Soil Biology & Biochemistry 97 (2016) 157–167

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

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

Long-term effects of elevated CO_2 on carbon and nitrogen functional capacity of microbial communities in three contrasting soils

Clayton R. Butterly [a, *], Lori A. Phillips [b], Jennifer L. Wiltshire [c], Ashley E. Franks [c], Roger D. Armstrong [a, d], Deli Chen [e], Pauline M. Mele [a, b], Caixian Tang [a, **]

[a] Department of Animal, Plant & Soil Sciences, Centre for AgriBioscience, La Trobe University, Melbourne, Vic 3086, Australia
[b] Department of Economic Development, Jobs, Transport & Resources, Bundoora, Vic 3083, Australia
[c] Department of Physiology, Anatomy and Microbiology, La Trobe University, Melbourne, Vic 3086, Australia
[d] Department of Economic Development, Jobs, Transport & Resources, Horsham, Vic 3401, Australia
[e] Faculty of Veterinary and Agricultural Science, The University of Melbourne, Melbourne, Vic 3010, Australia

ARTICLE INFO

Article history:
Received 5 October 2015
Received in revised form
16 March 2016
Accepted 18 March 2016
Available online 24 March 2016

Keywords:
Elevated CO_2
Free-air CO_2 enrichment (FACE)
Carbon and nitrogen cycling
ARISA
Community fingerprinting
Functional capacity

ABSTRACT

Elevated atmospheric CO_2 (eCO_2) affects soil-plant systems by stimulating plant growth, rhizosphere processes and altering the amount and quality of organic matter inputs. This study examined whether these plant-mediated processes indirectly influence the structure and function of soil microbial communities and soil carbon (C) and nitrogen (N) cycling. Surface soils (0–5 and 5–10 cm) of Calcarosol, Chromosol and Vertosol were sampled after 5 years' exposure to either ambient CO_2 (aCO_2; 390 ppm) or eCO_2 (550 ppm) using free-air CO_2 enrichment (SoilFACE). Changes in microbial community structure were not detected using automated ribosomal intergenic spacer analyses (ARISA). However, quantitative PCR of targeted organic C decomposition (*cu*, *cbh*), N mineralisation (*apr*, *npr*), nitrification (*amoB*, *amoA*, *norA*) and denitrification (*nirK*, *narG*, *nosZ*) genes showed that eCO_2 reduced the abundance of half of the functional genes in the Chromosol and Vertosol and their abundance was tightly coupled with total C and N pools. In the Chromosol, total N and C of soil (<2 mm particles) was reduced by up to 20% and was associated with enhanced microbial activity (soil respiration). Soil C was also reduced in the Vertosol (15%, 5–10 cm); however greater laccase, reduced cellulase and lower microbial activity indicated that organic matter decomposition was currently limited by N. The loss of soil organic N and C under eCO_2 was likely driven by greater N demand. This study highlighted that the indirect effects of eCO_2 on functional capacity of soil microbial communities in dryland agricultural system depended on the soil type.

1. Introduction

Increases in the carbon dioxide (CO_2) concentration in the atmosphere are expected to alter the productivity and sustainability of agricultural cropping systems. Elevated CO_2 (eCO_2) directly influences plant productivity by enhancing photosynthesis, reducing transpiration and increasing both transpiration and N-use efficiency (Morison and Gifford, 1984; Torbert et al., 2000; Ainsworth and Long, 2005; de Graaff et al., 2006; Tausz-Posch et al., 2012). Plant-induced changes in the amount, composition and distribution of organic matter inputs occur (Rogers et al., 1994) and these may indirectly affect the composition of soil microbial communities and their functional capacity associated with C and N cycling (Bardgett et al., 2008). Direct impacts of eCO_2 on soil microbes are unlikely since CO_2 concentrations in the soil are already orders of magnitude greater than those of the atmosphere. A better understanding of the long-term effects of eCO_2 on microbial communities and associated key processes, particularly N cycling and C mineralization, is required.

