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Studies of properties of microorganisms in bulk and rhizosphere soils following the application of cover crops

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1. Research Background

Sustainable agriculture is an agriculture in which the methods are environmentally friendly and the effects on improving plant production would be sustained. Cover crop has been introduced as one of tools to achieve sustainable agriculture. The cover crops are e.g., rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa* Roth, HV), which can be applied alone or in combination prior to the cultivation of subsequent plant. Application of cover crop for sustainable horticulture system becomes popular regarding to cover crop effects on increasing vegetable products to satisfy progressing demand of the vegetables.

One of cover crop functions is as a sink of available plant nutrients, such as inorganic nitrogen (N). In the process, cover crop residue is decomposed and organic N contained in the residue is transformed into inorganic N. This process drives biochemically by soil microorganisms, i.e., bacteria and fungi. The increases of activity of β-glucosidase enzyme (BG) and soil microbial biomass (SMB) in the soil can be indicators of microbial contribution to the residual decomposition process. However, roles of the soil microorganisms in the N supply during the decomposition period are barely known.

The inorganic N supplied from cover crop residue is taken up by the subsequently cultivated vegetable plants for their growth. The absorbed value is measurable as N uptake (N_{up}). Soil bacteria and fungi can inhabit plant rhizosphere and interact with the plant. One of the interactions is assisting the process of N utilization from the soil to the plants. Nonetheless, plant–microbe interactions related to N_{up} in plant rhizosphere soil during cover crop decomposition is still not well understood.

2. Research Aim

The research was conducted to understand the effects of cover crops on horticulture systems. The research aims were (1) to clarify the N availability from cover crop residue and its utilization by the subsequent plant, (2) to identify microorganisms in bulk soil

contributing to the N availability from cover crop residue, and (3) to evaluate the plant–microbe interactions related to the N_{up} in rhizosphere soil of the subsequent plant.

3. Materials and Methods

The research was carried out in field and pot experimental levels for two years (i.e., 2017 and 2018). Treatments were soil without any cover crops (control) and soils with fresh residues of rye, HV, and mixed (rye+HV) cover crops. Cover crops were prepared in the field in late autumn and incorporated into the soil at late spring. In the field experiment, the plot was split for two application rates of N fertilizer as ammonium sulfate, i.e., 0 and 2.5 g N m^{-2} in 2017 and 0 and 6 g N m^{-2} in 2018. This was aimed to compare the effects of cover crop and synthetic N fertilizer. The effects of cover crop were clarified in the pot experiment with the same four treatments.

Red leaf lettuce (*Lactuca sativa* L. var. *crispa* cv. Red fire) was the subsequent plant, in which the seedlings were transplanted at 5 days after incorporation (DAI) of cover crop. Lettuce plant was harvested at mature stage in the field and at mid growth and mature stages in the pot to evaluate the N_{up} and yield. Bulk and rhizosphere soils were collected during the experiments (i.e., 5–38 DAI in 2017 and 3–31 DAI in 2018). The bulk soil was analyzed for concentration of soil inorganic N (i.e., NO_3^--N + NH_4^+-N), activity of BG, and carbon-based SMB. Bulk and rhizosphere soils from the pot experiment were subjected to DNA-based molecular analysis to quantify and identify bacteria and fungi. Influenced microbial taxa, i.e., microbial groups whose the relative abundance was affected by cover crops, were selected; and their relative abundances were correlated with the values of BG activity and SMB or lettuce N_{up} and yield.

4. Result and Discussion

The N availability from the cover crops residue as concentration of soil inorganic N was as follows HV > rye+HV > rye = control from the highest to the lowest. This result was related to the N contents of the residues, which was HV > rye+HV > rye. In addition, carbon to nitrogen ratio (C:N ratio) of rye, rye+HV, and HV residues was 37.8, 21.6, and 10.7, respectively. Unarguably, the lower C:N ratio of residue, the faster rate of the residual decomposition. This general statement was clarified in this study that the residual decomposition rate was HV > rye+HV > rye. In consequence, HV residue supplied the highest concentration of inorganic N in the treated soil among other residues and control.

In term of the activity of soil microbes, BG activity, SMB, quantity of microbial DNA, and relative abundance of specific microbial groups in bulk soil were changed during the residual decomposition process of cover crops. HV residue significantly increased 1.15–

8.09 folds BG activity and 1.37–2.02 folds SMB compared with other residues and control. This indicated that soil microorganisms actively play roles in the residual decomposition by producing and activating a high level of BG enzyme to degrade HV residue and increasing microbial biomass. Due to the high decomposition rate of HV residue and the high concentration of soil inorganic N derived from HV residue, BG activity and SMB were the indicators of microbial roles in N availability from cover crop.

