

Dear Editor and Reviewer,

First of all, we thank the reviewer and editorial staff for comments on our manuscript. Within the possibilities, we will clarify doubts and, as far as possible, respond to the reviewer's comments. In places, it seemed appropriate to provide several paragraphs of detail and provide references.

Sincerely yours,

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Response to Ccomments by Reviewer 2

1. *Comment 1.: General comments* The study by Valadares and co-authors proposes a mechanistic model (ForPRAN) for the prediction of rhizosphere C and N cycles in Eucalyptus plantations. It is based on different belowground processes and uses (i) the 3-PG ecophysiological process model to simulate fine growth and rhizodeposition release on the basis of PAR radiation. Rhizosphere dimensions were estimated on the basis of root length and diameter.

Note 1: Fine roots biomass was estimated using an empiric model developed by us and mentioned on page 21. We did not directly use PAR, but instead shoot biomass, and also soil depth and clay content. The reasons for choosing these variables is explained on the pages 4 and 11.

2. *Comment 2.: The MCNiP model was refined by considering climatic factors as well as microbial movement.*

Note 2: We also considered a soil protection effect on substrate availability (page 4), as well as soil porosity (page 5) and other nutritional limitations using the soil fertility index (page 28).

3. *Comment 3: The development of modelling approaches for the prediction of rhizosphere processes and especially of rhizodeposition and microbial priming effects is an urgent task. However, I am not completely convinced that the current model is actually approaching this task. For one, rhizodeposition is modelled from PAR, which is used for representing root length growth (P. 5, l. 27): what is the basis of the assumption that there is a direct connection between these two players? Exuded C of trees can also originate from C storage in trees.*

In addition, the rate of exudation is highly responsive to different environmental conditions.

Answer 3: As mentioned in Note 1 above, fine roots biomass was estimated using an empiric model developed by us and mentioned on page 21. We did not directly use PAR, but instead shoot biomass, and also soil depth and clay content. The reasons for choosing these variables is explained on the pages 4 and 11. The links between light, photosynthesis, respiration, shoot biomass, root biomass, and exudates is well established in the literature, e.g. KUZYAKOV (2001), which explains the inner control by plants of the rhizodeposition process. Please, also see the their Figure 3, which corroborates the light-shoot-root C relationship.

When we consider Equations 7 and 8 in the Supplementary section (page 22), root length is just one element; others are the rate of efflux at the root apex (α) and the relative influx of C (β), etc. Hence, the efflux rate can be parameterized to consider environmental factors (external control factors), like microorganisms, soil texture, nutrient availability, etc. It is important to keep in mind that proposing a model does not imply that it will be fully parameterized for all the existing variability in nature. An experimental effort will have to be made to quantify rhizodeposition under contrasting environmental conditions for the eucalypts plantations. However, the current version allows calculation of an average value for trees (NEWMAN, 1985; PAUSCH; KUZYAKOV, 2017), which is a good start. In future, with additional research and understanding, we expect improvements to the model will include additional or refined controls on rhizodeposition, but it is reasonable not expect all such details to be included in the first version of this model.

4. *Comment*

4:

Secondly,

priming effects seem to be a central focus of this study, but the definition of it is rather vague. Priming effects are defined as change in native C mineralization in response to increased labile C input, e.g. from rhizodeposition. Priming effects in its narrower sense describe the increase in soil respiration or N mineralization that originates neither from background respiration or mineralization nor from respiring or mineralizing the additional organic C, but rather from microbial mining of additional recalcitrant SOM. The model in its current formulation does not describe this effect.

Answer 4: The priming effect (PE) was first reported by Löhnis (1926) by studying the decomposition of green manure of legume plants in soil, where there was intensified mineralization of the humus by the addition of fresh organic residues to the soil. Nowadays, it is well known that PE is common in most plant species and may be caused by other compounds besides plant residues, like dead microorganisms, high-molecular and low-molecular organic substances, or even mineral N (KUZYAKOV, 2002; BLAGODATSKAYA; KUZYAKOV, 2008; CHENG *et al.*, 2014; DIJKSTRA *et al.*, 2013; ZHU *et al.*, 2014). Therefore, the priming effect definition that we some other authors use is: a short-term change in the SOM turnover caused by the addition of plant residues, dead microorganisms, high and low-molecular organic substances or mineral N (KUZYAKOV, Y.; FRIEDEL; STAHR, 2000).

The mechanism of priming effect can be seen in the following way: 1- after the addition of a source of C/energy, the microbial succession begins, where the activation of several previously inactive microorganisms occurs and many of which respond to specific substrates (BLAGODATSKAYA; KUZYAKOV, 2008); 2- the increased activity of this microorganism increases soil organic matter degradation as a result of **co-metabolism** and **increased enzyme production**, ie, "real" priming effect (RPE) (Cadded <Cprimed>CBM) (BLAGODATSKAYA; KUZYAKOV, 2008).

