto, E. Agathokleous, N. Masui, F. Satoh, T. Koike, Leaf defense capacity of Japanese elm (*Ulmus davidiana* var. *japonica*) seedlings subjected to a nitrogen loading and insect herbivore dynamics in a free air ozone-enriched environment. *Environ. Sci. Pollut. Res.* **27**, 3350–3360 (2020).
87. S. A. Abu ElEla, E. Agathokleous, T. Koike, Growth and nutrition of *Agelastica coerulea* (Coleoptera: Chrysomelidae) larvae changed when fed with leaves obtained from an O<sub>3</sub>-enriched atmosphere. *Environ. Sci. Pollut. Res.* **25**, 13186–13194 (2018).
88. S. A. Abu ElEla, E. Agathokleous, N. A. Ghazawy, T. R. Amin, W. M. ElSayed, T. Koike, Enzyme activity modification in adult beetles (*Agelastica coerulea*) inhabiting birch trees in an ozone-enriched atmosphere. *Environ. Sci. Pollut. Res.* **25**, 32675–32683 (2018).
89. N. Masui, T. Mochizuki, A. Tani, H. Matsuura, E. Agathokleous, T. Watanabe, T. Koike, Does ozone alter the attractiveness of Japanese white birch leaves to the leaf beetle *Agelastica coerulea* via changes in biogenic volatile organic compounds (BVOCs): An examination with the Y-tube test. *Forests* **11**, 58 (2020).
90. M. A. Jamieson, L. A. Burkle, J. S. Manson, J. B. Runyon, A. M. Trowbridge, J. Zientek, Global change effects on plant–insect interactions: The role of phytochemistry. *Curr. Opin. Insect Sci.* **23**, 70–80 (2017).
91. Q. S. McFrederick, J. D. Fuentes, T. Roulston, J. C. Kathilankal, M. Lerdau, Effects of air pollution on biogenic volatiles and ecological interactions. *Oecologia* **160**, 411–420 (2009).
92. P. Y. Oikawa, M. T. Lerdau, Catabolism of volatile organic compounds influences plant survival. *Trends Plant Sci.* **18**, 695–703 (2013).
93. S. Dötterl, M. Vater, T. Rupp, A. Held, Ozone differentially affects perception of plant volatiles in western honey bees. *J. Chem. Ecol.* **42**, 486–489 (2016).
94. S. Dötterl, N. J. Vereecken, The chemical ecology and evolution of bee-flower interactions: A review and perspectives. *Can. J. Zool.* **88**, 668–697 (2010).
95. J. Peñuelas, D. Asensio, D. Tholl, K. Wenke, M. Rosenkranz, B. Piechulla, J. P. Schnitzler, Biogenic volatile emissions from the soil. *Plant Cell Environ.* **37**, 1866–1891 (2014).
96. M. Heil, Herbivore-induced plant volatiles: Targets, perception and unanswered questions. *New Phytol.* **204**, 297–306 (2014).
97. A. Guenther, Biological and chemical diversity of biogenic volatile organic emissions into the atmosphere. *ISRN Atmos. Sci.* **2013**, 786290 (2013).
98. E. Agathokleous, M. Kitao, E. J. Calabrese, Emission of volatile organic compounds from plants shows a biphasic pattern within an hormetic context. *Environ. Pollut.* **239**, 318–321 (2018).
99. C. Calfapietra, E. Pallozzi, I. Lusini, V. Velikova, in *Biology, Controls and Models of Tree Volatile Organic Compound Emissions*, Ü. Niinemets, R. K. Monson, Eds. (Springer, 2013), pp. 253–284.
100. J. Llusà, J. Peñuelas, B. S. Gimeno, Seasonal and species-specific Mediterranean plant VOC emissions by Mediterranean woody plant to elevated ozone concentrations. *Atmos. Environ.* **36**, 3931–3938 (2002).
101. Z. Feng, X. Yuan, S. Fares, F. Loreto, P. Li, Y. Hoshika, E. Paoletti, Isoprene is more affected by climate drivers than monoterpenes: A meta-analytic review on plant isoprenoid emissions. *Plant Cell Environ.* **42**, 1939–1949 (2019).
102. P. S. Giron-Calva, T. Li, J. D. Blande, Volatile-mediated interactions between cabbage plants in the field and the impact of ozone pollution. *J. Chem. Ecol.* **43**, 339–350 (2017).
103. Q. S. McFrederick, J. C. Kathilankal, J. D. Fuentes, Air pollution modifies floral scent trails. *Atmos. Environ.* **42**, 2336–2348 (2008).
104. D. M. Pinto, J. D. Blande, S. R. Souza, A.-M. Nerg, J. K. Holopainen, Plant volatile organic compounds (VOCs) in ozone (O<sub>3</sub>) polluted atmospheres: The ecological effects. *J. Chem. Ecol.* **36**, 22–34 (2010).
105. T. Li, J. D. Blande, Associational susceptibility in broccoli: Mediated by plant volatiles, impeded by ozone. *Glob. Chang. Biol.* **21**, 1993–2004 (2015).
106. J. D. Fuentes, T. H. Roulston, J. Zenker, Ozone impedes the ability of a herbivore to find its host. *Environ. Res. Lett.* **8**, 014048 (2013).
107. G. Farré-Armengol, J. Peñuelas, T. Li, P. Yli-Pirilä, I. Filella, J. Llusia, J. D. Blande, Ozone degrades floral scent and reduces pollinator attraction to flowers. *New Phytol.* **209**, 152–160 (2016).
108. R. D. Girling, I. Lusebrink, E. Farthing, T. A. Newman, G. M. Poppy, Diesel exhaust rapidly degrades floral odours used by honeybees. *Sci. Rep.* **3**, 2779 (2013).

109. D. M. Pinto, J. D. Blande, R. Nykänen, W.-X. Dong, A.-M. Nerg, J. K. Holopainen, Ozone degrades common herbivore-induced plant volatiles: Does this affect herbivore prey location by predators and parasitoids? *J. Chem. Ecol.* **33**, 683–694 (2007).
110. S. J. Himanen, A.-M. Nerg, A. Nissinen, D. M. Pinto, C. N. Stewart Jr., G. M. Poppy, J. K. Holopainen, Effects of elevated carbon dioxide and ozone on volatile terpenoid emissions and multitrophic communication of transgenic insecticidal oilseed rape (*Brassica napus*). *New Phytol.* **181**, 174–186 (2009).
111. E. Khaling, T. Li, J. K. Holopainen, J. D. Blande, Elevated ozone modulates herbivore-induced volatile emissions of *Brassica nigra* and alters a tritrophic interaction. *J. Chem. Ecol.* **42**, 368–381 (2016).
