Soil Biology & Biochemistry 114 (2017) 279–294

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

The responses of shoot-root-rhizosphere continuum to simultaneous fertilizer addition, warming, ozone and herbivory in young Scots pine seedlings in a high latitude field experiment

Muhammad Usman Rasheed [a, *], Anne Kasurinen [a], Minna Kivimäenpää [a], Rajendra Ghimire [a], Elina Häikiö [a], Promise Mpamah [b], Jarmo K. Holopainen [a], Toini Holopainen [a]

[a] *Department of Environmental and Biological Sciences, University of Eastern Finland, P. O. Box 1627, FI-70211, Kuopio, Finland*
[b] *Department of Biological and Environmental Sciences, University of Jyväskylä, PL 35 (YA), FI-40014, Jyväskylä, Finland*

ARTICLE INFO

Article history:
Received 26 June 2017
Accepted 25 July 2017
Available online 4 August 2017

Keywords:
Biogenic volatile organic compounds (BVOCs)
Climate change
Ectomycorrhiza
Growth allocation
Great web-spinning pine sawfly
Soil microbes

ABSTRACT

It is not clear how climate change in combination with increasing soil nitrogen availability and herbivory affects boreal forests, the largest terrestrial biome in the world. In this study, Scots pine (*Pinus sylvestris*) seedlings were exposed to moderate warming (ca. 1 °C), 1.5 × ambient ozone (O_3) concentration, fertilizer addition (120 kg N ha^{-1} yr^{-1}) and shoot herbivory by pine sawfly (*Acantholyda posticalis*) alone and in combination. We measured fine root morphology, mycorrhizal colonization level, root fungal biomass (ergosterol), rhizosphere emission of biogenic volatile organic compounds (BVOCs), and microbial biomass (PLFAs) in the rhizosphere soil as well as seedling above- and below-ground growth. Warming and fertilization effects on fine root proportions or root fungal biomass occurred in combination with other factors, combination effects being usually negative on the studied variables, or then warming and fertilization in combination cancelled some other factors' effects. O_3 effects on needle growth, root fungal biomass and BVOCs were more often seen after the third exposure year, and sometimes only in combination with other studied factors. Of abiotic factors, fertilizer addition had reducing effect on rhizosphere BVOCs. Though increased nitrogen availability and warming increased both shoot and root dry masses, growth allocation to above- and below-ground parts was not equally increased in the combined exposures. Thus, we conclude that climate change factors together with increased nitrogen availability and herbivory are likely to affect the below-ground compartments negatively, more often than shoots, and ultimately change in growth allocation pattern which may affect overall seedling growth and survival in later years.

1. Introduction

Boreal forests, a significant carbon (C) pool, comprise almost 1200 million hectares over Alaska, Canada, Northern Europe and Russia (Olson et al., 1997; Conard et al., 2002; Janssens et al., 2003), are already experiencing the climate change (IPCC 2013). According to the fifth IPCC assessment report (2013), the past three decades have been the warmest over the last 1400 years. The short-term projected (2016–2035) increase in the atmospheric temperature due to increasing emissions of greenhouse gases is expected to be in the range 0.3–0.7 °C globally, and 1–2 °C for northern latitudes, relative to the temperatures prevailing in the period 1986–2005 (IPCC 2013). Nitrogen (N) input to the soils has also increased, mainly due to increased emissions of nitrogenous oxides from combustion of fossil fuel, fertilizer production and aggressive agricultural practices (Dentener et al., 2006; Galloway et al., 2008; Reay et al., 2008; Gundale et al., 2014). Warming is also expected to increase the decomposition rate of the soil organic matter, especially in the boreal zone, where it may further increase N levels in soils due to increased N mineralization (Rustad et al., 2001). The N inputs remain low in natural boreal forests (1–12 kg N ha^{-1} yr^{-1}) compared to the industrialized regions, but in managed forests trees can be exposed to much higher N levels (up to 200 kg N ha^{-1} yr^{-1}) due to fertilization (Saarisalmi and Mälkönen, 2001; Dentener

