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Transient negative biochar effects on plant growth are strongest after microbial species loss

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A B S T R A C T

Biochar has been explored as an organic amendment to improve soil quality and benefit plant growth. The overall positive effects of biochar on crop yields are generally attributed to abiotic changes, while the alternative causal pathway via changes in soil biota is unexplored. We compared plant growth effects of legumes in sterile soil inoculated with dilutions of soil and soil microbial suspensions to determine the direct effects of biochar-induced changes in soil biota on plant growth. Suspensions and soil were from soil amended with biochar and soil without biochar. By comparing consecutive plant growth phases on the same inoculated soils, we also determined the temporal effects of soil biota from biochar-amended and control soils. Biota from biochar-amended soil was less beneficial for *Medicago sativa* growth, especially with small amounts of inocula. Flowering was delayed in the presence of biota from biochar plots. Inoculum with either soil or soil suspension gave similar results for plant biomass, indicating that microorganisms play a major role. *Vicia villosa* growth did not respond to the various inocula, even though the inoculum quantity strongly affected nematode community composition and protozoan abundance. In a later growing phase the negative effect of biochar-associated biota on *Medicago* growth mostly disappeared, which leads to the conclusion that the benefits of biochar application via abiotic changes may outweigh the negative effects of biochar on soil biota.

1. Introduction

Incorporation of different forms of organic material to soils can increase carbon storage capacity and simultaneously stimulate plant growth, plant health and nutrition. Biochar, a rest product after burning organic material at high temperature in the absence of oxygen (pyrolysis), is one of the materials explored for this purpose (Sohi et al., 2010). Meta-analyses of the effects of biochar addition to soil on crop growth demonstrate overall positive effects on yield (Jeffery et al., 2011; Biederman and Harpole, 2013). This could be explained by the effects of biochar on abiotic characteristics of the soil such as pH, water holding capacity and nutrient availability (Cao et al., 2014; van de Voorde et al., 2014). However, biochar addition can also influence the abundance of soil organisms such as earthworms, nematodes, or soil microorganisms (Lehmann et al., 2011). Here we aim to assess to what extent the positive effects of biochar addition on yield could also be mediated via changes in soil biota; this requires disentangling the direct effects of biochar on soil biota from the indirect effects via changes in abiotic soil parameters.

Legumes are a popular intercrop or cover crop in agricultural production systems, due to their nitrogen fixing abilities. They interact with both rhizobial bacteria and mycorrhizal fungi. How biochar affects legumes via its effect on soil microorganisms is poorly understood. In a biochar amelioration field experiment in a species rich grassland (van de Voorde et al., 2014) legume biomass strongly increased in the first growth season just after biochar amendment and this was related to increased potassium availability due to biochar addition (Oram et al., 2014). Biochar can

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disturb the chemical communication between plants and microorganisms due its high sorption characteristics (Masiello et al., 2013). Several other studies also report increases in legume biomass (Berek and Hue, 2016) or nodulation (Mollinedo et al., 2016; George et al., 2012) after biochar application (Schimmelpfennig et al., 2015; but see Tammeorg et al., 2014). The role that soil biota play in biochar-mediated effects on legumes are unknown, and experimental approaches are needed to study whether shifts in microbial communities persist following biochar application, in isolation of abiotic effects of biochar.

Most knowledge about biochar effects on plant and soil characteristics is based on short-term studies. There are few longer-term studies, but these do not inform on the persistence of biochar effects on soil biota due to yearly repeated additions of biochar (Zhang et al., 2014; He et al., 2016; Xiao et al., 2016). Other studies found that abiotic changes after biochar addition are short-lived, with abiotic effects such as enhanced nitrogen or potassium availability disappearing after a single growth season, while the effects on functions such as plant productivity remained enhanced, suggesting that microbial changes rather than abiotic changes could have led to the persistent effects of biochar addition (Mukherjee et al., 2014; Oram et al., 2014). One way to assess long-term indirect effects via changes in soil biota is the use of repeated growth cycles. For example, Butnan et al. (2015) used two consecutive corn-cropping cycles to test the temporal effect of biochar addition on corn growth. When biochar addition would stimulate plant pathogen pressure, a negative plant-soil feedback would be expected where plant growth would decrease the growth of subsequent plants in presence of biochar, and vice versa stimulation of mutualists should result in positive feedback. Biochar-initiated changes in microbial communities could continue beyond the short-lived abiotic changes due to density-dependent processes. Here we study the persistence of biochar effects on microbial-mediated plant growth using the experimental plant-soil feedback approach (Kulmatiski et al., 2008; van der Putten et al., 2013).