Bacterial and fungal DNA quantity was relatively higher in all cover crop-treated bulk soils than in control soil within 5–10 DAI, which was concomitant with active cover crop residual decomposition periods, i.e., up to 15 DAI. The high quantity of microbial DNA showed a high microbial growth and was assumed as a positive response of soil microorganism to the cover crop residues. Identification of bulk soil microorganism showed that HV and rye+HV residues increased relative abundance of bacteria belonging to phyla Proteobacteria and Bacteroidetes and relative abundance of fungi belonging to phylum Basidiomycota. These three microbial groups have been identified to comprise broad plant decomposers. Thus, the results implied that HV and rye+HV residues promoted composition of plant decomposers, which was suggested to contribute to the residual decomposition. The further analysis showed that bacteria member of family *Parachlamydiaceae* and unidentified bacteria member of class SAR202 positively correlated with BG activity and SMB in HV and rye+HV or negatively correlated in rye and control. Moreover, fungi member of families *Leucosporidiaceae* positively and strongly correlated with soil inorganic N in HV, positively and moderately correlated in rye and rye+HV, and negatively correlated in control. These results indicated that each cover crop enhances specific microbial groups to contribute to the N availability.

The N utilization by lettuce plants was maximized in HV-0N and rye+HV-0N over control-0N in the field resulted in the improvement of lettuce N_{up} and yield. This results in the field experiment were clarified in the pot experiment at the mature stage of lettuce plant. Conversely, lettuce N_{up} and yield in rye depended on 2.5N or 6N fertilizer addition. Thus, HV and rye+HV, but not rye, could alternate application of synthetic N fertilizer in lettuce cultivation system. The plant–microbe analysis for rhizosphere microorganism showed larger number of positive interactions than negative interactions in HV and rye+HV, indicated that lettuce roots in HV and rye+HV recruited more beneficial interactions of the rhizosphere microorganism. Further, bacteria member of families *Alteromonadaceae* and *Chthoniobacteraceae* may be associated with the improvement of lettuce N_{up} and yield in HV and rye+HV. In rye, bacteria member of family *Haliangiaceae* negatively correlated with lettuce N_{up} or yield. Those bacteria were potentially associated with the non-improved lettuce growth in rye.

In the 2017 pot experiment, a suppression of lettuce N_{up} and yield in HV treatment at the mid growth stage of the plant was observed. This showed a suppression of utilization of soil inorganic N, which was abundantly derived from HV residue. Allelopathic effects of HV residue to lettuce plant was assumed as the reason behind the suppression. HV residue contains allelochemicals, which toxically interfere or kill plants growing in the allelopathic zone of HV. The allelochemicals were assumed to be over-expressed during the decomposition process of HV residue. In consequence, lettuce plant growth was interfered. In addition, in the plant–microbe analysis, bacteria member of family *Cystobacterineae* and *Cytophagaceae* showed negative interactions with lettuce yield. Those bacteria were suggested to be associated with the suppression of N utilization in HV treatment at the mid growth stage of lettuce plant.

5. Conclusion, Limitation, and Recommendation for Further Research

In the conclusion, HV and rye+HV cover crops demonstrated the superior effects on lettuce cultivation system. Specifically, HV and rye+HV residues derived high and intermediate concentration of soil inorganic N, respectively, which was maximally utilized by lettuce plants to improve their N_{up} and yield. As the mechanism of the N availability, bulk soil microorganism responded HV residue by promoting BG activity and SMB. Moreover, HV and rye+HV residues promoted relative abundance of phyla Proteobacteria, Bacteroidetes, and Basidiomycota, in which the members in family taxonomical level positively correlated with BG activity, SMB, or soil inorganic N. In the N utilization process, rhizosphere microbial groups, e.g., families *Chthoniobacteraceae*, *Rhizobiaceae*, *Haliangiaceae*, *Cystobacterineae*, and *Cytophagaceae*, were recruited by lettuce roots and assumed to be associated with lettuce N_{up} and yield indicating the significance of plant–microbe interactions.

The results of this study lead to three limitations, i.e., application of cover crops (1) in one soil type (i.e., Calcaric, Eutric Fluvisol light clay) and (2) using one method (i.e., incorporation of fresh residue) and (3) unclear N budget. Cover crops have been applied worldwide using various methods (e.g., mulch, living mulch, and dry residue). It is necessary to evaluate the effects of cover crop and the response of soil microorganism to cover crop residue in various soil types and application methods to broaden cover crop benefits. The forms of N are biochemically changeable in a complex way and lost to the environment. Tracking the absolute value of N derived from cover crop residue through incubation and isotopic experiments will be informative to understand the N budget.