C | A | U Christian-Albrechts-Universität zu Kiel Agrar- und Ernährungswissenschaftliche Fakultät

Christian-Albrechts-Universität zu Kiel, 24118 Kiel

Institut für Phythopathologie
Dept. Soil- Plant- Microbiome

Prof.Dr. Bahar S. Razavi
Hermann-Rodewald-Str. 9, 24118 Kiel

Mail, Telefon, Fax

brazavi@phytomed.uni-kiel.de
tel. +49(0)431 880 1717
fax +49(0)431 880 2940

Datum
17.07.2022

To:

Head of PhD Defence Committee

University of South Bohemia
Branisovska 1760

Re: Report of PhD Thesis Evaluation for Mrs. Julian Cardenas-Hernandez

Dear colleagues,

Here by I am writing the evaluation report of thesis *"Effect of C:N:P Stoichiometry on Plant-Soil Microbe Interaction"*, pursed by Mrs. Cardenas-Hernandez.
The thesis consists of 3 studies which in 2 of them contribution of Mrs. Cardenas-Hernandez was about 75% while she contributed actively about %40 in last study by contributing in sampling, analysis, statistics, discussing of results and writing.

The chapters are started with a comprehensive and detail introduction about rhizosphere, detritusphere and root exudation, pattern of specific compounds release and their effect on soil C and nutritional state as well as microbial respond. The introduction also discusses efficient methods for relevant analysis such as stable isotopes 15N and 13C labeling as well as mechanisms occurring in the soil-plant-microbiome system such as priming effect. Also, how such phenomena would effect SOM decomposition and C:N:P ratio.

In listed studies she tried to answer to how changing nutrient availability alters plant-soil-microbial interactions over the ranges from resource-conservation to acquisitive species and identify key plant and soil drivers of exudation flux and its C:N:P composition.
What are the effect of live and dead roots of conservation and acquisitive plants on microbial activities and how this would control priming effect.

How changing nutrient availability, exudation and SOM dynamics would be reflected in plant traits such as growth, SLA, RS, etc.

In study 1: "The exudation of surplus products links plant functional traits and plant microbial stoichiometry", A total of eight grass species (from conservative to exploitative) were grown in pots under two different soil C:nutrient conditions for a month. As a result, a wide gradient of plant–microbial–soil interactions were created. The authors found that the plant–soil interactions were shaped by soil N availability. Faster-growing plants were associated with lower amounts of mineral N (and P) in the soil solution, inducing a stronger competition for N with microorganisms in the rhizosphere compared to slower growing plants. The plants responded by enhancing their N use efficiency and root:shoot ratio, and they reduced N losses via exudation. Root growth was supported either by reallocated foliar reserves or by enhanced ammonium uptake, which connected the specific leaf area (SLA) to the mineral N availability in the soil. Rapid plant growth enhanced the exudation flux. The exudates were rich in C and P relative to N compounds and served to release surplus metabolic products. The exudate C:N:P stoichiometry and soil N availability combined to shape the microbial stoichiometry, and N and P mining.

In the 2nd study C addition to the soil alters plant-microbial interactions and it is reflected in foliar 15N"), the role of the C addition in soil-plant interaction a greenhouse experiment considering 8 grass species were grown either in non-amendment or C-amendment soil conditions. The C addition enhanced dissolved OC, microbial DNA and specific respiration in planted and unplanted soils. The results also showed that the plant production was maintained after C addition to the soils due to the increased N use efficiency of both plant and microbiome.

In the last study (study number 3: "Priming effects in the rhizosphere and root detritusphere of two wet-grassland graminoids") Continuous labelling with 13C-depleted CO2 was applied for the partitioning of plant and soil C sources of CO2 efflux and the investigation of the priming effect in the rhizosphere and detritusphere of slow-growing conservative Carex acuta and fast-growing acquisitive Glyceria maxima. The results showed that Glyceria allocated more C into the soil, induced higher microbial activity and a larger portion of active microorganisms, and depleted mineral N stronger than Carex. Its rhizosphere priming was 2.5 times stronger than that of Carex. Root residues (detritusphere) induced negative priming at the early stage of decomposition (1–9 months). The depletion of available organic substances in the detritusphere of more easily decomposable Glyceria roots resulted in positive priming after 3 months. The priming in the detritusphere of N-poorer Carex roots was more intensive but started after 9 months

The authors found that the rhizosphere priming effect was positive and stronger than the detritusphere priming, which switched from initially negative to positive priming after depletion of available substances during few months. More productive species with faster N-uptake and higher belowground C input (here Glyceria) induce larger rhizosphere priming than slower-growing species (Carex).

