The impact of defense hormones on the interaction between plants and the soil microbial community

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Summary

Many plant species grow better in sterilized soil than in soil that contains a live microbial community. One hypothesis to explain this phenomenon is that the overall net pathogenic effect of soil microbial communities reduces plant performance. Induced plant defenses triggered by the application of the plant hormones jasmonic acid (JA) and salicylic acid (SA) may help to mitigate this pathogenic effect. However, little is known about how the activation of SA-induced resistance impacts the microbial composition and the expression of functional genes in the rhizosphere soil.

We manipulated and induced the plant defense system through foliar application of phytohormones (JA or SA), and examined whether the negative effect of live soil on plant growth was reduced. The growth of four plant species (*Jacobaea vulgaris*, *Cirsium vulgare, Trifolium repens* and *Daucus carota*) was affected differently in live soil and by the hormone treatments. Foliar application of SA increased plant growth in live soil for the species, *J. vulgaris* and *C. vulgare,* which were the two species that both produced less biomass in live soil than in sterilized soil, SA application slightly reduced plant growth in live soils for the species *T. repens* and *D. carota* that were not affected in live soil. Application of JA reduced plant growth in live and sterile soil for all species. For *J. vulgaris* the treatments were repeated for three more generations. In each generation, the live soil consisted of a mixture of 10% of soil collected from pots from the previous generation mixed with 90% sterilized soil. In all four generations, plant biomass was measured. The reduction in growth in live soil was consistent in each generation, and in each generation, this negative effect was mitigated by the application of SA to plants. Hence, we found no evidence for an increase in the negative plant-soil feedback over generations, but also no selection effect of SA application over time.

RNA extracted from the rhizosphere soil from each generation was subsequently sequenced. Soil microbial composition at genus level was studied and the expression of functional genes of live soils where plants grown under SA treatments and control were compared. Application of SA to *J. vulgaris* leaves altered the composition of bacterial communities in the rhizosphere soil but only in the second, third and fourth

growth cycle. However, the SA effects on bacterial community composition were small, while there was a substantial temporal effect on rhizosphere bacterial composition. As there were no genera of bacteria that responded to SA application in the first generation this suggests that there are no immediate responses of bacteria in the rhizosphere to SA application to plants.

Subsequently, the effects of the application of SA to *J. vulgaris* on the gene expression and functions of the soil-borne microbial community were examined for each of the four plant generations. Gene expression and functions of the soil-borne microbial community responded to the exogenous application of SA but these effects differed per generation. The number of differentially expressed genes tended to increase over generations, but remarkably there was no overlap for these annotated genes among the four generations. Moreover, we found that foliar application of SA upregulated GO terms of biological processes that were related to viral RNA genome replication, to interactions with host cells, to organelles of the host cells and to RNA polymerase activities. There were six GO terms of which the expression decreased in the second, third and fourth generation, and these were associated with processing nitrogen and macromolecules.

Finally, in a series of experiments, we examined for *J. vulgaris*, how plant responses to live soil changed over time, by repeatedly harvesting plants over time. In all experiments, plant growth was worse in live soil than in sterilized soil and this effect on plant biomass was consistent over time. However, relative growth rates of plants in the sterilized soil and live soil only differed for young plants and a reverse pattern was even observed during the latest stage where relative growth rates were higher for plants in live soil. This shows that while the soil treatment may result in plant biomass being consistently lower, this could have been caused solely by initial effects of the treatment on plant growth. Hence, to better understand plant-soil interactions, it is important to examine not only plant biomass but also plant growth rates. In a third growth experiment, we also examined the effect of the timing of soil inoculation prior to planting on the relative growth rate of *J. vulgaris* plants with four different timing treatments. Biomass was reduced in all inoculated soils and there was a negative relationship between time since inoculation and plant biomass. Again, in all inoculated soils the negative effect of the soil microbial community on plant growth

disappeared two weeks after planting. Overall, these results suggest that young plants or seedlings are most sensitive to soil pathogens.

In conclusion, our research shows that aboveground activation of defenses in the plant affects soil microbial communities and as soil microbes can greatly influence plant performance, this implies that induction of plant defenses, can lead to complex above-belowground feedbacks.