Effect of Arbuscular mycorrhizal fungi and biochar on soil seed bank viability

under the supervision of

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Foreword

This dissertation is a cumulative work of manuscripts, either , accepted, submitted or will be published at a later date. Therefore, this thesis is based on following paper. As we will try to publish the rest of the parts later.

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DEDICATION

I dedicate this work humbly to my wife and my kids, and to the spirit of my father and to my mother, and to all my friends in Berlin and Libya.

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Chapter 1

General introduction

1. The soil seed bank

Plant species have a wide range of strategies that allow them to be successful in unique circumstances. This diversity ensures that some plant species are able to survive under changing environmental conditions or climate. The life cycle of plants begins from the seeds; these seeds must survive and mature, germinate and then produce new plants and new seeds to complete the life cycle of the plant (Mordecai, 2012). Seed germination may happen immediately or could be delayed for some time. Thus a large number of seeds may remain dormant but viable in the soil. During this time, the seeds on or in the soil form a soil seed bank (Warr et al., 1993; Fenner and Thompson, 2005).

The soil seed bank is defined as all the viable seeds present in the soil or mixed with soil debris (Roberts, 1981; Simpson et al., 1989), and also consist of fruits and of vegetative parts of plants and in the case of mosses and ferns also of spores. Seeds are able to remain viable under the soil for a period of time, depending on the species and soil conditions (Priestley, 1986; Poschlod et al., 2004; Fernández-Quintanilla et al., 1991). The soil seed bank is the key to understand the dynamics of plant populations, and species in different ecosystems (Silvertown, 1982; Kalisz, 1991; Kalisz and McPeek, 1992; Guünter, 1997; Bekker et al., 1998a; Cabin et al., 1998). Seed banks are an important component in the establishment and development of plant communities and may be found in all ecosystems (Baker, 1989), including wetlands (van der Valk, 1981; Angeler and García, 2005; Yang and Li, 2013), and desert ecosystems (Kemp, 1989), or annual grasslands (Major and Pyott, 1966). They may play an important role in plant community development during succession (van der Valk and Davis, 1978, Grime, 1989, Leck et al., 1989 and Thompson, 2000).

The pioneer studies on soil seed banks commenced in 1859 and were carried out by Darwin when the emergence of seedlings was observed. Also, Darwin studied the phenomenon of seeds occurrence at different soil depths by using samples of soils from the bottom of a lake. In 1882, the first scientific paper was published by Putersen (Roberts, 1981).

Seed banks of the persistent seeds are important sources for the regeneration of plant communities (Fenner, 1992), and protect plants from extinction (Williams-Linera, 1993; Willems, 1995). The persistence of seeds in the soil depends on the maintenance of their viability (Murdoch and Ellis, 1992). This phenomenon is especially important in arid environments, where a large part of the flora consists of annual plants. Their seeds need to stay in the soil for many years in order to outlive the dry period (Kemp, 1989; Inouye, 1991; Guo et al., 1999 (Kemp, 1989; Inouye, 1991; Guo et al., 1999).

Investigations on soil seed bank primarily focused on aspects of maintenance through decreasing seed predation as well as by restoration of terrestrial ecosystems, given the fact that they provide a source for reestablishment of species, lost from the aboveground vegetation (Wellstein et al., 2007). The configuration of seed banks depends on the contribution of current and previous plant communities, seed rain and seed longevity. (Rice, 1989; Hutchings and Booth, 1996). However, seed survival prior to the germination is affected during primary and secondary seed dispersal and seed-microsite interaction during the seed dormancy which can lead to a seed losswhen through abiotic or biotic factors (Chambers and MacMahon, 1994).

Fungal saprophytes and pathogens are ubiquitous in soils, and are one of the main causes of mortality of many seeds in the seed bank in most terrestrial ecosystems (Leishman et al., 2000; Gilbert, 2005; Bell et al., 2006).

Some experiments revealed that longevity was increased by treatment with fungicides, (Fenner and Thompson, 2005). This conclusion is also supported by other evidence, namely that fungicide treatment contributed to improving the survival of buried seeds in a wet meadow in Canada but did not improve in sites that were drier (Blaney and Kotanen, 2001). The impact of fungal pathogens varies between sites, there are some studies that

suggest that the interactions between abiotic conditions, such as soil moisture, and the soil fungal community may have a role in explaining some of this variation (Schafer and Kotanen, 2003). Seed viability in the soil seed bank is influenced by the interaction between abiotic conditions in the environment and biotic conditions the seed (Fenner and Thompson, 2005).

2. Viability of seeds

Previous studies have established many different methods to investigate soil seed banks by assessing the presence and abundance of seeds in the soil. The majority of these studies have been done by extracting seeds from the soil, planting them under conditions suitable in terms of temperature and humidity, and counting the seedlings that germinated (Rabinowitz, 1981; Kitajima and Tilman, 1996; Carrington, 1997; Schott and Hamburg, 1997; Butler and Chazdon, 1998; Leckie et al., 2000).

There are two common and well-known methods for estimating the seed stock in the soil (Boulet, 1985; Valbuena and Trabaud, 2001). The first method is the direct technique where seeds are extracted, isolated, and identified by trained analysts using the high-quality microscopic Ergovision system; finally, they are tested for viability (Malone, 1967; Shaw, 1968). The second method is seed bank quantification by germination from soil in greenhouse trays, seed extraction, or a combination of these. The second method is considered more difficult and time consuming (Gross, 1990; Malone, 1967; Standifer, 1980).

The previous studies classified seed longevity based on the dormancy type and state of the seeds into three types. The first type is ‘transient’ in which the seeds remain viable in the soil for less than a year (Thompson, 1992). The second type is ‘persistent short term’ in which the seeds remain in the soil for 1-5 years; this type provides a buffer of low seed production in lean years (Thompson, 1992). The third type is ‘long-term persistent’ in which the seeds can last in the soil for more than five years (Baskin and Baskin, 1989; Thompson et al., 1993; Thompson and Baster, 1992). In the classical burial experiments of Beal and Duvel in the 19th century some seeds have germinated after up to 80–100

years of dormancy (Poschlod, 1991; Murdoch and Ellis, 1992). It is well established that fungal species as well as environmental factors may have harmful effects on seed vitality in the soil seed bank. Indeed, a large number of fungal species that regularly associate with seeds or are seed-borne infect the developing seeds while still attached to the mother plant (Neergaard, 1977; Meyer et al., 2007).

Causal factors responsible for mortality of seeds in the soil seed bank

- *Physical and chemical characteristics of soil*

This includes the pH of soil (Gardarin et al., 2010; Saatkamp et al., 2011a,b), soil water content (Mickelson and Grey 2006; Schafer and Kotanen 2003), soil temperature (Akinola et al., 1998., Griffin, 1972), soil moisture, and hypoxia (Voesenek and Blom, 1992; Bekker et al., 1998d; Murdoch and Ellis, 2000; Nicol et al., 2003; Webb et al., 2006). Factors that cause seed mortality include soil nutrients (Bekker et al., 1998c; Davis, 2007), such as the relative levels of soil carbon and nitrogen (Davis, 2007). Change in the chemical and physical properties of the soil may occur as a result of the use of certain components such as biochar (Brockhoff et al., 2010), which will be discussed later.

- *Biological characteristics of seeds and soil*

First, the biological characteristics of seeds, such as size, or seed coat thickness, as well as seed germination traits, all influence the longevity of the soil seed bank; importantly, these traits may vary within a species (Thompson et al., 1993; Bekker et al., 1998b; Thompson et al., 2003; Gardarin et al., 2010; Saatkamp et al., 2011b). Moreover, germinating too deep, aging, and loss of viability also factor into seed survival (Blaney and Kotanen, 2001).

Second, the biological characteristics of the soil, such as how soil fungi respond to moisture, plant litter (Blaney and Kotanen.2001; Schafer and Kotanen.2003), and soil microorganisms (Chee-Sanford et al., 2006; Kremer, 1993), as well as soil animals (Meisner at, el., 2013), may contribute to seed survival.