Overall, the main focus of all studies is measurement of C, N fluxes in the rhizosphere. The main strength of this thesis is multiple applications of labelling approaches as well as interactive application of destructive sampling and molecular analysis for instance DNA analysis.
Mrs. Cardenas-Hernandez performed series of experiment and reported the results and findings in frame of high quality publications with a clear sense and stepwise, smooth development from basic focus to mechanistically center. Therefore, I recommend this thesis to be accepted for defense.

Please do not hesitate to contact me for any further questions.

With kind regards,

Prof. Dr. Bahar S. Razavi

Jun.-Prof. Dr. Bahar S. Razavi
Dept.of Soil and Plant Microbiome
Christian-Albrechts-University of Kiel
Hermann-Rodewald-Str. 9, 24118 Kiel

a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

Department of Forest and
Conservation Sciences
Faculty of Forestry
3040 – 2424 Main Mall
Vancouver, B.C. Canada V6T 1Z4
T. 604 822 4701 F. 604 822 9133
cindy.prescott@ubc.ca

18 May, 2022

Opponent's Report on Dissertation "Effect of soil C:N:P stoichiometry on plant-microbe-soil interactions" by MSc Julian Cardenas-Hernandez

The dissertation explores an important topic that is at the forefront of research on plant-soil interactions – how the supply and composition of exudates released from plant roots influences microbial communities and processes in the soil. The research reported in the dissertation examines the complex interplay of contributing influences through the lens of three current theoretical frameworks – element stoichiometry, plant functional traits and plant surplus carbon. Within this scope, three distinct experiments were carried out.

The first experiment examined inter-relationships between plant growth rate, N availability and the abundance and nutrient stoichiometry of plant exudates. Relative to slow-growing (conservative) plants, fast-growing (exploitative) plants depleted available N in the soil, resulting in lower N concentrations in plant tissues, and increased root:shoot ratio. Root exudation from fast-growing plants was greater and was higher in C and P relative to N, indicating a link between the relative growth rate of plants and nutrient availability in the rhizosphere that is created through rates of uptake, and magnified through changes in the element stoichiometry of root exudates. An important implication of this finding is that rhizosphere microbes of fast-growing and moderately N-deficient plants will be even more deficient in N, which may cause them to release more enzymes to assist in liberating organic N. This possible effect is followed up on in other experiments in the dissertation.

The second experiment explores the influence of carbohydrate-rich rhizodeposits on N availability in soil, and resulting effects on growth and N content of plants. Agar – an analogue for root mucilage – was added to soil and it was hypothesized that (relative to unamended controls) the decline in soil N availability in agar-amended soil would induce N deficiency (especially in fast-growing plants) and promote release of microbial enzymes that 'mine N' from organic matter. Agar amendment reduced N concentrations in plant and microbial tissues, and increased respiration and production of enzymes that liberate C and N from soil organic matter. Effects were magnified in pots with plants, especially fast-growing plants, suggesting an influence of greater flux of C-rich but N-poor exudates. Production of the 'N-mining' enzyme Ala-aminopeptidase increased in C-amended soils but only those with plants, again suggesting a link to the flux of C-rich and N-poor exudates. These findings provide further evidence of a positive feedback loop through which relative availabilities of C and N in the rhizosphere influence plant nutrition and the flux and stoichiometry of root exudates, which intensify conditions in the rhizosphere and microbial responses. The experiment also demonstrated the efficacy of foliar δ15N measurements for tracking plant N limitation.

In the third experiment, influences of root exudation on mineralization of SOC are examined by growing a fast-growing plant species and a slow-growing one with continuous CO_2 labeling to track the fate of plant C released by roots. The fast-growing plant contributed more C to the soil via its roots, and more root-derived C ended up in microbial biomass. In contrast, the slow-growing plant exuded less, and most of this remained as DOC rather than generating microbial biomass. This effect was magnified when the plant was enriched in N via foliar fertilization. This finding demonstrates a positive relationship between plant growth rates, root exudation and microbial biomass production, suggesting that rapidly growing plants exude more and create a larger and more dynamic rhizosphere microbial community. Rates of CO_2 evolution from the soil, the proportion derived from the plant, and the release of C from SOC were all somewhat larger in the fast-growing plant. Although it is concluded that the fast-growing plant induced a larger priming effect of SOC, I am surprised by how small the difference was.