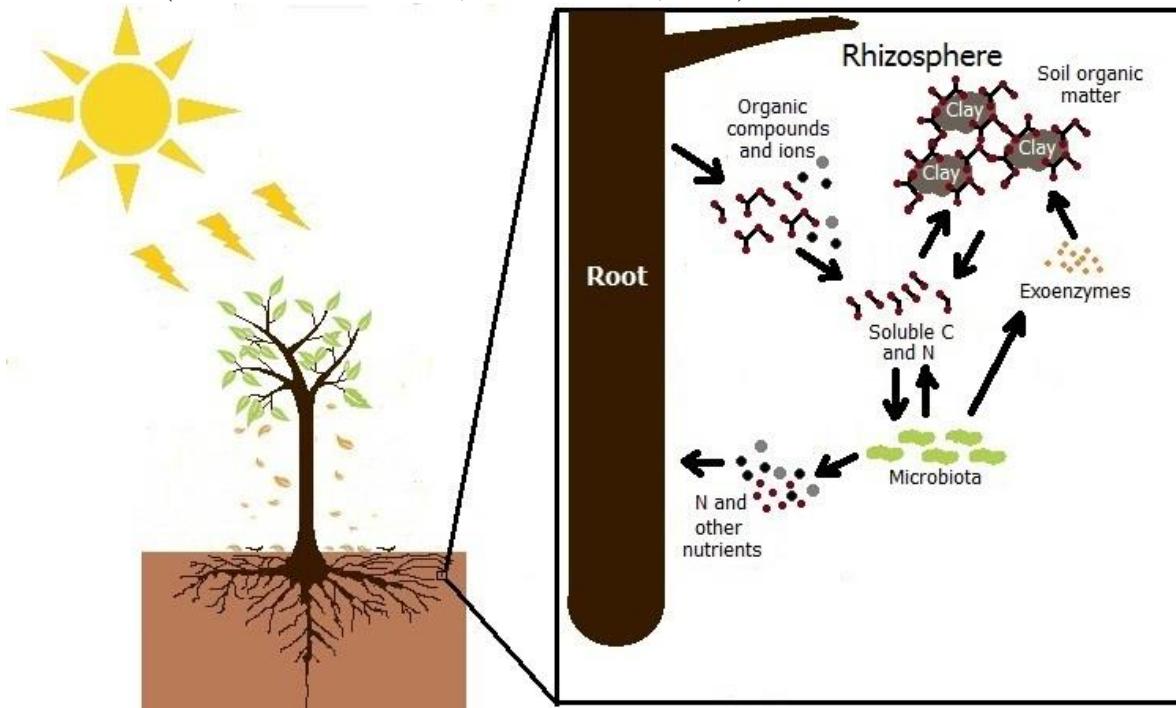


Figure 1. Illustration of rhizosphere C and N cycling processes in the ForPRAN model

The figure above that illustrates our model also describes the phenomenon of co-metabolism, which is one of the most important hypotheses of the priming effect.

But it is still important to know other definitions before concluding our answer: The term rhizosphere was first used by Hiltner (1904) (so, before Löhnis (1926)) to refer to the soil zone under the influence of legume roots. Current definitions of rhizosphere are generally close to this idea, with only minor modifications such as i) zone of soil immediately adjacent to the roots of the plants, where the quantities or activities of microorganisms differ from the rest of the soil, ii) soil volume influenced by root activity, and iii) soil adjacent to the roots with a different physical, chemical and biological environment from the rest of the soil (FAGERIA; STONE, 2006).

Thus, the rhizosphere is comprised of the microbiome influenced by the roots, a region that is constantly altered by organic compounds and ions and is therefore associated with a microbial population of unique characteristics of diversity and metabolism. However, the priming effect description came later than the definition of the rhizosphere effect. Thus, the description of the C and N cycle in the rhizosphere soil is enough to know that the environment is under the constant influence of C/energy and with a SOM turnover rate different to bulk soil. Considering the precedence of the description of the rhizospheric effect compared

with rhizosphere priming effect on microbial dynamics and soil organic matter turnover, we can say that the rhizosphere effect definition already includes the definition of priming effect with respect to C and N near the roots. See lines 19-24, page 2, where we described the rhizosphere effect.

To summarize:

1. the definition of the rhizospheric effect on C and N cycling presented by us coincides with the hypothesis of co-metabolism, which explains the rhizospheric priming effect. Our model is a mathematical description of the phenomenon of co-metabolism inside the rhizosphere, in which microbes uptake carbon and nitrogen from rhizodeposition and causes depolymerization of and metabolises soil organic matter, generating what we call a positive priming effect and net mineralization (so-called N gain – resulting in a positive N balance (net N mineralized and rhizodeposited)).
2. Hence, the model is already consistent with reviewer's comment, e.g. Table 3 for the effect on N supply.
3. We have added a definition of the rhizosphere priming effect.