Fungal pathogens are one of the main causes of the mortality of buried seeds. Some studies reveal that seed longevity increased when treated with fungicides (Fenner and Thompson, 2005). Other studies have addressed the effects of pathogens on the soil seed bank in ecosystems (Chambers and MacMahon, 1994; Thompson, 2000; Gilbert, 2005). Various pathogenic fungi have different impact in different sites, some studies suggesting that interactions between abiotic conditions, such as soil moisture and this variation may be goes back to the role of soil fungal community (Schafer and Kotanen, 2003). A number of studies support the hypothesis that using biological controls may reduce the incidence of fungal pathogens, e.g., the use of mycorrhizal fungi (Vaast et al., 1998; Kathiresan, 2006).

3. *Arbuscular mycorrhizal fungi (AMF)*

Arbuscular mycorrhizal fungi (AMF) are the most widespread root symbioses of terrestrial plants (Smith and Read, 2008). AM fungi are found in 80-90% of plant families, including most crop plants (Read et al., 1976; Harley and Smith, 1983; Schwarzott and Walker, 2001) They are thought to be ecologically important to most vascular plants (Harley and Smith, 1983) because of its role in increasing the absorption of immobile nutrients, principally phosphorus from the soil (Harrison, 1999), and because they mediate resistance to drought and pest tolerance (Nelsen and Safir, 1982). They account for up to 50% of the total soil microbial biomass (Olsson, 1999) and are thought to have an important role in the creation and maintenance of the soil aggregate structure (Rillig, 2004). In addition to the role of AM fungi in reciprocal nutrient fluxes, there are other functions, such as pathogen protection (Newsham et al., 1995; Borowicz, 2001; Wehner et al., 2010; Veresoglou and Rillig, 2012).

Mycorrhizal plants may even sustain a greater attack by pathogens, yet grow better than their non-mycorrhizal counterparts (Vaast et al., 1998).

The impact of AM fungi on pathogens occurs probably indirectly through improved nutrition or altered physiology of the host (Dehne, 1982; Smith, 1988; Lingua et al., 2002). AM fungi can suppress pathogen growth by competing with pathogens for infection sites or photosynthesis products, or by promoting the growth of soil microbes that are antagonistic to pathogens (Linderman, 1992; Thomas et al., 1994)

Some studies support the hypothesis that AM fungi enhance host plants, and as such, have been used for biocontrol of pathogens (e.g., Dehne and Schönbeck, 1979; Davis and Menge, 1981; Berg et al., 2007; Veresoglou and Rillig, 2012). Some studies have indicated that mycorrhizal plants may even sustain a greater attack by pathogens yet grow better than their non-mycorrhizal counterparts (Vaast et al., 1998). Tosi and Zazzerini (2000) found that the AM fungi can also confer a protection against fungal pathogens, such as *Plasmopara helianthii*, which infects sunflowers.

4. *Biochar and its effect on the physical and chemical parameters of soil and plant growth*

Biochar can be defined as a carbon-rich product (charcoal-like) by heating organic materials in a closed system with little or no air (Lehmann and Joseph, 2009; Burges, 2009). Natural Biochar is present in soil around the world deposited by natural events such grassland and forest fires (Krull et al., 2008; Skjemstad et al., 2002). Biochar is produced through pyrolysis where the different organic material is heated in the absence of oxygen (Schahczenski, 2010). Biochar can be obtained from biomass materials of either plant or animal origin (Antal and Grønli, 2003; Lehmann and Joseph, 2009; Harris,1999). The quality of the biochar produced depends on the production temperature, and a type of organic materials used and biomass particle size (Li and Zhang, 2005; Özçimen and Ersoy-Meriçboyu 2008; Yao et al., 2011; Asadullah et al., 2011)

Biochar has effects on main soil characteristics, including cation exchange capacity (CEC) of soils (Glaser et al., 2002; Yamato et al., 2006; Liang et al., 2006; Cheng et al., 2008; Novak et al., 2009; Brockhoff et al., 2010), pH of soil (Tryon, 1948;Yamato et al.,

2006; Rondon et al., 2007; Steiner et al., 2007; Novak et al., 2009) and soil fertility (Steiner, 2007; Joseph, 2008). Aside from the improved retention of nutrients (Wardle et al., 1998; Lehmann et al., 2003), biochar can also enhance soil water holding capacity, soil aggregation, and soil strength (Chan et al., 2008; Laird et al., 2010). When adding biochar to agricultural soil it can decrease leaching of nutrients (Lehmann et al., 2003; Lehmann et al., 2006; Laird et al., 2010). Furthermore, biochar may be useful in overcoming the deleterious effects of allelopathic residues (Wade et al., 2011). However, these effects depend on the pyrolysis conditions and biochar feedstock (Chan et al., 2008; Gaskin et al., 2008), and the soil itself (Speir, 2008). Biochar also appears to be able to strongly adsorb phosphate, even though it is an anion (Lehmann et al., 2005).

5. *Possible interactions between biochar and AM fungi*

There have been some studies conducted to measure the impact of biochar on AM fungi (Yamato et al., 2006; Rondon et al., 2007; Warnock et al., 2007; Rillig et al., 2010) and on the stimulation of resistance against disease agents (Prithiviraj et al., 2007; Elad et al., 2010). It has been found that the addition of biochar can play a role in increase the ability of AMF to help their host plant in resisting infection by plant pathogens (Matsubara et al., 2002). Biochar generally has positive effects on mycorrhizal root colonization in soil (Warnock et al., 2007).

6. Effect of AMF and /or biochar on the soil seed bank

There are no studies on the relationship between the soil seed bank and AM fungi.

The impact of AM fungi on plant growth are very well documented (Smith & Read 2008), but no studies about their influence on soil seed bank. Only recent study carried out by (Varga 2015) found that AM fungi can negatively influence seed germination, while still improving plant growth afterwards.

There are no studies on the effect of biochar on the soil seed bank, with the exception of some studies conducted on the Dark Earth soils in the Amazon (Glaser et al., 2001; Major et. al., 2005). These studies are generally based on the extraction of seeds from the soil seed bank, then culturing the seeds in greenhouses.

Dark Earth soils contain a high percentage of carbonized materials (Glaser et al., 2001) Clement et al. (2003) found that Dark Earth soils had a positive impact on the seed bank, and Major et al (2005) found the seedlings from a greater number of species emerged from forested Dark Earth seed banks (2.1 per flat) than from forested adjacent soil (1.2 per flat), and the total number of emerged seedlings was greater for Dark Earth seed banks (9.1 per flat, 1,365 m^2) than adjacent soil (2.2 per flat, 330 m^2).

This study is trying to identify some of the aspects that have not been studied in the past in relation to the soil seed bank.

7. Objectives of the thesis

The main objective of this thesis was therefore to test if and how AM fungi affect the soil seed bank and seed viability. The second major aim was to evaluate the impact of carbonized materials, such as biochar, on seed viability. Furthermore, possible interactions between AM fungi and biochar on seed viability in the soil seed bank were assessed.

I investigated the effects of biotic (mycorrhiza) in combination with abiotic (biochar) factors on seed viability in soil seed bank, plant biomass performance and physical-chemical properties.

I started with an experiment under greenhouse and field conditions on the effects of mycorrhiza presence on viability of soil seed bank of seeds (*Taraxacum officinale* G. H. Weber ex Wiggers), (*Dactylis glomerata* L.) and (*Centaurea nigra* L.). The three plant species were selected because they have been used previously in similar experiments, they are characterized by marked fungal growth and reasonably low seed germination, and that their seeds remain mainly ungerminated when buried in soil (Mitschunas et al., 2006) and they can be obtained commercially. I tested impacts of AMF presence on viability of the soil seed bank under greenhouse and field conditions and to identify underlying mechanisms (chapter 2).

In the second experiment, I tested if different feedstock types of biochar and their concentration in the soil can impact on chemical, physical characteristics of soil and seed viability of seeds of three plant species (chapter 3).

In the third experiment I investigated the interactions between AM fungi and biochar and their combined effects on seed viability in the soil seed bank and. Since both biochars and AM fungi have strong effects on plants and herbivores, possible interactions may change the separate influence of one of them (chapter 4).