112. B. J. Kopper, R. L. Lindroth, Effects of elevated carbon dioxide and ozone on the phytochemistry of aspen and performance of an herbivore. *Oecologia* **134**, 95–103 (2003).
113. P. S. Girón-Calva, T. Li, J. D. Blande, Plant–plant interactions affect the susceptibility of plants by oviposition by pests but are disrupted by ozone pollution. *Agric. Ecosyst. Environ.* **233**, 352–360 (2016).
114. C. S. Awmack, R. Harrington, R. L. Lindroth, Aphid individual performance may not predict population responses to elevated CO<sub>2</sub> or O<sub>3</sub>. *Glob. Chang. Biol.* **10**, 1414–1423 (2004).
115. M. L. Hillstrom, R. L. Lindroth, Elevated atmospheric carbon dioxide and ozone alter forest insect abundance and community composition. *Insect Conserv. Divers.* **1**, 233–241 (2008).
116. M. L. Hillstrom, J. J. Couture, R. L. Lindroth, Elevated carbon dioxide and ozone have weak, idiosyncratic effects on herbivorous forest insect abundance, species richness, and community composition. *Insect Conserv. Divers.* **7**, 553–562 (2014).
117. S. Compant, C. Clément, A. Sessitsch, Plant growth-promoting bacteria in the rhizosphere and endosphere of plants: Their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* **42**, 669–678 (2010).
118. P. R. Hardoim, L. S. van Overbeek, G. Berg, A. M. Pirttilä, S. Compant, A. Campisano, M. Döring, A. Sessitsch, The hidden world within plants: Ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiol. Mol. Biol. Rev.* **79**, 293–320 (2015).
119. F. Bringel, I. Couée, Pivotal roles of phyllosphere microorganisms at the interface between plant functioning and atmospheric trace gas dynamics. *Front. Microbiol.* **6**, 486 (2015).
120. V. Imperato, L. Kowalkowski, M. Portillo-Estrada, S. W. Gawronski, J. Vangronsveld, S. Thijs, Characterisation of the *Carpinus betulus* L. phyllosphere microbiome in urban and forest areas. *Front. Microbiol.* **10**, 1110 (2019).
121. M. Wenig, A. Ghirardo, J. H. Sales, E. S. Pabst, H. H. Breitenbach, F. Antritter, B. Weber, B. Lange, M. Lenk, R. K. Cameron, J.-P. Schnitzler, A. C. Vlot, Systemic acquired resistance networks amplify airborne defense cues. *Nat. Commun.* **10**, 3813 (2019).
122. M. A. Hassani, P. Durán, S. Hacquard, Microbial interactions within the plant holobiont. *Microbiome*. **6**, 58 (2018).
123. P. Fincheira, A. Quiroz, Microbial volatiles as plant growth inducers. *Microbiol. Res.* **208**, 63–75 (2018).
124. L. Philippot, J. M. Raaijmakers, P. Lemanceau, W. H. van der Putten, Going back to the roots: The microbial ecology of the rhizosphere. *Nat. Rev. Microbiol.* **11**, 789–799 (2013).
125. P. Vandenkoornhuise, A. Quaiser, M. Duhamel, A. Le Van, A. Dufresne, The importance of the microbiome of the plant holobiont. *New Phytol.* **206**, 1196–1206 (2015).
126. F. Changey, M. Bagard, M. Souleymane, T. Z. Lerch, Cascading effects of elevated ozone on wheat rhizosphere microbial communities depend on temperature and cultivar sensitivity. *Environ. Pollut.* **242**, 113–125 (2018).
127. Z. Chen, M. R. Maltz, J. Cao, H. Yu, H. Shang, E. Aronson, Elevated O<sub>3</sub> alters soil bacterial and fungal communities and the dynamics of carbon and nitrogen. *Sci. Total Environ.* **677**, 272–280 (2019).
128. J. Dunbar, L. V. Gallegos-Graves, B. Steven, R. Mueller, C. Hesse, D. R. Zak, C. R. Kuske, Surface soil fungal and bacterial communities in aspen stands are resilient to eleven years of elevated CO<sub>2</sub> and O<sub>3</sub>. *Soil Biol. Biochem.* **76**, 227–234 (2014).
129. Z. He, J. Xiong, A. D. Kent, Y. Deng, K. Xue, G. Wang, L. Wu, J. D. Van Nostrand, J. Zhou, Distinct responses of soil microbial communities to elevated CO<sub>2</sub> and O<sub>3</sub> in a soybean agro-ecosystem. *ISME J.* **8**, 714–726 (2014).
130. J. Zhang, H. Tang, J. Zhu, X. Lin, Y. Feng, Effects of elevated ground-level ozone on paddy soil bacterial community and assembly mechanisms across four years. *Sci. Total Environ.* **654**, 505–513 (2019).
131. P. Wang, E. L. Marsh, E. A. Ainsworth, A. D. B. Leakey, A. M. Sheflin, D. P. Schachtman, Shifts in microbial communities in soil, rhizosphere and roots of two major crop systems under elevated CO<sub>2</sub> and O<sub>3</sub>. *Sci. Rep.* **7**, 15019 (2017).
132. J. Wang, F. Hayes, R. Turner, D. R. Chadwick, G. Mills, D. L. Jones, Effects of four years of elevated ozone on microbial biomass and extracellular enzyme activities in a semi-natural grassland. *Sci. Total Environ.* **660**, 260–268 (2019).
133. D. A. Grantz, S. Gunn, H.-B. Vu, O<sub>3</sub> impacts on plant development: A meta-analysis of root/shoot allocation and growth. *Plant Cell Environ.* **29**, 1193–1209 (2006).
134. N. Akhtar, M. Yamaguchi, H. Inada, D. Hoshino, T. Kondo, M. Fukami, R. Funada, T. Izuta, Effects of ozone on growth, yield and leaf gas exchange rates of four Bangladeshi cultivars of rice (*Oryza sativa* L.). *Environ. Pollut.* **158**, 2970–2976 (2010).
135. H. Tang, G. Liu, J. Zhu, K. Kobayashi, Effects of elevated ozone concentration on CH<sub>4</sub> and N<sub>2</sub>O emission from paddy soil under fully open-air field conditions. *Glob. Chang. Biol.* **21**, 1727–1736 (2015).
136. A. M. Betzelberger, K. M. Gillespie, J. M. McGrath, R. P. Koester, R. L. Nelson, E. A. Ainsworth, Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. *Plant Cell Environ.* **33**, 1569–1581 (2010).
