Soil Biology & Biochemistry 92 (2016) 1–15

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Effects of climate warming and elevated CO_2 on autotrophic nitrification and nitrifiers in dryland ecosystems

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ARTICLE INFO

Article history:
Received 7 May 2015
Received in revised form
10 September 2015
Accepted 12 September 2015
Available online xxx

Keywords:
Ammonia-oxidizing archaea
Ammonia-oxidizing bacteria
Climate warming
Elevated temperature
Elevated CO_2
Nitrification

ABSTRACT

Global climate change is predicted to enhance atmospheric temperature and CO_2, with important consequences on biogeochemical nitrogen cycling in dryland ecosystems, which are highly vulnerable and characterized by extremely low nutrient availability. Belowground nitrification processes, predominantly mediated by ammonia-oxidizing archaea (AOA) and bacteria (AOB), are central to plant nitrogen availability and soil N_2O emissions, but their responses to future climatic scenarios in drylands remain largely unknown. Here we investigated the impact of factorial combinations of elevated CO_2 (+200 ppm) and elevated temperature (+3 °C) on dynamics of ammonia oxidizers and nitrification in three dryland soils planted with *Eucalyptus tereticornis*. Soil properties (including total carbon, H_2O%, and nitrate) and potential nitrification rates were strongly impacted by elevated temperature after nine months, accompanied by significantly higher AOA abundance in two soils and a gradual decrease in AOB abundance under elevated temperature. DNA-stable isotope probing showed increased assimilation of $^{13}CO_2$ by AOA, but not AOB, under warming, indicating that AOA were actively growing and utilizing the $^{13}CO_2$ substrates, which was coupled with significantly higher net nitrogen mineralization and nitrification rates. High-throughput microarray analysis revealed temperature selections of particular AOA assemblages and a significant reduction in diversity and co-occurrence of the metabolically active AOA phylotypes. Although these responses were soil specific, structural equation modelling by compiling all the data together showed that warming had significant direct and indirect impacts on soil nitrification which were driven by changes in AOA community structure, but no obvious effects of elevated CO_2 could be identified. Our findings suggest that warming has stronger effects than elevated CO_2 on autotrophic nitrification, and AOA are more responsive to elevated temperature than AOB in the tested dryland ecosystems.

1. Introduction

The global averaged land surface temperature has experienced a successive warming of 0.85 °C over the period from 1880 to 2012, and is projected to exceed 3.7 °C by the end of the 21st century (IPCC, 2013). In the meantime, the atmospheric CO_2 concentrations have increased by 40% since the pre-industrial times to current levels of >400 ppm, primarily from fossil fuel combustion and land-use changes (IPCC, 2013). These global climate changes are expected to have direct consequences on aboveground plant communities (Ghannoum et al., 2010), or indirectly influence the net primary production (NPP) by enhancing the nutrient competition between plants and microorganisms (Rütting et al., 2010) and altering the microbial turnover rates of soil nutrients (Karhu et al., 2014). The elevated CO_2 could enhance the NPP of Earth's

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http://dx.doi.org/10.1016/j.soilbio.2015.09.008
0038-0717/

ecosystems to increase carbon and nitrogen sequestration in soil organic matter and plant biomass, which in the long term may drive progressive nitrogen limitation of natural settings (Luo et al., 2004). Such climatic impacts were found to vary across soils with different nutrient conditions (Karhu et al., 2014), and might be particularly relevant within dryland ecosystems, which are highly vulnerable and characterized by extremely low availability of nutrients (Delgado-Baquerizo et al., 2014).

Drylands (including arid, semi-arid, and dry sub-humid ecosystems) constitute the largest terrestrial biome on the planet, comprising 41% of the Earth's land surface (Schimel, 2010), and provide essential ecosystem services (*e.g.*, biofuel, biodiversity, food, and fibre) to over 38% of the global population (Reynolds et al., 2007). As a consequence of climate change, the total area of drylands is predicted to expand by 10% at the end of this century (Feng and Fu, 2013), causing a significant drop in primary productivity (Fraser et al., 2011). Microbial communities have been considered as principal drivers in nutrient cycling processes (Singh et al., 2010), and their competition with plants for limited nutrients will ultimately determine the sustainability of NPP in drylands. A number of Free-air CO_2 enrichment (FACE) experiments have been carried out to assess effects of climate change on dryland ecosystem functioning and nutrient cycling (Del Galdo et al., 2006; Jin and Evans, 2007; Steven et al., 2013), which revealed complex responses of soil microbial communities to the projected climate change. Despite the global distribution of drylands and their important ecosystem services, considerable uncertainty remains about how future climatic scenarios will affect the microbe-mediated nutrient cycling in these ecosystems.