Chapter 2

Arbuscular mycorrhizal fungal hyphae negatively affect soil seed bank viability

Abstract

Seed banks represent a reservoir of propagules important for understanding plant population dynamics. The viability of seeds in a soil seed bank depends on soil conditions (including moisture or pH), seed species and soil biota. Compared to the vast amount of data on plant growth effects, next to nothing is known about how arbuscular mycorrhizal (AM) fungi could influence viability of seeds in the soil seed bank. To test if and how AM fungi could influence seed bank viability, we conducted three two-factorial experiments using seeds of three herbaceous plant species (*Taraxacumofficinale, Dactylis glomerata*, and *Centaurea nigra*) under mesocosm (experiments 1 and 2) and field conditions (experiment 3). To allow only hyphae to grow in and to prevent root penetration, paired root exclusion compartments (RECs) were used in experiments 2 and 3, which were either rotated (interrupted mycelium connection) or kept static (allows mycorrhizal connection). After harvesting, seed viability, soil water content, soil available phosphorus, soil pH and hyphal length in RECs was measured. A significant effect of mycorrhizal hyphaeon viability of seeds of different species was observed in experiments 1 and 3, but not in experiment 2. All three experiments showed that water content, soil pH and AMF extra radical hyphal lengths were increased in the presence of AM fungi, but available P was decreased significantly. Viability of seeds in the soil seed bank correlated negatively with water content, soil pH, AMF extra radical hyphal lengths and soil P availability.

Synthesis: Our results suggest that AM fungi can have a negative impact on soil seed viability, which is in contrast to the often-documented positive effects on plant growth. Such effects should be included in our conceptual models on AM symbiotic effects.

Keywords: Soil seed bank; Arbuscular Mycorrhiza; Seed viability; Soil seed bank, *Taraxacumofficinale*; *Dactylisglomerata*;*Centaureanigra*

Introduction

The soil seed bank comprises all viable seeds present on or in the soil or in the associated litter (Simpson, Leck, & Parker 1989). Being present in nearly all terrestrial ecosystems (Baker 1989),the seed bank plays a prominent role in the ecology of many plant species (Roberts 1981; Thompson 1987;Leck, Parker & Simpson 1989; Thompson, Bakker &Bekker1997; Baskin &Baskin 1998). Seeds can remain viable in soil for different periods of time depending on plant species and soil conditions (Priestley 1986; Buhler& Hartzler 2001;Poschlod,Tackenberg& Bonn 2005; Conn, Beattie& Blanchard 2006). Depending on the dormancy type and state of the seeds, the soil seed bank is traditionally classified as: transient (less than 1 year), short-term persistent (1-5 years), and long term persistent (larger than 5 years) (Baskin &Baskin 1989; Thompson &Baster 1992; Thompson 1993). The soil seed bank plays an important role in the composition and succession of many plant communities (Thompson 1992), for example in wetlands (Van der Valk 1981) or desert ecosystems (Kemp 1989).Seed banks can be an important component for understanding the dynamics of plant populations, communities and ecosystem functioning(Silvertown 1982; Kalisz 1991; Kalisz &McPeek 1992; Günter 1997; Bekker et al. 1998; Cabin, Mitchell, & Marshall1998). Persistent seeds in the soil seed bank can also represent a reserve of genetic potential accumulating over time (Simpson, Leck&Parker 1989).

Soil organisms can have a direct effect on the soil seed bank; for example, seeds may be affected by the activity of soil biota, such as the transfer and burial of seeds by earthworms (Grant 1983; Van der Reest&Rogaar 1988; Thompson, Green &Jewels 1994) or other soil animals (Grant, 1983; Shumway &Koide 1994; Willems &Huijsmans 1994; Bernhardt 1995). Furthermore, fungal pathogens are a main cause of mortality of buried seeds(Leishman et al2000), and abiotic conditions, such as soil moisture, moderate their effect on seeds(Schafer &Kotanen 2003). Other soil biota, such as arbuscular mycorrhizal (AM) fungi can be responsible for changes in abiotic conditions(Read & Perez-Moreno 2003).

AM fungi are a key component of soil ecosystem, especially in grasslands. They provide numerous services to plants, including enhanced nutrient uptake (particularly P), or increased plant resistance against pathogens and abiotic stressors(Smith &Read 2008). AM fungi also have an impact on plant diversity patterns in a variety of ecosystems (Van der Heijden et al. 1998; Hartnett &Wilson1999), for example by providing differential benefits to members of the plant community. Mycorrhizal plant growth responses range from positive to negative, suggesting that mycorrhizae operate along a mutualism-parasitism continuum, depending on the relative benefits and costs of the symbiosis (Johnson, Graham & Smith 1997; Johnson &Graham 2013); such effects may differ for different plant life history stages (Varga 2015).

Effects of AM fungi on plant growth are very well documented (Smith &Read 2008), but almost nothing is known about their influence on the seed bank, most likely because this is a plant life history stage generally viewed to not be influenced by AM fungal infection. In general the early stages of plants appear to be neglected with respect to effects of arbuscular mycorrhiza. Recently, Varga (2015) did showed that AM fungi can negatively influence seed germination, while still improving plant growth after wards. Thus, there is a pressing need to know if AM fungi can influence plant seeds and the soil seed bank.

Therefore, the main goal of this research is to explore if and how AM fungal mycelium could influence the seed bank, and specifically seed viability. To address this goal we carried out three experiments in the greenhouse and in the field, using seeds of three grassland species.

Material and Methods

Seeds and soil

In all our experiments, seeds of three herbaceous plant species (*Taraxacum officinale* G. H. Weber ex Wiggers), *Dactylis glomerata* L, and *Centaurea nigra* L. were used; these were obtained from a commercial supplier (Albert Treppens& Co Samen GmbH, Berlin, Germany). We chose these species because their seeds do not germinate when buried in soil at a temperature generally permitting fungal growth (Mitschunas, Wagner &Filser 2006). Seeds of *C. nigra* generally had quite high viability, whereas seeds of the other

two species had low viability in preliminary trials; since the direction of a potential effect of AM fungi is not clear *a priori*, we thus also represented different inherent seed viabilities.

The soil used in the greenhouse experiments was an AlbicLuvisol from a meadow in Dahlem (Berlin, Germany). It was a fresh loamy and sandy soil having the following properties: N = 0.12%, C = 1.87%, 74% sand, 18% silt and 8% clay and the soil pH was 7.1 (Rilliget al. 2010). The soil was obtained at a depth of 10-40 cm below the surface, then air-dried and passed through a 2 cm-sieve to remove plant material and stones, and to homogenize it. We chose this soil due to its high AM inoculum potential(Rillig et al. 2010).

Preparation of root exclusion compartments

Two out of the three experiments were carried out in the greenhouse and one (experiment 3) was set up in the field. A modified in-growth core design (Johnson, Leake& Read 2001) was used for experiments 2 and 3 only. Paired root exclusion compartments (RECs) were used in experiments 2 and 3, which were either rotated (interrupted mycelium connection) or kept static (mycorrhizal connection intact); thus providing a soil volume with or without AM fungal mycelium in which to place seeds.

The RECs (diameter 3 cm, height 12 cm) were prepared by covering the sides and bottom of the core with 30 μm nylon mesh (SefarNitex 03-30/18, Sefar GmbH, Edling, Germany) in order to allow only hyphae to grow in and to prevent root penetration. The RECs were filled at the beginning of the experiment with non-sterilized soil (see above).

Experiments

A series of three experiments, described below, were performed with the aim to explore the effects of AM fungal mycelium on the viability of seeds in the soil seed bank. Each experiment had a two-factorial design, where each treatment was replicated ten times.

The first factor was species identity, consisting of three species of plants (*T. officinale, D. glomerata,* and *C. nigra*).The second factor was presence or not of AM fungi with two levels (without and with AM fungal mycelium); this was achieved in experiment 2 and 3 with the REC arrays. Half of the RECs were kept static after placing them in the soil with the purpose to allow hyphal in-growth, and the other half were rotated by 1-2 mm three times a week around their vertical axes in order to sever any hyphae crossing the mesh barrier. We previously showed that in the same soil, rotating cores for excluding AM fungi had no confounding effects on soil abiotic properties (Leifheit, Verbruggen& Rillig 2014).