137. D. Simpson, A. Arneth, G. Mills, S. Solberg, J. Uddling, Ozone — The persistent menace: Interactions with the N cycle and climate change. *Curr. Opin. Environ. Sustain.* **9–10**, 9–19 (2014).
138. M. Vitale, W. Amirano, Y. Hoshika, E. Paoletti, Plant species-specific litter decomposition rates are directly affected by tropospheric ozone: Analysis of trends and modelling. *Wat. Air, Soil Pollut.* **230**, 311 (2019).
139. H. Wu, Q. Li, C. Lu, L. Zhang, J. Zhu, F. A. Dijkstra, Q. Yu, Elevated ozone effects on soil nitrogen cycling differ among wheat cultivars. *Appl. Soil Ecol.* **108**, 187–194 (2016).
140. H. Rennenberg, M. Dannenmann, A. Gessler, J. Kreuzwieser, J. Simon, H. Papen, Nitrogen balance in forest soils: Nutritional limitation of plants under climate change stresses. *Plant Biol.* **11**, 4–23 (2009).
141. B. Gielen, M. Löw, G. Deckmyn, U. Metzger, F. Franck, C. Heerd, R. Matyssek, R. Valcke, R. Ceulemans, Chronic ozone exposure affects leaf senescence of adult beech trees: A chlorophyll fluorescence approach. *J. Exp. Bot.* **58**, 785–795 (2007).
142. Z. Feng, S. Wang, Z. Szantoi, S. Chen, X. Wang, Protection of plants from ambient ozone by applications of ethylenediurea (EDU): A meta-analytic review. *Environ. Pollut.* **158**, 3236–3242 (2010).
143. A. Kasurinen, P. A. Peltonen, R. Julkunen-Tiitto, E. Vapaavuori, V. Nuutinen, T. Holopainen, J. K. Holopainen, Effects of elevated CO<sub>2</sub> and O<sub>3</sub> on leaf litter phenolics and subsequent performance of litter-feeding soil macrofauna. *Plant Soil* **292**, 25–43 (2007).
144. E. Agathokleous, M. Kitao, C. Qingnan, C. J. Saitanis, E. Paoletti, W. J. Manning, T. Watanabe, T. Koike, Effects of ozone (O<sub>3</sub>) and ethylenediurea (EDU) on the ecological stoichiometry of a willow grown in a free-air exposure system. *Environ. Pollut.* **238**, 663–676 (2018).
145. C. Shi, T. Watanabe, T. Koike, Leaf stoichiometry of deciduous tree species in different soils exposed to free-air O<sub>3</sub> enrichment over two growing seasons. *Environ. Exp. Bot.* **138**, 148–163 (2017).
146. N. T. Edwards, Root and soil respiration responses to ozone in *Pinus taeda* L. seedlings. *New Phytol.* **118**, 315–321 (1991).
147. J. K. McCrady, C. P. Andersen, The effect of ozone on below-ground carbon allocation in wheat. *Environ. Pollut.* **107**, 465–472 (2000).
148. W. F. J. Parsons, J. G. Bockheim, R. L. Lindroth, Independent, interactive, and species-specific responses of leaf litter decomposition to elevated CO<sub>2</sub> and O<sub>3</sub> in a northern hardwood forest. *Ecosystems* **11**, 505–519 (2008).
149. X. Li, Y. Deng, Q. Li, C. Lu, J. Wang, H. Zhang, J. Zhu, J. Zhou, Z. He, Shifts of functional gene representation in wheat rhizosphere microbial communities under elevated ozone. *ISME J.* **7**, 660–671 (2013).
150. T. Dolker, A. Mukherjee, S. B. Agrawal, M. Agrawal, Responses of a semi-natural grassland community of tropical region to elevated ozone: An assessment of soil dynamics and biomass accumulation. *Sci. Total Environ.* **718**, 137141 (2020).
151. J. L. Larson, D. R. Zak, R. L. Sinsabaugh, Extracellular enzyme activity beneath temperate trees growing under elevated carbon dioxide and ozone. *Soil Sci. Soc. Am. J.* **66**, 1848–1856 (2002).
152. Y. Feng, X. Lin, Y. Yu, H. Zhang, H. Chu, J. Zhu, Elevated ground-level O<sub>3</sub> negatively influences paddy methanogenic archaeal community. *Sci. Rep.* **3**, 3193 (2013).
153. C. Decock, J. Six, Effects of elevated CO<sub>2</sub> and O<sub>3</sub> on N-cycling and N<sub>2</sub>O emissions: A short-term laboratory assessment. *Plant Soil* **351**, 277–292 (2012).
154. W. E. Holmes, D. R. Zak, K. S. Pregitzer, J. S. King, Soil nitrogen transformations under *Populus tremuloides*, *Betula papyrifera* and *Acer saccharum* following 3 years exposure to elevated CO<sub>2</sub> and O<sub>3</sub>. *Glob. Chang. Biol.* **9**, 1743–1750 (2003).
155. W. E. Holmes, D. R. Zak, K. S. Pregitzer, J. S. King, Elevated CO<sub>2</sub> and O<sub>3</sub> alter soil nitrogen transformations beneath trembling aspen, paper birch, and sugar maple. *Ecosystems* **9**, 1354–1363 (2006).
156. T. Kanerva, A. Palojärvi, K. Rämö, K. Ojanperä, M. Esala, S. Manninen, A 3-year exposure to CO<sub>2</sub> and O<sub>3</sub> induced minor changes in soil N cycling in a meadow ecosystem. *Plant Soil* **286**, 61–73 (2006).
157. E. I. Pujol Pereira, H. Chung, K. Scow, M. J. Sadowsky, C. Van Kessel, J. Six, Soil nitrogen transformations under elevated atmospheric CO<sub>2</sub> and O<sub>3</sub> during the soybean growing season. *Environ. Pollut.* **159**, 401–407 (2011).
158. D. K. L. Hewitt, G. Mills, F. Hayes, S. Wilkinson, W. Davies, Highlighting the threat from current and near-future ozone pollution to clover in pasture. *Environ. Pollut.* **189**, 111–117 (2014).

159. T. Kanerva, K. Regina, K. Rämö, K. Ojanperä, S. Manninen, Fluxes of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub> in a meadow ecosystem exposed to elevated ozone and carbon dioxide for three years. *Environ. Pollut.* **145**, 818–828 (2007).
160. Y. Qiu, Y. Jiang, L. Guo, K. O. Burkley, R. W. Zobel, H. D. Shew, S. Hu, Contrasting warming and ozone effects on denitrifiers dominate soil N<sub>2</sub>O emissions. *Environ. Sci. Technol.* **52**, 10956–10966 (2018).