Nitrogen, as an essential nutrient element, is recognized as the most limiting factor affecting the NPP and organic matter decomposition in natural dryland ecosystems (Delgado-Baquerizo et al., 2014). Among all the nitrogen cycling processes, ammonia oxidation is considered to be the rate-limiting step for nitrification (Kowalchuk and Stephen, 2001), exerting significant control over the balance between relatively immobile ammonium and more mobile nitrite and nitrate, and thus is crucial for plant nitrogen availability, groundwater nitrate leaching, and greenhouse gas N_2O production (Singh et al., 2010; Hu et al., 2015a). Ammonia oxidation is catalyzed by two functional microbial guilds: ammonia-oxidizing archaea (AOA) affiliated within the novel *Thaumarchaeota* phylum (Brochier-Armanet et al., 2008), and ammonia-oxidizing bacteria (AOB) belonging to the β- or γ-proteobacteria (Purkhold et al., 2000). The slow-growing ammonia oxidizers obtain their sole energy source through oxidizing ammonia, and therefore ammonia substrate availability is considered to be the principal determinant shaping the differential growth, metabolic divergence, and ecological niches of AOA and AOB (He et al., 2012; Prosser and Nicol, 2012). Given the extremely low nutrient levels in drylands, competition for limited substrates among ammonia oxidizers and plants would be intense in these ecosystems (Hu et al., 2015b).

There is increasing evidence suggesting the cellular, genomic, and physiological differences between AOA and AOB, for example: (1) AOA had a significantly high affinity for ammonia than AOB (Martens-Habbena et al., 2009), and were reported to prefer the ammonia-poor and acidic environments (He et al., 2012; Zhang et al., 2012), by contrast, AOB tended to play a more important role in nitrification in the nitrogen-rich and alkaline environments (Verhamme et al., 2011; Xia et al., 2011); (2) AOA's pathway of assimilating inorganic carbon via a hydroxypropionate/hydroxybutyrate cycle is far more energy efficient than AOB, and requires one third less energy than AOB via the costly Calvin–Benson cycle (Könneke et al., 2014). AOA's high efficiency of metabolism perfectly suites the lifestyle of AOA to thrive in oligotrophic environments (Könneke et al., 2014); and (3) the size of AOA cells are generally 10-fold smaller than that of AOB, and the increased surface-to-volume could offer AOA a better adaptation under nutrient-poor conditions (Martens-Habbena and Stahl, 2011). Therefore, the contrasting substrate affinity and divergent nitrification pathways between AOA and AOB might lead to differential responses to above- and below-ground resource partitioning induced by climate change, and will eventually modulate the fate of nitrogen resources for plant.

To date, there has been a large body of studies addressing how climate changes influence aboveground plant communities (Ghannoum et al., 2010) and soil physiochemical dynamics (Barnard et al., 2005; Rütting et al., 2010; Phillips et al., 2011) across various humid tropical, temperate and polar regions (Schimel, 2010; Lamb et al., 2011; Van Groenigen et al., 2011; Brown et al., 2012). Although climate impacts on belowground processes and microbial communities have been frequently reported in recent years (He et al., 2010; Dijkstra et al., 2012; Rousk et al., 2012; Zhou et al., 2012; Frey et al., 2013), relatively less effort was devoted to decipher the responses of nitrogen cycling microorganisms (Pereira et al., 2011, 2013), particularly ammonia oxidizers, to climate changes in dryland ecosystems. Among multiple environmental and climatic factors, temperature is recognized as one of the most important drivers influencing nitrification (Tourna et al., 2008) and shaping the large-scale distribution patterns of AOB in soils (Fierer et al., 2009). By conducting a meta-analysis of publicly-deposited AOA *amoA* gene sequences across a wide spectrum of habitat types, the distribution patterns of AOA was also found to be strongly driven by temperature on a global scale (Cao et al., 2013). Although no significant effects of CO_2 on the abundance of nitrifiers and denitrifiers were observed in laboratory microcosms (Zhang et al., 2010) and the FACE experiment of agricultural soils (Pereira et al., 2011, 2013), soil ammonia oxidizers could respond significantly to changes in root exudates and plant residues induced by elevated CO_2 in grassland ecosystems (Horz et al., 2004). Few studies have investigated the interactive effects of elevated temperature and elevated CO_2 on ammonia oxidizers, and we still lack a mechanistic understanding of how climate changes interact to mediate the autotrophic nitrification in dryland ecosystems, adding major uncertainties in projecting the future nitrogen cycles.

The main objective of this study was to examine the effects of elevated temperature and CO_2 on the abundance, community composition, and metabolic activity of ammonia oxidizers in dryland ecosystems during early stages of *Eucalyptus tereticornis* establishment. Intact soil monoliths taken from three sites differing in basic characteristics were subjected to factorial combinations of temperature and CO_2 treatment for nine months in climate controlled growth chambers (Fig. S1). The final harvested soils from the controlled growth chamber experiment were immediately used for a short-term $^{13}CO_2$-DNA-stable isotope probing (SIP) microcosm, which allowed deciphering detailed community structures of the metabolically active nitrifiers by a high-throughput functional gene microarray (Abell et al., 2012). We tested the following hypotheses: (1) elevated temperature will directly increase the nitrification rates by stimulating the activity of nitrifiers, or indirectly affect the nitrification activity by modifying mineralization, and plant and soil properties, (2) elevated CO_2 can only indirectly affect the nitrification rates by impacting the plant biomass, but such effects might be trivial in a short-term experiment of nine months; and (3) AOA and AOB will respond differently to climate changes, due to their significant divergence in substrate affinity, energy use efficiency, and genetic features.