Experiments were set up under controlled (experiment 1 and 2) and field conditions (experiment 3).Fifty seeds of each species were enclosed in plastic mesh bags (2×2 cm, mesh pore size 500 µm) to protect them from seed predators and facilitate harvest at the end of the experiment. The mesh bags were placed inside the RECs equidistantly (2±1mm, distance of mesh bag from side of core; 5±1cm deep from the surface). We selected this depth because it is an appropriate depth for the presence of viable seeds in the soil seed bank and mycorrhizal fungi in soil (Korb et al. 2004). As host plants for the mycorrhizal network in the pot experiments we used *Trifolium repens*in experiment 1 and Sudangrass (*Sorghum x drummondii*)in experiment 2. Both species are frequently used in mycorrhizal studies. Seeds of these host plants were sown on wet paper in plastic containers in a climate chamber at 20 °C and 16h duration of light. Seedlings were then transplanted four weeks after germination into the experimental microcosm.

Experiment 1: Greenhouses inoculation-based study

In this two-factorial experiment, the first factor (seed species identity) consisted of three different seed species while the second factor was the addition of AM fungi with two levels (without and with AM fungi). Half of the pots were filled with autoclaved soil (to eliminate any AM fungal propagules), mixed with 10g mycorrhizal pellets (AM fungi treatment); containing the AM fungus *Rhizophagus irregularis* (Blaszk., Wubet, Renker&Buscot) C.Walker &Schuessler (formerly *Glomus intraradices*) (Biomyc®

Germany). The other half of the pots received the same autoclaved soil but with autoclaved pellets for the non-mycorrhizal control (no-AM fungi treatment); a microbial wash was prepared and added to all pots as described by Achatz et al. (2014).

Experiment 2: Greenhouse study using rotated RECs

For confirming the results of experiment 1 and to eliminate the possibility that results were driven by autoclaved soil and a single added AM fungal species, we carried out another experiment with a rotated REC design. This two-factorial experiment with species identity and AM fungal mycelium presence as factors was carried out in the greenhouse. AM fungal mycelium presence consisted of the levels rotated (interrupted mycelium connections) or kept static (AM fungal mycelium present inside RECs). Each pot (3L per pot) at the beginning of the experiment was filled with non-sterilized field soil containing an AM fungal community.

Experiment 3: Field study using rotated RECs

This experiment was conducted in the field with a semi-natural plant community, consisting predominantly of *Lolium perenne* and *Poa annua,* during April to June 2013 at experimental garden plots of FreieUniversität Berlin; this general site was used in a previous experiment using RECs (Achatz and Rillig, 2014). Weused non-sterilized soil inside the RECs; we filled into the RECs the same soil as in the pot experiments. Twenty RECs were placed in the field, always with a distance of 5 cm between the cores. To enable a connection to the existing mycorrhizal network in the field plot, half of the compartments were kept static after placing them in the soil (depth: ca. 12 cm), the others were rotated three times per week by 1-2 mm severing the hyphae attempting to cross into the RECs (Achatz et al.2014). Fifteen weeks after planting, the seeds were taken out of the RECs and a soil sample from each REC was taken for further analysis.

Post-harvest measurements

All measurements were carried out with soil from RECs (experiments 2 and 3), or the experimental soils in pots (experiment 1). In order to determine the available phosphorus (P) content in the soil, the calcium-acetate-lactate soluble phosphorus content was determined spectrophotometerically according to the German standard method DIN 3.4.1.30.2a (Blume, Deller &Leschber,2000).Soil pH was assessed at the end of the experiment with a pH-meter (Knick 761 Calimatic) in a 1:5 (w/v) aqueous dilution. Soil water content was determined as weight loss after drying at 70°C for 72 hours.

Hyphal length of AM fungi was determined in 4.0 g of fresh soil by an aqueous extraction and membrane filter technique modified after Jakobsen, Abbott & Robson(1992). Hyphae of AM fungi were distinguished microscopically at (200X) from other fungal hyphae as described by Rillig, Field&Allen (1999).

Seed were extracted from the RECs or soils in pots. Fifty seeds of every species were counted and tested by the modified method of Malone (1967) staining them with a solution of2,3,5-Triphenyltetrazolium chloride(TTC; Sigma-Aldrich, St. Louis). The dicotyledonous species, (*C. nigra*, *T. officinale*) and the grass (*D. glomerata*) were exposed to 0.1% and 1% solution of TTC, respectively. After keeping the seeds in darkness for 48 hours at 20ºC and rinsing five times in sterile distilled water, the seeds were agitated between cover slides to remove the seed coat (testa)and then they were observed using a light microscope. Embryos which were completely pink to red were considered viable, while those embryos which were partially white, yellow or brown were categorized as not viable (Van Waes &Deberg 1986).

Statistical analysis

Seed survival data were analyzed in R (Version 2.14.1) through mixed-effects generalized linear models. We used the function (*glmer)* in the package lme4 for this purpose (Zuur et al. 2009). Errors were assumed to follow a binomial distribution. In all three experiments we used mycorrhizal status and plant species as categorical predictors

and we considered their interaction. Block effects were accounted through a random effects factor. In experiments two and three, we assumed each pot to be a different block. In experiment three each neighboring REC pair (rotated and non-rotated RECs) was a different block.

For pH, hyphal length and available phosphorus we implemented two-way ANOVAs with the same predictors as for seed survivorship. Data on soil pH, hyphal length and available P in soil were log-transformed and seed survival were arcs in-transformed as necessary to meet the assumptions of normality and homoscedasticity

Differences between the hyphal connection/presence treatments were analyzed by single factor ANOVA including all the data. We used Tukey-Kramer HSD to conduct multiple comparison tests. The relationships among hyphal length, water content, seed viability, soil P concentrations and soil pH were tested via Pearson correlation coefficients.

Results

Demonstration of treatment effectiveness

In all three experiments, irrespective of field or greenhouse or RECs or inoculation-based approaches, we found significant differences in AM fungal hyphal abundance between the AMF and no-AMF treatments (Fig. 1). Hyphal abundances were always clearly higher in the AMF treatments.

Effect AM fungi on seed viability

In our experiments we investigated the impact of AM abundance on seed viability. We found significant main effects for the factor “mycorrhiza” and the factor “seed species” in all three experiments(Table 1), with the interaction term significant in experiments 2 and 3, but not in experiment 1. There were consistently negative effects of AM fungal presence on seed viability of *C. nigra* in all three experiments, but there were no such effects for seeds of *T. officinale* and *D. glomerata* in any experiment (Fig.2). Overall seed

viability, irrespective of treatment was much lower for *T. officinale* and *D .glomerata* than for *C. nigra* in all three experiments(Table 1, Fig. 2).

Soil properties

We assessed the impact of AM fungi on soil characteristics to gain insight into potential AM fungal mediated effects on seed viability. We found that AM fungi had significantly negative effect on available P content in soil as compared to the control(Figure 3).In addition, we found that water content, soil pH had significantly increased with AM fungi as compared to the control (without AMF) (Fig.3).In the field experiment, seed viability was negatively related with soil AM fungal hyphal length, pH and water content, but positively with soil P (Table 2).

Discussion

We showed through our three complementary experiments, which employed different means of manipulating AM fungal abundance, and which were carried out in the field and in pots, that AM fungi had a clear and negative impact on soil seed viability for one of the three species of plants we examined. The fact that this result was robust to the particularities of experimental design, each of which has its advantages and drawbacks, increases confidence in our findings. For example, in one case (experiment 1) only one AM fungal species was involved (added as inoculum), whereas in the other experiments, communities of AM fungi were likely active. Importantly, we observed this effect in the field as well as in pots.