161. A. Bhatia, A. Ghosh, V. Kumar, R. Tomer, S. D. Singh, H. Pathak, Effect of elevated tropospheric ozone on methane and nitrous oxide emission from rice soil in north India. *Agric. Ecosyst. Environ.* **144**, 21–28 (2011).
162. T. J. Kou, X. H. Cheng, J. G. Zhu, Z. B. Xie, The influence of ozone pollution on CO<sub>2</sub>, CH<sub>4</sub>, and N<sub>2</sub>O emissions from a Chinese subtropical rice–wheat rotation system under free-air O<sub>3</sub> exposure. *Agric. Ecosyst. Environ.* **204**, 72–81 (2015).
163. T. Kou, X. Hang, S. K. Lam, D. Chen, J. He, Ozone pollution increases CO<sub>2</sub> and N<sub>2</sub>O emissions in ozone-sensitive wheat system. *Agron. J.* **110**, 496–502 (2018).
164. X. Bao, J. Yu, W. Liang, C. Lu, J. Zhu, Q. Li, The interactive effects of elevated ozone and wheat cultivars on soil microbial community composition and metabolic diversity. *Appl. Soil Ecol.* **87**, 11–18 (2015).
165. T. Kanerva, A. Palojärvi, K. Rämö, S. Manninen, Changes in soil microbial community structure under elevated tropospheric O<sub>3</sub> and CO<sub>2</sub>. *Soil Biol. Biochem.* **40**, 2502–2510 (2008).
166. R. L. Phillips, D. R. Zak, W. E. Holmes, D. C. White, Microbial community composition and function beneath temperate trees exposed to elevated atmospheric carbon dioxide and ozone. *Oecologia* **131**, 236–244 (2002).
167. S. K. Mörsky, J. K. Haapala, R. Rinnan, P. Tiiva, S. Saarnio, J. Silvola, T. Holopainen, P. J. Martikainen, Long-term ozone effects on vegetation, microbial community and methane dynamics of boreal peatland microcosms in open-field conditions. *Glob. Chang. Biol.* **14**, 1891–1903 (2008).
168. P. S. Nikolova, C. P. Andersen, H. Blaschke, R. Matyssek, K.-H. Häberle, Belowground effects of enhanced tropospheric ozone and drought in a beech/spruce forest (*Fagus sylvatica* L./*Picea abies* [L.] Karst). *Environ. Pollut.* **158**, 1071–1078 (2010).
169. Y. Ueda, K. Frindte, C. Knief, M. D. Ashrafuzzaman, M. Frei, Effects of elevated tropospheric ozone concentration on the bacterial community in the phyllosphere and rhizosphere of rice. *PLOS ONE*. **11**, e016317 (2016).
170. A. Kasurinen, M. M. Keinänen, S. Kaipainen, L.-O. Nilsson, E. Vapaavuori, M. H. Kontro, T. Holopainen, Below-ground responses of silver birch trees exposed to elevated CO<sub>2</sub> and O<sub>3</sub> levels during three growing seasons. *Glob. Chang. Biol.* **11**, 1167–1179 (2005).
171. T. Mrak, I. Straus, T. Grebenc, J. Gričar, Y. Hoshika, G. Carriero, E. Paoletti, H. Kraigher, Different belowground responses to elevated ozone and soil water deficit in three European oak species (*Quercus ilex*, *Q. pubescens* and *Q. robur*). *Sci. Total Environ.* **651**, 1310–1320 (2019).
172. J. M. Fraterrigo, T. C. Balsler, M. G. Turner, Microbial community variation and its relationship with nitrogen mineralization in historically altered forests. *Ecology* **87**, 570–579 (2006).
173. T. Rudrappa, K. J. Czymmek, P. W. Paré, H. P. Bais, Root-secreted malic acid recruits beneficial soil bacteria. *Plant Physiol.* **148**, 1547–1556 (2008).
174. J. Sasse, E. Martinoia, T. Northen, Feed your friends: Do plant exudates shape the root microbiome? *Trends Plant Sci.* **23**, 25–41 (2018).
175. M. Szoboszlai, A. White-Monsant, L. A. Moe, The effect of root exudate 7,4'-dihydroxyflavone and naringenin on soil bacterial community structure. *PLOS ONE*. **11**, e0146555 (2016).
176. C. Preece, J. Peñuelas, A return to the wild: Root exudates and food security. *Trends Plant Sci.* **25**, 14–21 (2020).
177. C. Preece, G. Farré-Armengol, J. Llusà, J. Peñuelas, Thirsty tree roots exude more carbon. *Tree Physiol.* **38**, 690–695 (2018).
178. C. Preece, J. Peñuelas, Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. *Plant Soil* **409**, 1–17 (2016).
179. Z. Chen, X. Wang, Z. Feng, Q. Xiao, X. Duan, Impact of elevated O<sub>3</sub> on soil microbial community function under wheat crop. *Wat. Air. Soil Pollut.* **198**, 189–198 (2009).
180. Z. Chen, X.-k. Wang, F.-x. Yao, Z.-z. Zheng, Z. Feng, Elevated ozone changed soil microbial community in a rice paddy. *Soil Sci. Soc. Am. J.* **74**, 829–837 (2010).
181. A. B. Dohrmann, C. C. Tebbe, Effect of elevated tropospheric ozone on the structure of bacterial communities inhabiting the rhizosphere of herbaceous plants native to Germany. *Appl. Environ. Microbiol.* **71**, 7750–7758 (2005).
182. X. Wang, L. Qu, Q. Mao, M. Watanabe, Y. Hoshika, A. Koyama, K. Kawaguchi, Y. Tamai, T. Koike, Ectomycorrhizal colonization and growth of the hybrid larch F<sub>1</sub> under elevated CO<sub>2</sub> and O<sub>3</sub>. *Environ. Pollut.* **197**, 116–126 (2015).
183. Y. Feng, Y. Yu, H. Tang, Q. Zu, J. Zhu, X. Lin, The contrasting responses of soil microorganisms in two rice cultivars to elevated ground-level ozone. *Environ. Pollut.* **197**, 195–202 (2015).
184. Q. Li, Y. Yang, X. Bao, F. Liu, W. Liang, J. Zhu, T. M. Bezemer, W. H. van der Putten, Legacy effects of elevated ozone on soil biota and plant growth. *Soil Biol. Biochem.* **91**, 50–57 (2015).