The findings from the experiments in this dissertation make an important contribution to our collective understanding of the factors that influence plant root exudation and in turn its influences on microbial processes in the rhizosphere. The experiments were elegantly designed to simultaneously test several possible influential factors. The experiments were remarkably comprehensive in the large number of important variables measured, in order to develop a broad-based assessment of effects and the underlying mechanisms. The treatments were well-replicated, allowing statistical analyses to uncover a variety of effects of the treatments. Methods were state-of-the-art and included some novel and clever twists such as C and N labeling of plants hence exudates, foliar fertilization to avoid confounding soil effects through N addition, agar amendment to imitate rhizodeposition, and measuring C mineralization from root systems following severing of aboveground plant tissues. Two papers are already published in good scientific journals and the third is near publication. The research described in the dissertation is therefore first-rate and highly deserving of a doctoral degree. The candidate has demonstrated his ability to design and conduct scientific experiments, analyze and interpret the results and present the findings in the context of current knowledge in the discipline.

Questions and comments:

My questions for the candidate stem from some of the interpretations of research findings as well as presentation of the current state of knowledge, particularly in the introductory chapter.

1. Re: the effect of plant growth rate on SOC stock. Throughout the dissertation, there seems to be an assumption that soil microbes serve only to degrade organic matter, so any processes that stimulate soil microbial biomass will cause SOM to decline. For example, early in the General Introduction is the statement: "rhizodeposition fuels microbial activity which in turn accelerates decomposition of soil organic matter". This view omits current thinking based on several lines of evidence that microbial biomass

(including necromass and microbial products) are key *sources* of SOM. So any measurements of SOC loss due to rhizosphere priming need to be compared with rates of SOC production via microbial biomass to determine net effects on SOM stocks. Do any of the measurements in your dissertation experiments allow you to address *net* SOC accumulation or loss?

2. There is an assumption that 'activating' the microbial community via energy-rich exudates will generate additional demand for N, which will cause microbes to mine it from SOM (bottom of page 8, top of page 9). However, it is only *increased* microbial biomass that requires additional N as, unlike energy, the demand for nutrients can be satisfied by turnover of the existing microbial biomass. So the microbial demand for nutrients may not be as great as envisioned.

3. It is also possible that microbes simply remain constrained by the most growth-limiting factor, and are not necessarily compelled to generate enzymes to mine N from soil in order to generate more microbial biomass. They may also be constrained by the need to acquire N in order to generate the enzymes needed to release N from SOM. The common assumption that organisms are constantly trying to increase their individual or collective biomass can lead to expectations that are not borne out in evidence. Perhaps the relatively small differences in priming effects reflects the microbes not being compelled to increase their collective biomass.

4. The dissertation, particularly the introductory chapter, is steeped in optimality thinking, which presumes that every characteristic and process in plants is optimized. While this is a common lens through which to view such patterns, it is not the only way of interpreting findings, and simply invoking an optimality or adaptation explanation may limit our ability to deduce underlying mechanism. For example, on page 1, "Acquisitive plants rapidly take up available nutrients *to support their rapid growth* and preferentially allocate the resources above ground *to improve access to light and to maximize photosynthesis*". Because we cannot know 'why' plants do what they do, we can only theorize as to the reasons, so these potential explanations need to be phrased as theories, lest their repetition can convert them into just-so stories. Are there other mechanisms that could result in the same outcome, *i.e.* a positive relationship between plant aboveground growth rate and aboveground biomass?

   Likewise, "Under nutrient limitation, plants reduce their growth rate, build long-lived nutrient-poor tissues and increase biomass allocation to roots *to explore a larger soil volume"*. This last explanatory phrase is a just-so story. And the previous phrases suggest that plants intentionally grow more slowly and put less nutrients in their tissues when supply is limited. If it were an undernourished person who was smaller with symptoms of poor nutrition, would you assume that their body had *optimized* their growth and development? Or are these simply unavoidable physiological outcomes of inadequate nutrition? Could the same be true for the plants and microbes in your experiments? (Just removing explanations beginning with "to" goes a long way to keeping our minds open as to underlying mechanisms).

Sincerely

Dr. Cindy Prescott, *Professor*