Since we assumed that AM fungi would be unlikely to directly affect seed viability, we measured a number of soil parameters known to influence soil seed viability, which could also be influenced by AM fungal hyphae. Seed viability can be affected by soil physicochemical properties (Pakeman, Small &Torvel, 2012), such as soil pH and soil water content (Bekker et al. 1998; Wagner &Mitschunas2008), and perhaps nutrients. Other factors include the soil microbial community(Leishman et al. 2001; Shafer

&Kotane, 2003; Dalling et al 2011), which could in turn be influenced by the soil physicochemical parameters. For example, soil water content can affect the vitality of seeds in the soil both directly and indirectly due to its interrelation with other parameters such as aeration and temperature. Soil moisture potentially affects germination of fungal spores and growth of soil fungi(parasitic or saprobic)colonizing seeds, in addition to affecting change in the soil microbial community, which may affect seed viability(Wagner &Mitschunas 2008).

In this study, we found a close relationship between the increase in (local) soil water content, affected by the AM fungal treatment, and decreased seed vitality. Perhaps the reason for this relates to water transport along AM fungi hyphae (Querejeta, Egerton-Warburton& Allen 2003) into the compartment containing the seeds, or perhaps the effect is due to effects on water content due to potential AM hypha-mediated effects on soil aggregation (Rillig &Mummey 2006). Irrespective of the mechanism, which our study was not designed to disentangle, the higher water content could then have facilitated microbial growth, leading to the degradation of seeds.

AM fungi are functionally mostly associated with an increased uptake of phosphorus from the soil, but other nutrients can also be taken up and taken to the plant host (Smith &Smith 2011).Our results accordingly showed decreased soil P availability with AM fungal presence in all three experiments (Fig. 3). This decreased phosphorus in the soil perhaps also contributed to decreased seed viability, perhaps via effects on the soil microbial community. So, also Van der Walk &Rosburg (1997) collected seed bank samples in the northern Everglades along a phosphorus gradient with three vegetation zones, where they found the highest seed numbers in the zone with the highest available P. This also is in accordance to Iannucci(2014), who showed that additional mineral fertilization can have positive effects on the seed-bank size of ungrazed natural pastures, where mineral fertilizer applications increased the seed-bank size notably, whereas the author suggest to use it to improve degraded Mediterranean pastures.

Our results, besides adding novel, basic data on AM fungal effects on an important plant life history stage, could also have applied relevance, for example in restoration. The seeds of desirable species could be rare and seeds of less desirable exotic species could be very

abundant in the seed bank (St. John1998); in the beginning of the restoration process, AM fungi may confer an advantage to certain seed types by inhibiting viability of others. Harnessing such relationships could thus aid in encouraging successional trajectories through the addition or management of mycorrhizal inoculum, e.g. by helping to control weeds(Jordan, Zhang&Huerd2000).

Conclusion

Our results suggest that AM fungi can have a negative impact on soil seed viability, which is in contrast to the often-documented positive effects on plant growth. This result highlights how symbionts may have different or even contrasting effects on different life history stages of their host. These results invite further investigations on the generality of this finding in other plant species and ecosystems, and our findings should be included in our conceptual models of AM fungal effects on plant populations and communities.

Table II. 1: ANOVA F values for the effects of AM fungi (AMF) and seed species (Sp), and their interaction on viability of seeds of three species (* = p<0.05; **= p<0.01; *** = p<0.001)(n=10). In experiment 1, the AMF treatment was achieved by inoculation or not inoculating, whereas in the experiments 2 and 3 this was achieved using rotated/static RECs.

Experiment	***Mycorrhiza (AMF)***	***Species (Sp)***	***Interaction (AMF×Sp)***
Experiment 1	52.80***	1.41***	4.81
Experiment 2	137.83***	2.55***	24.45***
Experiment3	183.21***	5.33***	106.66***

Table II.2: Pearson's correlation coefficients for all variables measured in the field experiment (* = p<0.05; ** p<0.01) (n=10).

	Viability of seeds	Hyphal length	Soil pH	Water content
Viability of seeds	-	-	-	-
Hyphal length	**-0.657****	-	-	-
Soil pH	**-0.616***	NS	-	-
Water content	**-0.714****	***0.***549***	NS	-
Phosphorus	**0.803****	-***0.***692****	***0.***773****	-0.**564***

Figure II.1: Demonstration of treatment effectiveness. Effects of RECs (no AMF), and static core (AMF) on hyphal length of AM fungi in soil in all experiments. Means and standard deviation (n = 10) are shown. Different letters indicate significant differences between the treatments at $p< 0.05$ according to the Tukey-Kramer HSD test.

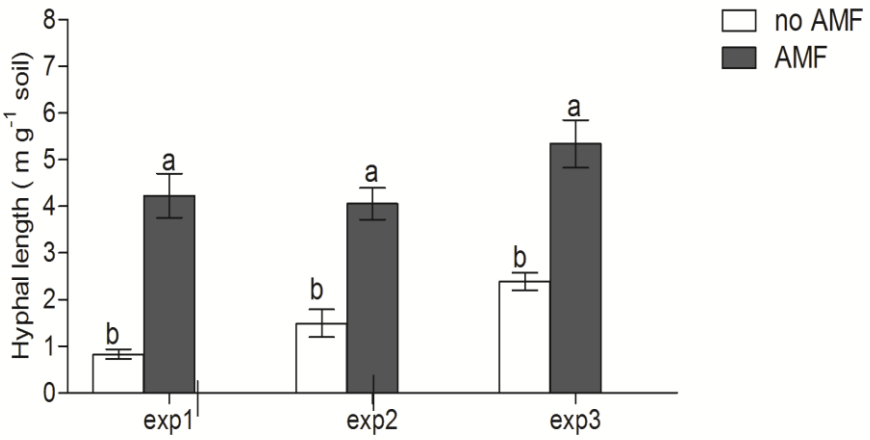


Figure II.2: Effects of AM fungi seed viability (%) of *C. nigra*, *D. glomerata* and *T. officinale*. AM fungi presence was either achieved (a) by adding inoculum to an autoclaved soil in experiment 1; (b) using rotated/static RECs in the greenhouse (experiment 2); or (c) with rotated/static RECs in field plots (experiment 3). Means and standard deviation (n = 10) are shown. Different letters indicate significant differences between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.