* Corresponding author.
E-mail address: usman.rasheed@uef.fi (M.U. Rasheed).

http://dx.doi.org/10.1016/j.soilbio.2017.07.024

et al., 2006; Galloway et al., 2008; Gundale et al., 2014). Increased emission of nitrogenous oxides and unburnt hydrocarbons into the atmosphere have also led to increased concentrations of tropospheric ozone (O_3) (Ehhalt, 2001; Vingarzan, 2004). Based on the four representative concentration pathways (RPCs) the near term (year 2050) global tropospheric O_3 concentration may decrease slightly, remain the same as or even increase compared to the year 2000 values (Cionni et al., 2011; Lamarque et al., 2011; IPCC 2013; Kim et al., 2015). While decadal (2000–2009) mean tropospheric O_3 concentration in the northern boreal forests (>60 °N) is in the range 40–45 ppb, it will probably remain above 35 ppb until year 2059 (Cionni et al., 2011), which is likely to reduce tree growth and survival (Wittig et al., 2009).

Moderate warming and increased N inputs to the soil have been reported to increase net primary production of the forests if there are no other growth limiting factors (Kellomäki et al., 2008; de Vries et al., 2009; Wu et al., 2011; Vicca et al., 2012). Increase in mean annual temperature of up to 4 °C and up to 140 kg N ha^{-1} yr^{-1} of N fertilization have been shown to increase shoot growth in northern boreal forests (Saarisalmi and Mälkönen, 2001; Utriainen and Holopainen, 2001b; Reich and Oleksyn, 2008; Gundale et al., 2014). However, if acidification of soil occurs due to high or long-term N inputs, then N addition may limit tree growth (Nellemann and Thomsen, 2001). In contrast to shoot response, increased N availability is expected to cause a decrease in root biomass, especially in the fine root biomass (Yuan and Chen, 2010). Warming effects on roots can be direct (environmental change) or indirect (mediated through plant responses). This might partly explain why there are more mixed results of root responses to warming, as warming response have been reported to range from decrease (Bergner et al., 2004; Bronson et al., 2008) to increase (Kasurinen et al., 2012; Leppälammi-Kujansuu et al., 2013) of root dry mass. Warming and N can have opposite effects on below-ground microbial profiles. Warming can increase (Clemmensen et al., 2006; Kasurinen et al., 2012; Leppälammi-Kujansuu et al., 2013) while N fertilization can decrease the abundance of soil and root fungal biomass (Frey et al., 2004; Högberg et al., 2007; Blasko et al., 2013; Leppälammi-Kujansuu et al., 2013); and increased N availability in soil can also reduce the gram-negative bacterial population in the rhizosphere (Bååth, 2003; Högberg et al., 2003, 2007; Koranda et al., 2014). Warming may also lead to increased insect herbivore outbreaks (Zhou et al., 1997; Bale et al., 2002). Above-ground herbivore infestation can affect foliage N concentrations and reduce annual needle production, radial and vertical shoot growth directly, whereas root growth, ectomycorrhizas (ECM) and soil microbes are all affected via plant physiology and chemistry (Gehring et al., 1997; Lyytikäinen-Saarenmaa, 1999; Kurkela et al., 2005; Gange, 2007; Lindroth, 2010). However, there is no information how above-ground herbivory affects roots, ECM and soil microbes in combination with these three abiotic factors.