185. J. Liang, T. W. Crowther, N. Picard, S. Wiser, M. Zhou, G. Alberti, E.-D. Schulze, A. D. McGuire, F. Bozzato, H. Pretzsch, S. de-Miguel, A. Paquette, B. Hérault, M. Scherer-Lorenzen, C. B. Barrett, H. B. Glick, G. M. Hengeveld, G.-J. Nabuurs, S. Pfautsch, H. Viana, A. C. Vibrans, C. Ammer, P. Schall, D. Verbyla, N. Tchekabakova, M. Fischer, J. V. Watson, H. Y. H. Chen, X. Lei, M.-J. Schelhaas, H. Lu, D. Gianelle, E. I. Parfenova, C. Salas, E. Lee, B. Lee, H. S. Kim, H. Bruehlheide, D. A. Coomes, D. Piotta, T. Sunderland, B. Schmid, S. Gourlet-Fleury, B. Sonké, R. Tavani, J. Zhu, S. Brandl, J. Vayreda, F. Kitahara, E. B. Searle, V. J. Neldner, M. R. Ngugi, C. Baraloto, L. Frizzera, R. Balazy, J. Oleksyn, T. Zawila-Niedzwiecki, O. Bouriaud, F. Bussotti, L. Finér, B. Jaroszewicz, T. Jucker, F. Valladares, A. M. Jagodzinski, P. L. Peri, C. Gonmadje, W. Marthy, T. O'Brien, E. H. Martin, A. R. Marshall, F. Rovero, R. Bitarho, P. A. Niklaus, P. Alvarez-Loayza, N. Chamuya, R. Valencia, F. Mortier, V. Wortel, N. L. Engone-Obiang, L. V. Ferreira, D. E. Odeke, R. M. Vasquez, S. L. Lewis, P. B. Reich, Positive biodiversity-productivity relationship predominant in global forests. *Science* **354**, aaf8957 (2016).
186. J.-F. Lamarque, D. T. Shindell, B. Josse, P. J. Young, I. Cionni, V. Eyring, D. Bergmann, P. Cameron-Smith, W. J. Collins, R. Doherty, S. Dalsoren, G. Faluvegi, G. Folberth, S. J. Ghan, L. W. Horowitz, Y. H. Lee, I. A. MacKenzie, T. Nagashima, V. Naik, D. Plummer, S. O. Righi, S. T. Rumbold, M. Schulz, R. B. Skeie, D. S. Stevenson, S. Strode, K. Sudo, S. Szopa, A. Voulgarakis, G. Zeng, The Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP): Overview and description of models, simulations and climate diagnostics. *Geosci. Model Dev.* **6**, 179–206 (2013).
187. G. Myhre, D. Shindell, F.-M. Bréon, W. Collins, J. Fuglestedt, J. Huang, D. Koch, J.-F. Lamarque, D. Lee, B. Mendoza, T. Nakajima, A. Robock, G. Stephens, T. Takemura, H. Zhang, Anthropogenic and natural radiative forcing, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P. M. Midgley, Eds. (Cambridge University Press, 2013), pp. 659–740.
188. B. Kirtman, S. B. Power, J. A. Adedoyin, G. J. Boer, R. Bojariu, I. Camilloni, F. Doblas-Reyes, A. M. Fiore, M. Kimoto, G. Meehl, M. Prather, A. Sarr, C. Schär, R. Sutton, G. Van Oldenborgh, G. Vecchi, H. J. Wang, Near-term climate change: Projections and predictability, in *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, T. F. Stocker, D. Qin, G.-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex, P. M. Midgley, Eds. (Cambridge Univ. Press, 2013), pp. 953–1028.
189. P. J. Young, A. T. Archibald, K. W. Bowman, J.-F. Lamarque, V. Naik, D. S. Stevenson, S. Tilmes, A. Voulgarakis, O. Wild, D. Bergmann, P. Cameron-Smith, I. Cionni, W. J. Collins, S. B. Dalsoren, R. M. Doherty, V. Eyring, G. Faluvegi, L. W. Horowitz, B. Josse, Y. H. Lee, I. A. MacKenzie, T. Nagashima, D. A. Plummer, M. Righi, S. T. Rumbold, R. B. Skeie, D. T. Shindell, S. A. Strode, K. Sudo, S. Szopa, G. Zeng, Pre-industrial to end 21st-century projections of tropospheric ozone from the Atmospheric Chemistry and Climate Model Intercomparison Project (ACCMIP). *Atmos. Chem. Phys.* **13**, 2063–2090 (2013).
190. N. Boussez, J. L. Attié, V. H. Peuch, M. Michou, G. Pfister, D. Edwards, L. Emmons, C. Mari, B. Barret, S. R. Arnold, A. Heckel, A. Richter, H. Schlager, A. Lewis, M. A. Avery, G. W. Sachse, E. V. Browell, J. W. Hair, Evaluation of the MOCAGE chemistry transport model during the ICARTT/ITOP experiment. *J. Geophys. Res. Atmos.* **112**, D10S42 (2007).
191. H. Teyssède, M. Michou, H. L. Clark, B. Josse, F. Karcher, D. Olivé, V.-H. Peuch, D. Saint-Martin, D. Cariolle, J.-L. Attié, P. Nédélec, P. Ricaud, V. Thouret, R. J. van der A, A. Volz-Thomas, F. Chéroux, A new tropospheric and stratospheric Chemistry and Transport Model MOCAGE-Climat for multi-year studies: Evaluation of the present-day climatology and sensitivity to surface processes. *Atmos. Chem. Phys.* **7**, 5815–5860 (2007).
192. E. Agathokleous, M. Kitao, Y. Kinose, A review study on ozone phytotoxicity metrics for setting critical levels in Asia. *Asian J. Atmos. Environ.* **12**, 1–16 (2018).
193. A. Anav, A. De Marco, C. Proietti, A. Alessandri, A. Dell'Aquila, I. Cionni, P. Friedlingstein, D. Khvorostyanov, L. Menut, E. Paoletti, P. Sicard, S. Sitch, M. Vitale, Comparing concentration-based (AOT40) and stomatal uptake (PODY) metrics for ozone risk assessment to European forests. *Glob. Chang. Biol.* **22**, 1608–1627 (2016).
194. G. Mills, A. Buse, B. Gimeno, V. Bermejo, M. Holland, L. Emberson, H. Pleijel, A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. *Atmos. Environ.* **41**, 2630–2643 (2007).
195. LRTAP Convention, *Manual on Methodologies and Criteria for Modelling and Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends. Chapter 3 Mapping Critical Levels for Vegetation* (UNECE Convention on Long-range Transboundary Air Pollution, 2017); <https://icpvegetation.ceh.ac.uk>.
