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Soil microbial biomass and enzyme activity under elevated CO₂ in Central Amazon: how global changes can affect tropical forests belowground

Luciana Bachega¹, Laynara Lugli^{1,2}, and the Carlos Quesada^{1*}

¹National Institute of Amazonian Research (INPA), Brazil (bachegalr@gmail.com)

²Technical University of Munich, School of Life Sciences, Freising, Germany

*A full list of authors appears at the end of the abstract

It is projected that the Amazon Forest could act as a carbon (C) sink in future climate change scenarios by efficiently storing extra biomass produced. Under atmospheric dioxide carbon (CO₂) elevation, the forest would experience an effect of C fertilization that could enhance nutrient requirements resulting in increased rates of nutrient cycling, soil enzymes activity, and soil microbial biomass stocks. However, we can expect that the potential effects of elevated CO₂ (eCO₂) could be restricted by soil nutrient limitation, especially in the low phosphorus (P) conditions found in central Amazonia. We aimed to estimate the effect of eCO₂ belowground, focusing on soil microbial biomass and enzymes activity on bulk soil and rhizosphere in central Amazonia, Brazil. In 2019 we set up the AmazonFACE program a CO₂ fertilization in a central Amazon rainforest in a factorial design experiment with eight Open-Top Chambers (OTC): four controls with ambient CO₂ concentration (aCO₂), and four with eCO₂ (200 ppm above the control chambers). We grew six pots with *Inga edulis*, a native N-fixing species, per OTC; additionally, we added 600 mg/kg of P in three pots per OTC in a total of four treatments: aCO₂, eCO₂, aCO₂+P and, eCO₂+P. In 2021 we harvested the plants and evaluated total soil microbial biomass carbon (MBC) and the potential activity of extracellular enzymes acid phosphatase (AP), β-glucosidase (BG), N-acetyl-β-glucosaminidase (NAG), enzymatic stoichiometry (BG/AP, and BG/NAG), and the microbial biomass specific enzyme rate (the ratio of each enzyme/MCB) in the bulk soil and in the soil attached to the roots, that we considered the rhizosphere. We hypothesized that the effect of eCO₂ and P addition would increase MBC and enzyme activity; higher MBC and enzymes activity would be found in the rhizosphere instead of bulk soil. We found that the effects of eCO₂ were only present with the interaction with P addition: higher MBC, but lower AP and BG/MBC in eCO₂+P compared to controls. We also found an interaction effect of eCO₂ regarding bulk soil and rhizosphere: higher NAG activity on bulk soil, and higher BG/NAG on rhizosphere. We found a difference between bulk soil and rhizosphere in almost all variables, except for MBC and BG/MBC. Enzyme activity and AP/MBC and NAG/MBC were higher for bulk; nevertheless, the enzymatic stoichiometry was greater in the rhizosphere. As we expected, eCO₂+P increased MBC, although we found a higher microbial biomass specific enzyme rate in controls, which can suggest nutrient limitation, such as P. In contrast to our assumption, the bulk soil showed higher enzymes activity

and microbial specific enzyme rates than the rhizosphere. However, the higher ratio of BG/AP on the rhizosphere can indicate lower P investment. We also found that the effect of eCO₂ on soil enzymes can be different between bulk soil and rhizosphere (high rhizosphere BG/NAG), potentially decreasing nitrogen investment on soil near the roots. Our results suggest that under eCO₂, the Amazon Forest could increase soil C stock due to MBC and this effect can change nutrients demand especially on the rhizosphere.

Carlos Quesada¹: Bachega, Luciana R.¹; Lugli, Laynara F. ^{1,2}; Martins, Nathielly P.¹; Leite, Pamela¹; Pereira, Ana Caroline, M.¹; Pereira, Iokanan¹; Guedes, Alacimar¹; Garcia, Sabrina¹; Santana, Flavia¹; Aleixo, Izabela¹; Portela, Bruno T. T. ¹; Damasceno, Amanda¹; Ushida, Gabriela¹; Ferrer, Vanessa¹; Menezes, Juliane C. G. ¹; Santos, Yago R ¹; Souza, Cassio¹; Moraes, Anna C. M. ¹; Souza, Vinicius F. ³; Hartley, Iain P.⁴; Lapola, David⁵; Fuchslueger, Lucia⁶; Valverde-Barrantes, O.⁷; ¹National Institute of Amazonian Research, Manaus, Brazil; ²Technical University of Munich, School of Life Sciences, Freising, Germany ³Universidade Estadual do Amazonas, Manaus, Brazil ⁴College of Life and Environmental Sciences, University of Exeter, UK; ⁵University of Campinas, SP, Brazil;