Since O_3 is phytotoxic gas, it can reduce stomatal conductance and photosynthesis, advance leaf senescence, and thereby reduce C sequestration capacity of forests (Andersen, 2003; Grantz et al., 2006; Sitch et al., 2007; Wittig et al., 2009; Lindroth, 2010). Ozone-caused changes in C fixation and allocation may ultimately reduce shoot and root growth, and like O_3 effects on roots, effects ECM and soil microbes are considered to be mainly indirect (Manning, 1995; Andersen, 2003; Wang et al., 2015, 2016). So far, effects of elevated O_3 on ECM have been ranging from no effect (Kainulainen et al., 2000; Pritsch et al., 2009) to decrease (Perez-Soba et al., 1995; Wang et al., 2015), or increase, sometimes increase being only transient (Rantanen et al., 1994; Manninen et al., 1998; Kasurinen et al., 1999; Häikiö et al., 2009). Similarly, soil microbe responses to O_3 have been ranging from decrease (Pritsch et al., 2009) to no effect (Phillips et al., 2002) in a few field experiments where O_3 effects on soil microbial abundance and profiles beneath temperate trees have been studied. It thus seems that ECM and soil microbe responses depend on the exposure level, forest type and tree species (Perez-Soba et al., 1995; Agathokeleous et al., 2016). Ozone interaction with warming (Kasurinen et al., 2012) as well as ozone interaction with N addition (Utriainen and Holopainen, 2001b; Häikiö et al., 2007) on Scots pine and deciduous boreal tree species have been previously studied, but so far there is no knowledge of Scots pine below-ground responses to ozone and warming in combination using an open-field exposure system. O_3 effects on defoliator-tree interactions has been widely studied with deciduous trees (Lindroth, 2010), but O_3 and herbivory interaction studies with terpene-storing conifers are less common (Kivimäenpää et al., 2016; Ghimire et al., 2017). In most of the studies, the effects of elevated O_3 on herbivore-tree interaction have been highly species-specific, general patterns difficult to find, and focus has been more on above-ground than below-ground responses (Valkama et al., 2007; Wittig et al., 2009; Lindroth, 2010; Ghimire et al., 2017).

Shoots, roots and rhizosphere soil are all emitting biogenic volatile organic compounds (BVOCs) to the atmosphere. BVOCs act as airborne communication signals within and between plants and other organisms, are essential in plant reproduction, and act as defense and protection against biotic and abiotic stresses (Holopainen and Gershenzon, 2010; Penuelas and Staudt, 2010). Via aerosol formation, BVOCs can also have a cooling effect in atmosphere giving a significant negative feedback to warming climate (Holopainen, 2004; Spracklen et al., 2008). Exposure to warming, N addition, O_3 exposure or moderate defoliation by sawfly species, all have been reported to increase shoot BVOC emissions from Scots pines (Ghimire et al., 2013, 2017; Kivimäenpää et al., 2016; Ghimire et al., 2017). Kivimäenpää et al. (2016) and Ghimire et al. (2017) also report that warming, O_3 and N availability effects on shoot BVOCs can be modified by herbivory treatment such that herbivory can increase the stimulation of BVOCs due to the abiotic factors. On the other hand, Ghimire et al. (2013) have reported that even a moderate defoliation of Scots pine due to feeding by pine sawfly (*Diprion pini*) can decrease rhizosphere BVOC emissions. As far as we know, there is no information about rhizosphere BVOCs responses to the above factors in combination.

We studied the single and interaction effects of warming, increased nutrient availability, increased tropospheric O_3 concentration and needle herbivory by great web-spinning pine-sawfly (*Acantholyda posticalis* M.) on the shoot-root-rhizosphere continuum in Scots pine seedlings in a three-year field experiment. Our hypothesis for shoot growth was that 1) warming and fertilizer addition can increase and O_3 exposure and above-ground herbivory reduce shoot growth, and therefore warming and increased nutrient availability effects on shoots can be cancelled when in combination with O_3 and herbivory. For root growth we hypothesized that 2) single and interaction effects of all factors on roots can be more often negative (reduce growth) than those on shoot growth. In addition, 3) effects of warming and increased nutrient availability on root fungal biomass and the abundance of soil microbes can be opposite, i.e., warming increases and fertilizer addition decreases them, while 4) O_3 effects on root fungal biomass and soil microbial abundance may be influenced by warming, fertilizer addition and herbivory. 5) Above-ground herbivory alone and in combination with the above abiotic factors has also a potential negative effect on below-ground compartments via shoot. Based on Kivimäenpää et al. (2016) and Ghimire et al. (2017), 6) if the rhizosphere BVOC responses follow the shoot BVOC responses at least to some extent, all above factors may affect rhizosphere BVOC emission rates when alone and there may be some interaction effects as well.