196. E. Paoletti, W. J. Manning, Toward a biologically significant and usable standard for ozone that will also protect plants. *Environ. Pollut.* **150**, 85–95 (2007).
197. G. Kier, H. Kreft, T. M. Lee, W. Jetz, P. L. Ibsch, C. Nowicki, J. Mutke, W. Barthlott, A global assessment of endemism and species richness across island and mainland regions. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 9322–9327 (2009).

198. M. M. Millán, E. Mantilla, R. Salvador, A. Carratalá, M. J. Sanz, L. Alonso, G. Gangoiti, M. Navazo, Ozone cycles in the western Mediterranean basin: Interpretation of monitoring data in complex terrain. *J. Appl. Meteorol.* **39**, 487–507 (2000).
199. P. Sicard, A. De Marco, F. Troussier, C. Renou, N. Vas, E. Paoletti, Decrease in surface ozone concentrations at Mediterranean remote sites and increase in the cities. *Atmos. Environ.* **79**, 705–715 (2013).
200. E. Oksanen, Responses of selected birch (*Betula pendula* Roth) clones to ozone change over time. *Plant Cell Environ.* **26**, 875–886 (2003).
201. E. A. Ainsworth, Understanding and improving global crop response to ozone pollution. *Plant J.* **90**, 886–897 (2017).
202. C. Vacher, A. Hampe, A. J. Porté, U. Sauer, S. Compant, C. E. Morris, The phyllosphere: Microbial jungle at the plant-climate interface. *Annu. Rev. Ecol. Evol. Syst.* **47**, 1–24 (2016).
203. J. A. Vorholt, Microbial life in the phyllosphere. *Nat. Rev. Microbiol.* **10**, 828–840 (2012).
204. V. E. Wittig, E. A. Ainsworth, S. L. Naidu, D. F. Karnosky, S. P. Long, Quantifying the impact of current and future tropospheric ozone on tree biomass, growth, physiology and biochemistry: A quantitative meta-analysis. *Glob. Chang. Biol.* **15**, 396–424 (2009).
205. R. Matyssek, G. Bahnweg, R. Ceulemans, P. Fabian, D. Grill, D. E. Hanke, H. Kraigher, W. Oßwald, H. Rennenberg, H. Sandermann, M. Tausz, G. Wieser, Synopsis of the CASIROZ case study: Carbon sink strength of *Fagus sylvatica* L. in a changing environment—experimental risk assessment of mitigation by chronic ozone impact. *Plant Biol.* **9**, 163–180 (2007).
206. A. S. Lefohn, H. Wernli, D. Shadwick, S. J. Oltmans, M. Shapiro, Quantifying the frequency of stratospheric-tropospheric transport affecting enhanced surface ozone concentrations at high- and low-elevation monitoring sites in the United States. *Atmos. Environ.* **62**, 646–656 (2012).
207. P. Sicard, R. Serra, P. Rossello, Spatiotemporal trends in ground-level ozone concentrations and metrics in France over the time period 1999–2012. *Environ. Res.* **149**, 122–144 (2016).
208. M. Legrand, S. Preunkert, B. Jourdain, H. Gallée, F. Goutail, R. Weller, J. Savarino, Year-round record of surface ozone at coastal (Dumont d’Urville) and inland (Concordia) sites in East Antarctica. *J. Geophys. Res. Atmos.* **114**, D20306 (2009).
209. R. G. Derwent, C. S. Witham, S. R. Utembe, M. E. Jenkin, N. R. Passant, Ozone in Central England: The impact of 20 years of precursor emission controls in Europe. *Environ. Sci. Policy* **13**, 195–204 (2010).
210. T. W. Walker, D. B. A. Jones, M. Parrington, D. K. Henze, L. T. Murray, J. W. Bottenheim, K. Anlauf, J. R. Worden, K. W. Bowman, C. Shim, K. Singh, M. Kopacz, D. W. Tarasick, J. Davies, P. Von Der Gathen, A. M. Thompson, C. C. Carouge, Impacts of midlatitude precursor emissions and local photochemistry on ozone abundances in the Arctic. *J. Geophys. Res. Atmos.* **117**, D01305 (2012).
211. J. L. Innes, J. M. Skelly, M. Schaub, *Ozone and Broadleaved Species. A Guide to the Identification of Ozone-Induced Foliar Injury* (Birmensdorf: Eidgenössische Forschungsanstalt WSL, 2001).
212. M. Schaub, P. Jakob, L. Bernhardt, J. L. Innes, J. M. Skelly, N. Kräuchi, *Ozone Injury Database* (Swiss Federal Research Institute WSL, 2002).
213. P. Sicard, N. Vas, V. Calatayud, F. J. Garcia-Breijo, J. Reig-Armiñana, M. J. Sanz, L. Dalstein-Richier, Dommages forestiers et pollution à l’ozone dans les réserves naturelles: Le cas de l’arolle dans le sud-est de la France. *Forêt Méditerranéenne*. **31**, 273–286 (2010).
214. K. O. Burkey, E. Agathokleous, C. J. Saitanis, A. M. Mashaheet, T. Koike, Y.-T. Hung, in *Handbook of Environmental and Waste Management Volume 3, Acid Rain and Greenhouse Gas Pollution Control*, Y.-T. Hung, L. K. Wang, N. K. Shammass, Eds. (World Scientific Publishing Co., 2020), p. 1055. ISBN-10: 9811207127.
215. L. Dalstein, X. Torti, D. Le Thiec, P. Dizengremel, Physiological study of declining cembro pines (*Pinus cembra* L.) in southern France. *Trees* **16**, 299–305 (2002).
216. L. Dalstein, N. Vas, F. Tagliaferro, A. M. Ferrara, F. Spaziani, Effets de l’ozone sur la forêt et la végétation dans les Alpes franco-italiennes. *Forêt méditerranéenne* **2**, 149–156 (2005).
217. G. Wieser, W. J. Manning, M. Tausz, A. Bytnerowicz, Evidence for potential impacts of ozone on *Pinus cembra* L. at mountain sites in Europe: An overview. *Environ. Pollut.* **139**, 53–58 (2006).
218. V. Calatayud, J. Cerveró, M. J. Sanz, Foliar, physiological and growth responses of four maple species exposed to ozone. *Water Air Soil Pollut.* **185**, 239–254 (2007).
219. M. S. Günthardt-Goerg, P. Vollenweider, Linking stress with macroscopic and microscopic leaf response in trees: New diagnostic perspectives. *Environ. Pollut.* **147**, 467–488 (2007).