Changes in microbial community composition and structure under eCO_2 are highly variable (Drigo et al., 2008) and are likely due to preferential stimulation of different groups of soil organisms (Pritchard, 2011). Increases in fungal abundance and diversity have been observed in semi-arid ecosystems (Drigo et al., 2007; Lipson et al., 2014; Procter et al., 2014). A greater influence of eCO_2 on

* Corresponding author.
** Corresponding author.
E-mail addresses: C.Butterly@latrobe.edu.au (C.R. Butterly), C.Tang@latrobe.edu.au (C. Tang).

http://dx.doi.org/10.1016/j.soilbio.2016.03.010
0038-0717/

fungi than bacteria has been proposed due to the reduction in N content of plant residues (Cotrufo et al., 1998) although enhanced bacterial abundance and diversity have also been observed (Norby et al., 2001; Drissner et al., 2007). Similarly, eCO_2 increased the abundance of actinobacteria and diversity of archaea in an Australian grassland (Hayden et al., 2012) but reduced species richness and did not alter the abundance of dominant organisms in another (He et al., 2012). Nevertheless, it is important to ascertain whether changes in soil microbial communities under eCO_2 alter their functional capacity. Drissner et al. (2007) suggested that increased microbial biomass and bacterial diversity buffered the potential increase in soil C of a grassland during 9 years of eCO_2. Similarly, increased fungal abundance and C mineralization could negate additional C inputs under eCO_2 or even reduce soil fertility in the long-term (Dijkstra et al., 2005; Carney et al., 2007; Drigo et al., 2013). While eCO_2 stimulated key genes involved in the C and N cycles in soybean (He et al., 2014), little information exists for N-limited environments. Furthermore, few studies have attempted to link soil microbial and chemical properties, particularly under field conditions (He et al., 2010, 2014).

Since belowground processes are mediated by the plant, microbial responses to eCO_2 are likely to be system specific (Carrillo et al., 2014). In dryland agricultural systems, crops are grown in monoculture and soil organisms endure long dry periods in the absence of a living plant. Although some organic-N cycle processes can continue under dry conditions (Phillips et al., 2015), N availability is generally limited by low N content. We have observed increased wheat and field pea biomass under free-air CO_2 enrichment (FACE) in a semi-arid environment (Butterly et al., 2015a,b) and reduced wheat residue quality (greater C:N ratio), consistent with other studies (Norby et al., 2001). Under eCO_2, greater microbial activity may not enhance residue decomposition (Lam et al., 2014), particularly when N is limited (Hu et al., 2006). Microbial release of N from legume residues is critical for N fertility, especially in soils that have a limited capacity to supply N from soil organic matter. Microbial responses to eCO_2 are likely to depend on the soil environment. However, few studies have considered more than one soil type (Procter et al., 2014).

This study aimed to quantify the effects of eCO_2 on changes in soil C and N, and microbial biomass size and activity, community composition and functional capacity after 5 years of exposure to free-air CO_2 enrichment (FACE). To our knowledge this is the first study to examine the effects of eCO_2 on microbial process in three contrasting soil types under the same agronomic and environmental conditions. In particular, we hypothesised that (i) eCO_2 would indirectly alter microbial community composition (favour fungi over bacteria) and the abundance of functional genes due to changes in C and N inputs, (ii) soil C would remain unchanged as altered microbial communities would be able to decompose residues with a greater C:N (additional C input offset by faster C mineralization), and (iii) eCO_2 would increase the abundance of functional genes which correlated with labile C and N pools (products of decomposition).