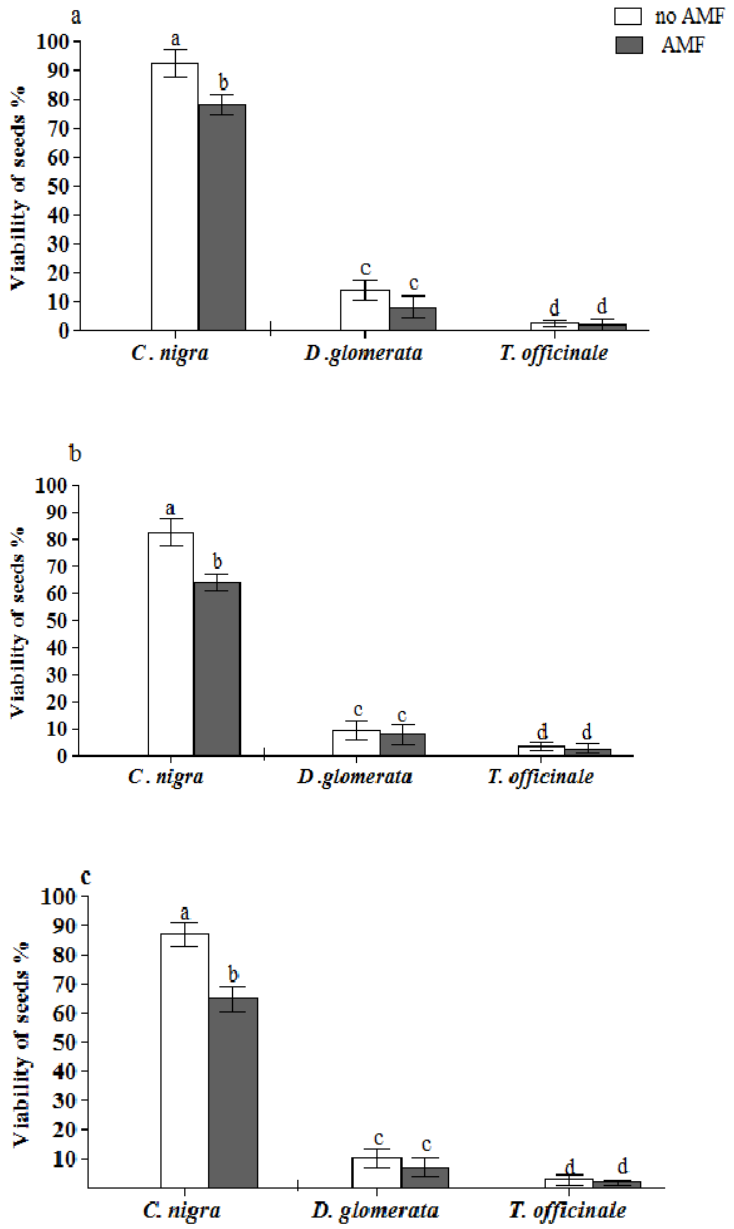
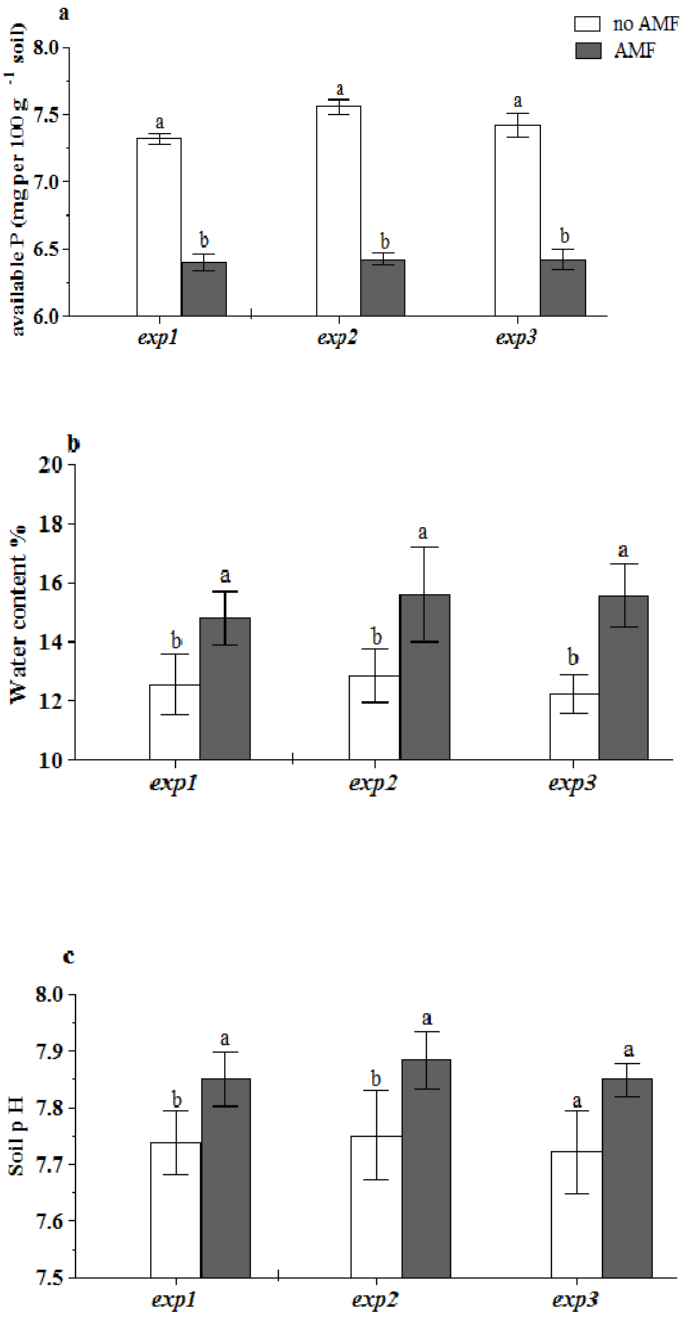


Figure II.3: Effects of rotated RECs as (no AMF) and static core (AMF) on(a) phosphorus concentration of soil, (b)water content and(c) soil pHin all experiments. Means and standard deviation (n = 10) are shown. Different letters indicate significant differences between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.



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Chapter3

Effects of two different types of biochar at three different concentrations on the viability of seeds of three plant species

Abstract

Biochar is produced by pyrolysis of different organic material with numerous suggested benefits as a soil amendment. Despite broad research interest in biochar effects, little is known about consequences for the viability of seeds in soil seed banks, which play an important role in the composition and ecology of different plant communities. The goal of the present study was to determine the effect of biochar on viability of plant seeds in a soil seed bank and to identify underlying mechanisms. In a greenhouse experiment, we investigated the effects of two types of biochar (from peanut shell pellets and plant twigs) at different addition rates (control, 1%, 5% and 10% v/v) on the viability of three types of plant seeds (*Taraxacum officinale*, *Dactylis glomerata* and *Centaurea nigra*). We observed a significant increase in the viability of *D. glomerata* and *T. officinale* seeds at 1% and 5% biochar addition compared to the control and 10% biochar. Differences among plant species may be related to seed traits, such as seed coat thickness. Applied at high doses biochar may have detrimental effects on viability of *C. nigra* seeds. Our results indicate that low doses of biochar may have positive impacts on seed viability in the soil, while the reverse may be true for high doses. These results have important implications for restoration efforts employing biochar.

Keywords: Biochar, Soil seed bank, Seed viability, *Taraxacum officinale*, *Dactylis glomerata*, *Centaurea nigra*.

Introduction

The soil seed bank includes all viable non-germinated seeds in the soil or on the surface (Thompson and Grime, 1979; Baker, 1989; Ooi, 2012).Persistence in the seed bank is determined by a combination of factors, including heritable traits such as size, nutritional status, thickness of the seed coat, and the biological, chemical, and physical properties of the soil (Gallagher and Fuerst, 2005), for example, soil properties (Long et al., 2009; Pakeman et al., 2012) and soil pH, soil water content can affect seed viability (Bekker et al., 1998a; Wagner and Mitschunas, 2008). and soil nutrients (Bekker et al., 1998b; Davis, 2007), soil temperature (Akinola et al., 1998). Also soil seed longevity is associated with soil microbial activity (Wagner and Mitschunas, 2008; Dalling et al., 2011) Due to its interrelation with other parameters such as aeration and temperature. which are particularly likely to play a key role in the loss of seed viability (Cook, 1980; Lonsdale, 1988; Pickett and McDonnell, 1989; Crist and Friese, 1993).

Biochar can be obtained from biomass materials of either plant or animal origin by heating it to less than 700°C in the absence of air (pyrolysis) (Harris, 1999; Antal and Grønli, 2003; Chan and Xu, 2009). The quality of the biochar produced depends on the production temperature, surface and pyrolysis conditions, and type of the organic materials used. Biochar is thought to be a stable source of carbon and it affects other soil characteristics including cation-exchange capacity (CEC) (Liang et al., 2006), soil pH (Warnock et al., 2007), fertility (Chanet al.,2008; Steiner, 2007a), and water retention (Glaser et al., 2002; Chan and Xu, 2009; Novak et al., 2009; Downie et al., 2009; Elad et al., 2011). Biochar increases soil organic carbon (SOC) making it desirable in soil and it can improve nutrients supply to plants, promoting growth (Glaser et al., 2002; Lehmann et al., 2003; Rondon et al., 2007).

Several studies conducted on the impact of biochar on crops reveal that it has a positive effect in most cases (Graber et al., 2010). Improved crop response can be attributed directly to the effects of biochar-supplied nutrients (Silber et al., 2010), besides many indirect effects. These include: increased nutrient retention (Chan et al., 2007, 2008; Chan and Xu, 2009), changes in soil pH (Yamato et al., 2006; Steiner et al., 2007b; Novak et al., 2009), enhanced soil aeration (Yanai et al., 2007; Downie et al. 2009; Van

Zwieten et al., 2010), improved physical properties including water retention (Iswaran et al., 1980; Ballestero and Douglas, 1996; Glaser et al., 2002; Chan et al., 2008; Laird et al., 2009; Novak et al., 2009), promotion of mycorrhizal fungi (Warnock et al., 2007) or N-fixing bacteria (Rondon et al., 2007), interactions with soil microarthropods (Salem et al., 2013) and modifications of soil physical character and creation of shelter for microorganisms (Sohi et al., 2009). Although there are many reports on the effects of biochar on plant growth, soil properties and also seed germination (Reyes and Casal, 1998; Reyes and Casal, 2006; Bargmann et al., 2013; Reyes et al., 2015), and seedling growth (Solaiman et al., 2012), to our knowledge, there has been no study to date on the impact of biochar on the viability of seeds in the soil seed bank. There is only one study, conducted in the Amazon, Manaus region, Brazil, studying seed banks in Amazonian Dark Earth (Terra Preta do Indio) soils (Major et al., 2005). They found that the seedlings germinated in a greater number of species in Terra Preta soil than in forested adjacent soil; however, Terra Preta observational results cannot be straightforwardly extended to biochar effects.