220. N. Conran, E. Paoletti, Visible foliar injury and physiological responses to ozone in Italian provenances of *Fraxinus excelsior* and *F. ornus*. *ScientificWorldJournal* **7**, 90–97 (2007).
221. N. E. Grulke, E. Paoletti, R. L. Heath, Comparison of calculated and measured foliar O<sub>3</sub> flux in crop and forest species. *Environ. Pollut.* **146**, 640–647 (2007).
222. K. Novak, M. Schaub, J. Fuhrer, J. M. Skelly, B. Frey, N. Kräuchi, Ozone effects on visible foliar injury and growth of *Fagus sylvatica* and *Viburnum lantana* seedlings grown in monoculture or in mixture. *Environ. Exp. Bot.* **62**, 212–220 (2008).
223. S. N. Singh, *Climate Change and Crops. Environmental Science and Engineering* (Springer-Verlag, 2009).
224. M. Schaub, V. Calatayud, M. Ferretti, G. Brunialti, G. Lövblad, G. Krause, M. J. Sanz, in *Manual on Methods and Criteria for Harmonized Sampling, Assessment, Monitoring and Analysis of the Effects of Air Pollution on Forests* (UNECE ICP Forests Programme, 2010), p. 22.
225. J. M. Skelly, J. L. Innes, J. E. Savage, K. R. Snyder, D. Vanderheyden, J. Zhang, M. J. Sanz, in *Water, Air, and Soil Pollution* (Springer, 1999), vol. 116, pp. 227–234.
226. C. J. Saitanis, S. M. Bari, K. O. Burkey, D. Stamatelopoulos, E. Agathokleous, Screening of Bangladeshi winter wheat (*Triticum aestivum* L.) cultivars for sensitivity to ozone. *Environ. Sci. Pollut. Res.* **21**, 13560–13571 (2014).
227. S. Brace, D. L. Peterson, D. Bowers, *A Guide to Ozone Injury in Vascular Plants of the Pacific Northwest* - [United States Department of Agriculture Forest Service, Pacific Northwest Research Station, General Technical Report PNW-GTR-446, September 1999 (1999)].
228. M. J. Sanz, G. Sanchez, V. Calatayud, M. T. Minaya, J. Cervero, *La Contaminación Atmosférica en los Bosques: Guía para la identificación de daños visibles causados por ozono* (Organismo Autónomo de Parques Nacionales, 2001).
229. C. J. Saitanis, M. G. Karandinos, Effects of ozone on tobacco (*Nicotiana tabacum* L.) varieties. *J. Agron. Crop Sci.* **188**, 51–58 (2002).
230. E. Agathokleous, C. J. Saitanis, K. O. Burkey, G. Ntatsi, V. Vougeleka, A. M. Mashaheet, A. Pallides, Application and further characterization of the snap bean S156/R123 ozone biomonitoring system in relation to ambient air temperature. *Sci. Total Environ.* **580**, 1046–1055 (2017).
231. K. Stolte, Symptomatology of Ozone Injury to Pine Foliage, in *Evaluating Ozone Air Pollution Effects on Pines in the Western United States*, P. R. Miller, K. W. Stolte, D. M. Duriscoe, J. Pronos, Eds. (USDA Forest Service General Technical Report PSW-GTR-155, Pacific Southwest Research Station, Forest Service US Department of Agriculture, 1996).
232. F. Tagliaferro, A. M. Ferrara, F. Spaziani, E. Viotto, Sintomi fogliari di tipo ozono like su vegetazione spontanea e ornamentale in Piemonte. *Linea Ecol.* **27**, 47–53 (2005).
233. G. Wieser, R. Häslér, B. Götz, W. Koch, W. M. Havranek, Role of climate, crown position, tree age and altitude in calculated ozone flux into needles of *Picea abies* and *Pinus cembra*: A synthesis. *Environ. Pollut.* **109**, 415–422 (2000).
234. P. Sicard, L. Dalstein-richier, N. Vas, Annual and seasonal trends of ambient ozone concentration and its impact on forest vegetation in Mercantour National Park (South-eastern France) over the 2000–2008 period. *Environ. Pollut.* **159**, 351–362 (2011).
235. M. S. Günthardt-Goerg, P. Vollenweider, C. J. McQuattie, Differentiation of ozone, heavy metal or biotic stress in leaves and needles. *Ekologia*. **22**, 110–113 (2003).
236. P. Vollenweider, M. Ottiger, M. S. Günthardt-Goerg, Validation of leaf ozone symptoms in natural vegetation using microscopical methods. *Environ. Pollut.* **124**, 101–118 (2003).
237. R. Kohut, *Handbook for Assessment of Foliar Ozone Injury on Vegetation in the National Parks* (National Park Service, U.S. Department of the Interior, Air Resources Division, 2005).
238. P. Vollenweider, M. S. Günthardt-Goerg, Diagnosis of abiotic and biotic stress factors using the visible symptoms in foliage. *Environ. Pollut.* **137**, 455–465 (2005).
239. S. Anttonen, J. Herranen, P. Peura, L. Kärenlampi, Fatty acids and ultrastructure of ozone-exposed Aleppo pine (*Pinus halepensis* Mill.) needles. *Environ. Pollut.* **87**, 235–242 (1995).
240. M. Kivimäenpää, S. Sutinen, V. Calatayud, M. J. Sanz, Visible and microscopic needle alterations of mature Aleppo pine (*Pinus halepensis*) trees growing on an ozone gradient in eastern Spain. *Tree Physiol.* **30**, 541–554 (2010).
241. T. Holopainen, S. Anttonen, A. Wulff, V. Palomäki, L. Kärenlampi, Comparative evaluation of the effects of gaseous pollutants, acidic deposition and mineral deficiencies: Structural changes in the cells of forest plants. *Agric. Ecosyst. Environ.* **42**, 365–398 (1992).
242. E. Pääkkönen, T. Holopainen, L. Kärenlampi, Ageing-related anatomical and ultrastructural changes in leaves of birch (*Betula pendula* Roth.) clones as affected by low ozone exposure. *Ann. Bot.* **75**, 285–294 (1995).
243. T. N. Mikkelsen, H. S. Heide-Jørgensen, Acceleration of leaf senescence in *Fagus sylvatica* L. by low levels of tropospheric ozone demonstrated by leaf colour, chlorophyll fluorescence and chloroplast ultrastructure. *Trees* **10**, 145–156 (1996).