5. *Comment 5: Finally, I think the general reasoning for the set-up of the study could be improved: Why is it importante to derive predictions on rhizosphere C and N cycling for Eucalyptus plantations specifically? Why is rhizosphere priming important?*

Answer 5: *Eucalyptus* plantations occupy an area of approximately 5 million hectares in Brazil and around 20-25 million hectares in the world. Nitrogen (N) is one of the most accumulated nutrients in *Eucalyptus* plantations, with values up to 1,300 kg/ha. Interestingly, stands in Brazil often show little or no N fertilization response (DE MELO *et al.*, 2016; PULITO *et al.*, 2015; SMETHURST *et al.*, 2015). The responses, when present, occur within the first two or three years and cease to exist during the time of the harvest (PULITO *et al.*, 2015). Therefore, *Eucalyptus* plants can meet its high N demand without fertilization or with the application of low doses, between 40 and 70 kg/ha of N, which shows that our understanding of N supply in these systems is incomplete. Within plant types, woody species showed the highest RPE followed by grasses, while crops had the lowest level of the RPE, indicating that plant traits and physiology may exert important controls on the RPE (HUO; LUO; CHENG, 2017). *Eucalyptus regnans* forests, for example, are able to uptake amounts of N that meet their nutritional requirements and maintain normal photosynthetic activity even in soils with low natural fertility to global standards exposed to fires (DIJKSTRA *et al.*, 2017). The key mechanism that explains this phenomenon is the increase of soil organic matter mineralization induced by root growth (also known as priming effect) (DIJKSTRA *et al.*, 2017). As experimentally proven by Dijkstra *et al.* (2017), *Eucalyptus* roots themselves induce greater microbiological activity in soil, and greater respiration and N mineralization. The authors observed two-fold higher microbial biomass and 5.5 higher gross N mineralization in the soil where *Eucalyptus* plantations grew their roots than in the same soil with root exclusion. As a result, a net reduction of 0.50 kg C m⁻² and 12 g N m⁻² was observed in the

total C and N contents in plots where roots grew in a relatively short period of time (April 2010 to March 2011) (DIJKSTRA *et al.*, 2017).

Experimental evidence for Brazilian sites in the Vale do Rio Doce region (Minas Gerais state) with a hybrid clone of *E. grandis* x *E. urophylla* also point to the existence of a link between the root activity and the increase in the rates of mineralization of the forest soil (DELVAUX, 2014). The author observed higher microbial biomass, metabolic quotient (q_{mic}), enzymatic activity (laccase and Mn-peroxidase), potential mineralization and, consequently, lower total N in the rhizosphere than in the bulk soil (DELVAUX, 2014). In a greenhouse experiment, Hurtarte (2017) observed that the growth of seedlings roots of a hybrid clone of *E. grandis* x *E. urophylla* in an oxisol reduced the stocks of C associated mineral fraction in the rhizosphere, especially when plants were under N nutritional limitation.

Therefore, the rhizosphere effect (or rhizosphere priming effect) in eucalypts is of particular interest to the forest plantaiton industry and to the broader scientific community. Despite this, there are still no models to measure the quantitative importance of this process for forest plantations of the genus *Eucalyptus*. That is why we formed a group of researchers from Brazil and Australia to present this model to Biogeosciences.

6. Comment 6: Specific comments

P. 1, l. 11: The unit for fine root length is unusual, please change to m m-2 or similar.

Answer: kg/ha, km/ha are units allowed by the international system of units;

P. 1, l. 14: : : :for the prediction of rhizosphere C and N cycling:

Accepted and appropriately changed;

P. 1, l. 19: Do you mean root or soil respiration? N mineralization? N immobilization? : : :immigration, and SOM formation.

Soil respiration and N mineralization, etc;

P. 1, l. 21: : : :variables that influenced N gain from rhizosphere N mineralization most were: : :

Accepted and appropriately changed;

P. 2, l. 6: I think the expectation that additional N is expected in deeper soil layers than in the topsoil is quite surprising: Where should this N come from? N enters the ecosystem from N fixation and N cycling with leaf litter, which are both processes that are mostly occurring in the topsoil.

It is the accumulated result of the net N mineralization minus N rhizodeposited (quantity), therefore the relation is proportional. The higher the soil layer, the greater the amount of N gain. I think the reviewer confused concentration with amount, concentration being higher in surface soil.

P. 2, l. 7: : : :higher than: : : : :observation that growth responses: : :

Accepted and appropriately changed;

P. 2, l. 14: : : : rhizosphere processes: : : : : N supply for some trees: : :
Accepted and appropriately changed;

P. 2, l. 16: : : : in the form of dead roots: : :
Accepted and appropriately changed;

P.2, l. 32: The abbreviation for the Microbial Carbon and Nitrogen Physiology model is 'MCNiP'.

Accepted and appropriately changed;

P. 2, l. 33-34: It remains unclear why mineralization rates need to be linked
Accepted and appropriately changed. The MCNiP model was not originally presented as part of a commonly used crop production model. To do so, we needed to link it to a number of other processes that operate at the broader ecosystem level.

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