Our study aimed to investigate (i) the effects of two types of biochar on seed viability, (ii) the effects of biochar concentration on seed viability, and (iii) the effects of biochar on soil characteristics potentially affecting the soil seed bank.

Materials and methods

Seeds, biochar and soil

Seeds of three different plant species (*Taraxacum officinale* agg. ,*Dactylis glomerata L.*, and *Centaurea nigra L.*) were used in this experiment. We selected these species because they are typical of Central European grasslands with relatively low seed germination, and they can be easily obtained (Mitschunas et al., 2006).

We used polyethylene cover material used to cover strawberry plants(Gardol. Made for BAHAG. AG, Mannheim, Germany) to make small mesh bags of 2-3 cm size and the following properties: thickness = 0.5 mm; pores number 124 cm^{-2}; pore size = 500µm)to

make contact with the soil, to protect seeds from seed predators, and to facilitate retrieval of seeds after incubation in the soil. We placed 50 seeds of each species in each small bag.

Biochar was prepared using the pyrolysis method described by Masulili et al. (2010). Two kinds of waste biomass feed stocks were used for biochar production:(i) peanut shell pellets (PS-char) of 6mm and 8mm and (ii) different plant twigs (TP-char) collected from the Botanical Garden Berlin. The collected samples were piled in the greenhouse for air-drying and were subsequently oven-dried overnight at 80°C. The material was then cut into small pieces less than 3mm. The peanut shell pellets and plant twigs were placed in metal containers, surrounded and covered by sand, and loosely sealed with aluminum foil. Each biochar was produced at the same temperature (500°C) for 5 h and under the same pyrolysis conditions (Hammer et al., 2014). After the pyrolysis process, the biochar was ground into small granules and passed through a 2 mm sieve. The biochar materials were mixed with soil at 1%, 5 %, and 10% v/v ratio. The pH of the biochar at equilibrium with water (1:5 w/w) was 7.2 for PS-char and 7.5 for the TP-biochar. The differences in pH between the biochars can likely be attributed to the different ash content (Liesch et al., 2010). The C content differed between TP-char (75.46 %) and PS-char (69.15%). The O-content (20.03 %) in PS-char, and lowest in TP-char (15.21 %), and P (1300 mg/kg) in PS-char and low in TP -char (170 mg/kg). The N content was higher in PS char (0.751%) than in TP-char (0.167 %) (Euro EA Elemental Analyzer, HekaTech, Germany).

The soil used in the experiment was a fresh loamy sandy material (AlbicLuvisol) with the following properties; N = 0.12%; C = 1.87%; C/N ratio 15.58; 74% sand, 18% silt, 8% clay; soil pH = 7.1 (analyses conducted by LUFA Rostock Agricultural Analysis and Research Institute, Germany; and on a Euro EA C/N analyzer, HEKAtech GmbH, Wegberg, Germany) (Rillig et al., 2010). The soil was collected from a meadow in Dahlem (Berlin, Germany) at a depth of 10–40 cm below the surface. It was air-dried and then sieved through a 2 cm-sieve to remove plant material and stones and to homogenize the soil (Rillig et al., 2010, Siddiky et al., 2012). Soil pH was assessed at the end of the experiment with a pH-meter (Knick 761 Calimatic) in a 1:5 (w/v) aqueous dilution. Approximately 2-3 mg soil samples was dried and crushed with a mill (Retsch GmbH,

Haan, Germany) and weighed into tin capsules to analyze nitrogen and carbon concentration by an Elemental Analyser (EuroEA, HekaTech, Germany) with acetanilide (Merck, Darmstadt, Germany) as internal standard.

Experimental set-up and measurements

The experiment had a factorial design. The first factor was seed identity (three different plant species), the second factor was the biochar type (two levels) and third factor the application of biochar with four levels (control (none), 1%, 5% and 10% v/v).

We placed three bags (one of each species with 50 seeds) at a depth of 5 cm in each plastic pot (pots; diameter 10 cm, height 15 cm) filled with soil differing in biochar type and concentration. Every treatment had eight replicates summing to a total of 56 (2x4x8) experimental units. Each of the 56 pots had three bags containing the seeds.

The position of pots was re-randomized once a week. The average temperature of the greenhouse was 22 °C during day and 16 °C during night, the relative humidity was 60%, and pots were watered as needed (about every two days 75 ml to each pot). After 15 weeks, we extracted the seeds from the soil bags and calculated the number of viable seeds using a modified Malone's method (1967). For viability testing, the seeds were stained with a solution of 2,3,5-triphenyl tetrazolium chloride.

The dicotyledonous species (*C. nigra* and *T. officinale*) were exposed to a 0.1%, and the grass (*D. glomerata*) to a 1% solution of 2,3,5-triphenyl tetrazolium chloride. The seeds were kept for 48 hours in darkness at 20ºC and were rinsed five times in sterile distilled water. The seeds were agitated between cover slides and were examined using a light microscope. Embryos completely colored pink to red were considered viable, while seeds with embryos partially colored or white, yellow, or brown were assumed to be not viable (Nachlas et al., 1960; Grabe, 1970; Van Waes and Deberg, 1986).

Statistical analyses

Data were analyzed in R (Version 2.14.1) using a general linear model (Zuur et al., 2009). The Shapiro test and Levine's test were conducted to test for normal distribution of residuals and the homogeneity of variance, respectively. Data regarding soil pH and available C/N in soil were log-transformed as necessary to meet the assumptions of ANOVA. The factor "Species" was the random effect accounting for data correlation within each pot

Differences between the viability of seeds were analyzed by a single factor ANOVA including all the data. We used Tukey-Kramer HSD to conduct multiple comparison tests. The relationships among water content, seed viability, soil nutrient concentrations (N, C), and soil pH were tested by Pearson correlation coefficients.

Results

Effects of the different biochar on seed viability

Assessing the effect of two different types of biochar on seed viability, we found significant positive interactions between species and biochar on seed bank viability (Fig. 1a, b and c,). There was a negative effect of biochar at high dosage on seed viability, while the low biochar addition treatments (1% and 5%) had significantly increased seed viability compared the control (without biochar). The percentage of seed viability of *C. nigra* was generally high (61 - 84 %), and much lower in *D. glomerata* and *T. officinale* (4.5 - 9.25 % and 2.7- 7.25%, respectively) (Fig. 1a, b and c)

Effect of biochar on soil water content, soil nutrients and soil pH

We assessed the impact of biochar on soil water content. We found that soil water content significantly increased with the biochar addition rate (Fig. 2 and Table 1). Soil water content was not correlated with soil pH. There was a positive effect of biochar at higher dosage (5% and 10%) on soil moisture compared the control (without biochar).

Assessing the effect of two different types of biochar on soil nitrogen, we found that for both biochars there were no differences in soil nitrogen (Fig 3a). There was no significant correlation between nitrogen and any other variables (Table 1).

Addition of biochar had a significantly positive effect on soil carbon concentration for both biochar types (Fig 3b). Soil carbon concentration was positively correlated with seed viability of D. glomerata and T. officinale ($r=0.472$, $p<0.05$ and $r= 0.322$ $p<0.05$, respectively), but not correlated with seed viability of C. nigra (table 2).

We observed a significant effect of biochar on soil pH; high doses of both biochars significantly increased soil pH compared to the control (Fig. 2 and Table 1).

