

## **The rhizosphere: A hotspot of nitrous oxide action**

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### **Extended Abstract**

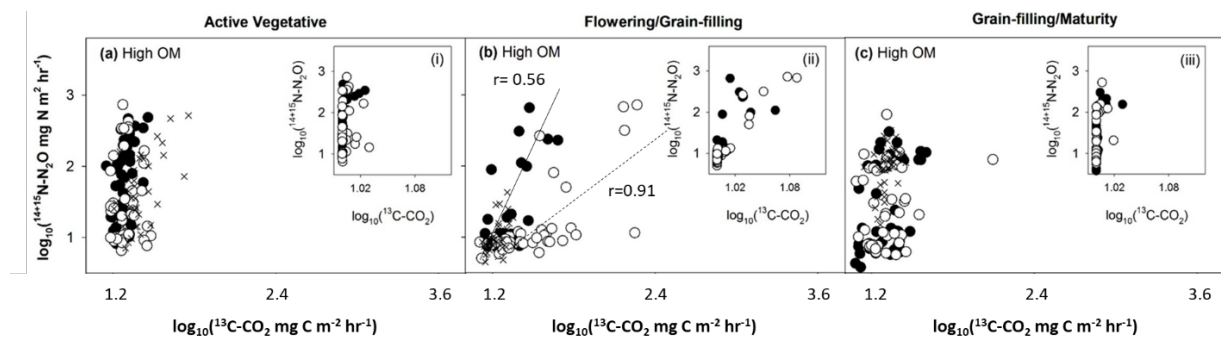
Hotspots of nitrous oxide (N<sub>2</sub>O) production in soil are usually attributed to interactions between resources such as oxygen, C, N and water, resulting in diverse habitats within the soil. This spatial variation can be considered at a range of scales, but to understand the influence of plants on the nature of hotspots of N<sub>2</sub>O production and reduction necessitates consideration at the scale of the rhizosphere. Here we explore the role of root-derived carbon in shaping hotspots of N<sub>2</sub>O in the rhizosphere.

What does theory tell us? In their review, Giles et al (2012) demonstrate that diffusion of resources from plant roots is spatially limited as it operates against the flow of water uptake by plants. This concentration of resources within the rhizosphere creates hotspots of activity of specialist organisms capable of utilising these high concentrations of resources. Rates of the nitrate reducing processes denitrification and nitrate ammonification are therefore likely to be high in the rhizosphere especially as they can utilise root-derived C as an electron donor for reduction to N<sub>2</sub>O and, in the case of denitrification, to N<sub>2</sub>. Such root inputs are transient, resulting in a rapidly responding N<sub>2</sub>O-genic microbial community and hotspots of N<sub>2</sub>O action. Morley et al (2014) have demonstrated the nature of this electron source to have a direct impact on denitrifier N<sub>2</sub>O-to-N<sub>2</sub> ratios. Using an artificial root (micro rhizon tube) simulating rhizodeposition of organic C into soil, they showed organic acids, major constituents of root exudates, to result in greater reduction of N<sub>2</sub>O to N<sub>2</sub> than amino acids or sugars. This highlights that the efficiency in the N<sub>2</sub>O reductase can be C compound type dependent, and opens up the possibility of plant-driven engineering of the rhizosphere.

Responses to plant-derived C may also reflect carbon catabolite repression (CCR) where bacteria selectively assimilate a preferred compound among a mixture of several potential carbon sources. This competition and preference within the soil heterotrophic community for rhizodeposited C will vary with distance away from the root source (Giles et al 2017), but it is unknown how other interacting factors influence CCR, or the role of CCR in shaping hotspots of N<sub>2</sub>O production and reduction. The respective contributions of denitrification and nitrate ammonification would be expected to vary spatially. We can hypothesise the spatial relationships of these processes based on our understanding of the regulation of these processes, but validating their contribution to N<sub>2</sub>O production within rhizosphere hotspots at the scale of the microbiology remains technically challenging.

There is evidence that plant regulation of N<sub>2</sub>O production can vary between different plant species, cultivars or lines, and with growth stage, and it is possible that this is a reflection of different exudate compositions. Isotope labelling of plants in a CO<sub>2</sub> headspace enriched or depleted in <sup>13</sup>C enables plant-derived <sup>13</sup>C in respired CO<sub>2</sub> to be quantified and related to N<sub>2</sub>O/N<sub>2</sub> production. The strength of this relationship can be used as indicative that plant-derived C is being utilised during N<sub>2</sub>O-genic processes,

and related to plant architectural and biochemical traits. Figure 1 gives an example of change in relationships between respired plant-derived  $^{13}\text{C}\text{-CO}_2$  and  $\text{N}_2\text{O}$  emissions, showing variation in relationships between cultivars and with growth stage, with the strongest relationship being at flowering/grain filling. There is scope to examine the physiological and genetic bases behind this regulation, that may provide the framework for future breeding initiatives, for example for crop plants that can lower net emission of  $\text{N}_2\text{O}$ , without compromising agronomic or quality characteristics. This means that understanding the nature and regulation of rhizosphere hotspots could result in strategies to engineer the rhizosphere to lower net emissions of  $\text{N}_2\text{O}$ , that may help set us on a path towards coupled environmental and food security.



**Figure 1.** Relationships between  $^{13}\text{C}\text{-CO}_2$  ( $\log_{10}$ ) and  $\text{N}_2\text{O}$  ( $\log_{10}$ ) emissions at (a) active vegetative, (b) flowering/grain filling and (c) grain filling/maturity growth stages of rice cultivars Brierley Dhan 28 (filled circle), cultivar Brierley BJ1 (open circle), compared with unplanted soil (cross) [4].

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