2. Materials and methods

2.1. Field experiment design and sampling details

This study was conducted using the soil free-air carbon dioxide enrichment (SoilFACE) facility at the Department of Economic Development, Jobs, Transport & Resources (DEDTR), Horsham, Victoria, Australia (36°44′57″S, 142°06′50″E). The facility was described in detail in Butterly et al. (2015a). Briefly, intact soil mesocosms (0.3 m diameter; 1.0 m depth) of Calcarosol, Chromosol and Vertosol (Isbell, 1996) or Calcisol, Luvisol and Vertisol (WRB, 2014), representing major soil types within dryland cropping systems of South-Eastern Australia were used. The Calcarosol had 6.4 g C kg^{-1}, 0.5 g N kg^{-1}, pH 5.9, 84.5% sand 10% silt and 5.5% clay, the Chromosol 46.6 g C kg^{-1}, 4.0 g N kg^{-1}, pH 4.5, 15.9% sand 65.8% silt and 18.3% clay and the Vertosol 11.0 g C kg^{-1}, 0.8 g N kg^{-1}, pH 7.7, 12% sand 36.9% silt and 51.1% clay. Mesocosms were arranged into bunkers sunk into the ground so that the soil surface of each mesocosm was at ground level and exposed to either ambient CO_2 (aCO_2; 390 ppm) or elevated CO_2 (eCO_2; 550 ppm) concentration for 5 years/growing seasons. Each treatment was replicated four times. The FACE system used to achieve eCO_2 was outlined in Mollah et al. (2009). Wheat (*Triticum aestivum* L. cv. Yitpi), field pea (*Pisum sativum* L. cv. PBA Twilight) and canola (*Brassica napus* L. cv. Hyola 50) were grown in the following rotation; field pea in 2009, wheat in 2010, field pea in 2011, wheat in 2012 and canola in 2013, except that canola was grown for the last two years in the Calcarosol. The facility is located in the Victorian Wimmera region characterised by a Mediterranean climate with a mean annual rainfall of 436 mm (range 544 in 2010 to 288 mm in 2012) over the experimental period. At maturity, plant shoots were cut-off at the base of the stems and transported to the laboratory. Following the removal of grain, the remaining residues were chopped (<2 cm) and returned to their respective mesocosms in the field. Mesh netting was used to ensure that residues were not lost during the summer period.

Surface soils (0–5 cm and 5–10 cm) were sampled in September 2013. Within each replicate, a composite sample for each soil type and layer was obtained by combining nine soil samples i.e. 3 soil cores (2.5 cm OD × 5 cm long) × 3 mesocosms. Soils were transported on ice and stored at 4 °C. On the following day, soils were sieved (≤2 mm) and subsamples for molecular work were transferred to sterile polybags (Twirl-em, Labplas, Canada), immediately frozen using liquid-N_2 and subsequently stored at −80 °C. The remaining samples were returned to 4 °C overnight while the gravimetric water contents (subsamples dried at 105 °C) were obtained. Overall, the study consisted of a randomised split-plot design with two CO_2 concentrations (main-plots), three soil types (sub-plot), two soil depths (sub-plots) with 4 replicates (n = 48).

2.2. Pre-incubation

A pre-incubation was performed prior to chemical and biological measurements (not involving DNA) to eliminate any confounding effects due to spatial and soil-type differences in soil water content. Soils were adjusted to 60% field capacity (θ_g) using reverse osmosis (RO) H_2O where θ_g was 0.12, 0.46 and 0.44 g g^{-1} for Calcarosol, Chromosol and Vertosol, respectively and incubated at 25 °C for 10 days. Bags were opened periodically to allow gas exchange with ambient air. At the end of the pre-incubation period, soils were destructively sampled for soil respiration, microbial biomass C and N and inorganic N. All other analyses were performed using air-dried soil.

2.3. Soil respiration

Soil respiration (SR) and substrate-induced respiration (SIR) were quantified using air-tight incubation jars (Butterly et al., 2009) and traditional alkali absorption (Zibilske, 1994). Briefly, 5 g dry weight equivalent (DW) of each soil was placed into individual jars (Ball Half Pint, Jarden Corporation, USA) with two vials containing 8 ml RO H_2O to maintain headspace humidity or 3 ml of 0.5 M NaOH to trap CO_2. Two sets of soil cores receiving the same volume (70% θ_g) of either RO H_2O or glucose solution (600 μg C g^{-1} soil) were included. A set of jars with no soil was included as a control. Jars were incubated at 25 °C in the dark for 16 days. The jars were