One way to examine how biochar-induced changes in soil microbial communities influence plant growth is to grow test plants in a standard sterile soil, and inoculate these soils with microorganisms collected from soils where biochar has been added or not. The use of sterilized soil allows for similar abiotic conditions while varying the microbial composition by adding small amounts of inocula. Sterile soil can be inoculated with pure live soil, or with a microbial suspension extracted from that soil. When both inoculation types affect plant growth similarly compared to the non-inoculated control soil, it is likely that microorganisms cause the effects on plant growth, since they are present in both types of inocula. If, on the other hand, only soil addition inhibits plant growth strongly, this points at an important role for soil meso- or macro-fauna that are excluded in the microbial suspension, such as plant-feeding nematodes or insects. For example, soil addition decreased biomass production of the weed *Jacobaea vulgaris* more than the addition of a microbial suspension of the same soil (van de Voorde et al., 2012).

The amount of inoculum influences the chance that beneficial/pathogenic partners will be present in the inoculum and thus can determine plant growth effects. Dilution of inoculum generally leads to declining diversity and results in mixed effects on ecosystem functioning (Roger et al., 2016). For example, plants responded with increased, decreased or no change in growth following species loss via dilution, depending on soil origin (Hol et al., 2015a). Inoculation of seedlings with several dilutions of soil suspensions resulted in huge variation in the diversity of *Rhizobium* recovered (Bala et al., 2001). The authors suggested that low abundant yet competitive strains, which are only present when large amounts of inoculum are used, will dominate during nodulation, while the abundant and competitively inferior strains will infest plants when only small amounts of inoculum are used. How soil inoculation will affect plant growth will also depend on the plant species and their interactions with meso-fauna and microorganisms. Plant species with strong mutualistic interactions, such as plants that depend on arbuscular mycorrhiza or rhizobia, will be more sensitive to microbial inoculation. For those microbial-dependent plant species, soil suspension inoculation should increase plant growth due to addition of mutualists, compared to inoculation with pure soil where herbivores may overrule the effects of the microbial mutualists. The origin of both plants and inoculum may play an important role, since plants can be adapted to the local soil conditions, at a scale of meters to kilometers (Smith et al., 2012). Thus, biochar-mediated changes in soil conditions may influence locally adapted plants more than plants from elsewhere. The predominant use of commercial seeds leaves only a marginal role for local adaption in agriculture.

We compared plant growth effects of legumes in sterile soil inoculated with dilutions of soil and soil microbial suspensions to determine the direct effects of biochar-induced changes in soil biota on plant growth. By comparing consecutive plant growth phases on the same inoculated soils, the microorganisms were allowed to acclimate over phase 1, and we could determine if acclimation over time had a subsequent effect on the same plant species in a later growing phase. We hypothesised that biochar addition will change the overall composition of the soil community, resulting in beneficial effects on legume growth, and that loss of soil diversity will negatively affect plant growth over multiple generations. We expected that microorganisms will respond faster to biochar addition than larger soil organisms due to their faster reproduction, and thus larger differences are expected between control and biochar treatments for the microbial suspensions. Different amounts of inocula were used to test whether effects would be robust to species loss (Hol et al., 2010). Given the higher amount of legumes in the biochar plots where the soil was collected from (van de Voorde et al., 2014), we assume a lower density of nitrogen-fixing bacteria in the control plots. Populations at low densities are more vulnerable to random extinctions (Gaston, 2008), and thus we expected the strongest dilution effects with inoculum from the control plots.

2. Material and methods

Soil collected from a field experiment with plots with and without biochar addition (van de Voorde et al., 2014) was used as inoculum in three greenhouse studies, to examine how biochar-mediated changes in soil biota affect plant growth. To study which part of the soil community is responsible, we inoculated whole soil or microbial suspensions from the field soil into experimental pots containing sterile soil. In the experiment we varied the size fraction of the soil community (all soil biota vs soil microorganisms (<45 μm) only) and the amount of soil inoculum.

2.1. Soil collection

Bulk soil (150 kg) was collected on July 18, 2011 from a semi-natural grassland that was previously used as arable land, (Mossel, Ede, The Netherlands 52°04′ N, 05°45′ E) by collecting the top 20 cm soil. This area is of glacial origin and on a sandy soil (93.9% sand, 5.3% silt, 3.4% clay). The soil is characterized as a "holtpodzol" on coarse sand with a soil organic C content of 2.8%. Soil was passed through a sieve (1 cm ø) to remove stones and roots, and divided over 20 bags of 7.5 kg each. The soil was autoclaved 3 times at 121 °C for 1 h, with 24 h in-between autoclaving events to also kill spore-forming bacteria. The autoclaved soil was stored at room