244. L. Nazarenko, G. A. Schmidt, R. L. Miller, N. Tausnev, M. Kelley, R. Ruedy, G. L. Russell, I. Aleinov, M. Bauer, S. Bauer, R. Bleck, V. Canuto, Y. Cheng, T. L. Clune, A. D. Del Genio, G. Faluvegi, J. E. Hansen, R. J. Healy, N. Y. Kiang, D. Koch, A. A. Lacis, A. N. Le Grande, J. Lerner, K. K. Lo, S. Menon, V. Oinas, J. Perlwitz, M. J. Puma, D. Rind, A. Romanou, M. Sato, D. T. Shindell, S. Sun, K. Tsigaridis, N. Unger, A. Voulgarakis, M.-S. Yao, J. Zhang, Future climate change under RCP emission scenarios with GISS ModelE2. *J. Adv. Model. Earth Syst.* **7**, 244–267 (2015).
245. Intergovernmental Panel on Climate Change, in *Climate Change 2014: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*, C. B. Field, V. R. Barros, D. J. Dokken, K. J. Mach, M. D. Mastrandrea, T. E. Bilir, M. Chatterjee, K. L. Ebi, Y. O. Estrada, R. C. Genova, B. Girma, E. S. Kissel, A. N. Levy, S. MacCracken, P. R. Mastrandrea, L. L. White, Eds. (Cambridge Univ. Press, 2014), 1132 pp.



246. D. J. Seidel, Q. Fu, W. J. Randel, T. J. Reiohler, Widening of the tropical belt in a changing climate. *Nat. Geosci.* **1**, 21–24 (2008).
247. J. P. Tuovinen, Assessing vegetation exposure to ozone: Properties of the AOT40 index and modifications by deposition modelling. *Environ. Pollut.* **109**, 361–372 (2000).
248. P. A. Evans, M. R. Ashmore, The effects of ambient air on a semi-natural grassland community. *Agric. Ecosyst. Environ.* **38**, 91–97 (1992).
249. K. Rämö, T. Kanerva, S. Nikula, K. Ojanperä, S. Manninen, Influences of elevated ozone and carbon dioxide in growth responses of lowland hay meadow mesocosms. *Environ. Pollut.* **144**, 101–111 (2006).
250. H. Pleijel, G. Pihl Karlsson, E. Sild, H. Danielsson, L. Skärby, G. Sellén, Exposure of a grass-clover mixture to ozone in open-top chambers - Effects on yield, quality and botanical composition. *Agric. Ecosyst. Environ.* **59**, 55–62 (1996).
251. M. Volk, P. Bungener, F. Contat, M. Montani, J. Fuhrer, Grassland yield declined by a quarter in 5 years of free-air ozone fumigation. *Glob. Chang. Biol.* **12**, 74–83 (2006).

**Acknowledgments:** We are grateful to H. Kreft, Professor at the University of Bonn, Germany, for providing the dataset of endemic richness and for helpful suggestions. This paper was prepared within the International Union of Forest Research Organizations (IUFRO) Research Group 8.04.00 “Air Pollution and Climate Change” and under the Working Party 8.04.05 “Ground-level ozone.” **Funding:** Z.F., E.A., X.Y., and Z.L. acknowledge funding from the National Natural Science Foundation of China (nos. 41771034, 31950410547, 41907383, and 31901159). E.A. and Z.F. acknowledge multiyear support from The Startup Foundation for Introducing Talent of Nanjing University of Information Science and Technology (NUIST), Nanjing, China (no. 003080 to E.A. and no. 002992 to Z.F.). E.O. acknowledges funding from the Academy of Finland (BETUMICS project 284931). J.D.B. acknowledges funding from Academy of Finland decision number 309425. V.C. thanks the project ELEMENTAL (CGL 2017-83538-C3-3-R, MINECO-FEDER). J.P. acknowledges the European Research Council Synergy grant ERC-SyG-2013-610028 IMBALANCE-P. The sponsors were not involved in the study design; the collection, analysis, or interpretation of the data; the preparation of the

manuscript; or the decision where to submit the manuscript for publication. **Author contributions:** E.A., Z.F., and E.P. initially designed the study. E.A. had a leading role, served as the hub of communication among the authors, and supervised the production of the manuscript. E.A., E.O., Q.W., V.A., and J.D.B. reviewed the literature. E.A. drafted Introduction. E.O. and E.A. each drafted a version of the plant section, with inputs from F.H., and generated the final section. Z.F., E.P., M.D., C.J.S., V.C., M.V., and H.H. reviewed the plant section. P.S. and A.D.M. conducted the analyses of global O<sub>3</sub> exposure and plant diversity and wrote the results with inputs from E.A. V.A., J.D.B., and E.A. drafted the section on insects, with inputs from Z.L. and X.Y. V.C., E.P., and C.J.S. reviewed the section on insects. Q.W. drafted the section on microbes, with inputs from E.A. S.D.V., J.P., and J.D.B. reviewed the section on the microbiota. E.O., Z.F., and E.A. drafted the “Unresolved questions” section. E.A. created Table 1 and table S1 and drafted Abstract and Conclusions. P.S. created Fig. 3 and Table 2. C.J.S. created Figs. 1 and 2 with inputs from E.A. E.A. and Z.F. edited all reviewed sections and produced an integrated manuscript. All co-authors reviewed the integrated manuscript, substantially contributed intellectual input, and approved the final version for publication. **Competing interests:** The authors declare that they have no competing interests. **Data and materials availability:** All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 7 April 2020

Accepted 29 June 2020

Published 12 August 2020

10.1126/sciadv.abc1176

**Citation:** E. Agathokleous, Z. Feng, E. Oksanen, P. Sicard, Q. Wang, C. J. Saitanis, V. Araminiene, J. D. Blande, F. Hayes, V. Calatayud, M. Domingos, S. D. Veresoglou, J. Peñuelas, D. A. Wardle, A. De Marco, Z. Li, H. Harmens, X. Yuan, M. Vitale, E. Paoletti, Ozone affects plant, insect, and soil microbial communities: A threat to terrestrial ecosystems and biodiversity. *Sci. Adv.* **6**, eabc1176 (2020).

## Ozone affects plant, insect, and soil microbial communities: A threat to terrestrial ecosystems and biodiversity

Evgenios Agathokleous, Zhaozhong Feng, Elina Oksanen, Pierre Sicard, Qi Wang, Costas J. Saitanis, Valda Araminiene, James D. Blande, Felicity Hayes, Vicent Calatayud, Marisa Domingos, Stavros D. Veresoglou, Josep Peñuelas, David A. Wardle, Alessandra De Marco, Zhengzhen Li, Harry Harmens, Xiangyang Yuan, Marcello Vitale and Elena Paoletti

*Sci Adv* 6 (33), eabc1176.  
DOI: 10.1126/sciadv.abc1176

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