Discussion

Although there are studies exploring the effects of biochar on seed germination (e.g., Deenik et al., 2010; Reyes et. al., 2015) and seedling growth (e.g., Solaiman et al., 2012), nothing is known on the influence of biochar on seed viability in the soil seed bank. Our study aimed to fill this gap, and we did find significant effects of two types of biochar on seed viability.

Biochar application had a positive effect on viability in two of the three tested seeds species, depending on the biochar concentration in the soil, but there were no significant differences among biochar types in our study. Probably this is due to the absence of significant differences in the mineral content of both types of biochar, and further studies should test a broader range of biochar properties. However, we found that the amount of biochar in the soil was important, as at low and moderate concentrations we observed in

both types of biochar an increase of seed viability of *T. officinale* and *D. glomerata*, but this percentage dropped again with increased dose in both types of biochar. PT biochar had even a negative impact in seed viability of *C. nigra* at the highest addition level.

Plausible explanations for the seed species dependence documented in our results likely relate tothe properties of the seeds themselves, such as size, seed coat thickness and other traits (Davis et al., 2008; Gardarin et al., 2010). The seed coat thickness in *C. nigra*perhaps had a crucial role in preventing the influence of soil fungi in the attack and decomposition of seeds, i.e. the thicker seed coat may have eliminated anypositive impact of biochar on survival of the seeds in soil, which already had a very high viability in the control.

The biochar at high doses caused reduced seed viability, but biochar had positive impact at low and moderate doses. Perhaps this is due to ability to absorb water and decreased soil aeration (Mickelson and Grey, 2006; Downie et al., 2009; Van Zwieten et al., 2010), which act on improve the microbial activity and disrupt metabolic processes and thus cause high seed mortality (Bekker et al., 1998a).

Biochar itself has a highly porous nature and thus can change physical properties of the soil by improving soil aeration, by reducing tensile strength and increasing field capacity of soil (Chan et al., 2007;Yanai et al., 2007; van Zwieten et al., 2010). On the other hand, a too high soil moisture can favor deleterious fungi and bacteria (Blaney and Kotanen, 2001; Schafer and Kotanen, 2003; Dalling et al., 2011), which perhaps explains that at a high concentration of biochar the positive effect on seed viability diminish or even has a negative effect on bigger seeds as *C. nigra*.

Our results showed that the addition of biochar at low doses can play an important role in improving seed viability in the soil seed bank, perhaps through improved soil properties such as soil pH (Wagner and Mitschunas, 2008) and soil aeration(Van Zwieten et al., 2010),

But the high doses of biochar had negative impact on seed viability in soil seed bank This was likely due to increased microbial activity (Rillig et al., 2010; Libra et al., 2011) which leads to reduced seed viability(Blaney and Kotanen, 2001).

Conclusions

We showed for the first time a positive effect of biochar on seed availability. Presence of biochar may reduce seed mortality in species with low seed viability probably due to a better aeration and soil pH .

Future research should include field experiments to assess appropriate concentrations, different soil conditions and interactions with soil biota.

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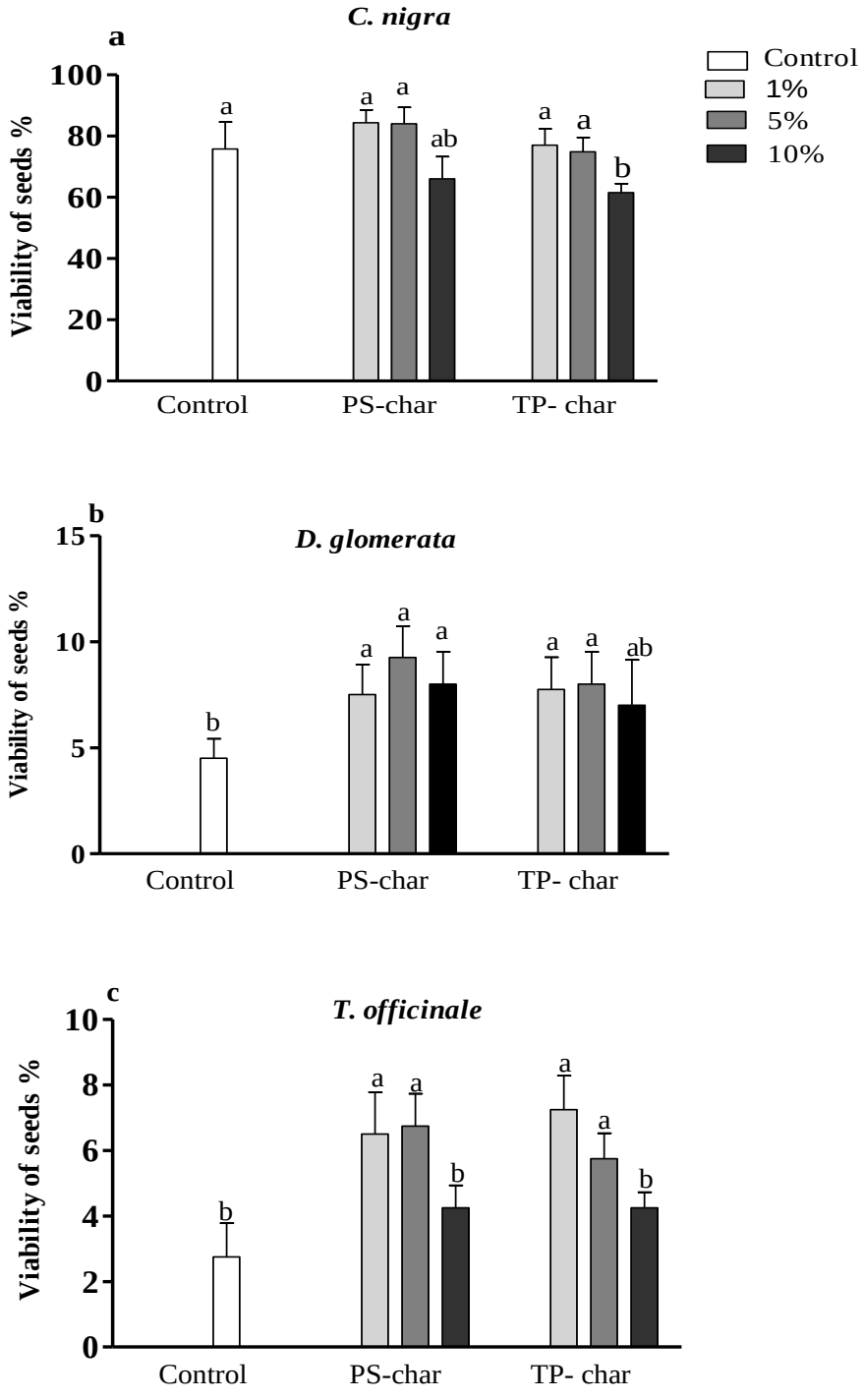
Table III.1 Pearson's correlation coefficients (r) for the effects of PS-char and TP-char on viability of seeds of three species and their interaction on all variables (* = p<0.05; **= p <0.01; *** = p <0.001)(n=8).

	Water content	*C %*	*N %*	*Soil pH*	Seed viability *C. nigra*	Seed viability *D .glomerata*	Seed viability *T.officinale*
Water content	-						
C %	**.574(**)**						
N %	NS	NS	-				
Soil pH	NS	NS	NS	-			
Seed viability *C.nigra*	NS	NS	NS	NS	-		
Seed viability *D. glomerata*	**472(**)**	**.472(**)**	NS	**.311(*)**	NS	-	
Seed viability *T. officinale*	**.486(**)**	**.322(*)**	NS	**.313(*)**	**.392(**)**	**.392(**)**	-

Figure III. 1 Effect of biochar (PS-char &TP-char) and different concentration of biochar on viability of *C. nigra* (a), *D. glomerata* (b) and *T. officinale* (c) seeds. Means and standard deviation are shown (n=8). Different letters indicate significant differences between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.



FigureIII.2 Effect of two type of biochar (PS-char &TP-char) and different concentration of biochar on and soil pH (a) and soil water content (b),as measured at the end of the experiments. Means and standard deviation are shown (n = 8). Different letters indicate significant differences between the treatments at $p< 0.05$ according to the Tukey-Kramer HSD test.

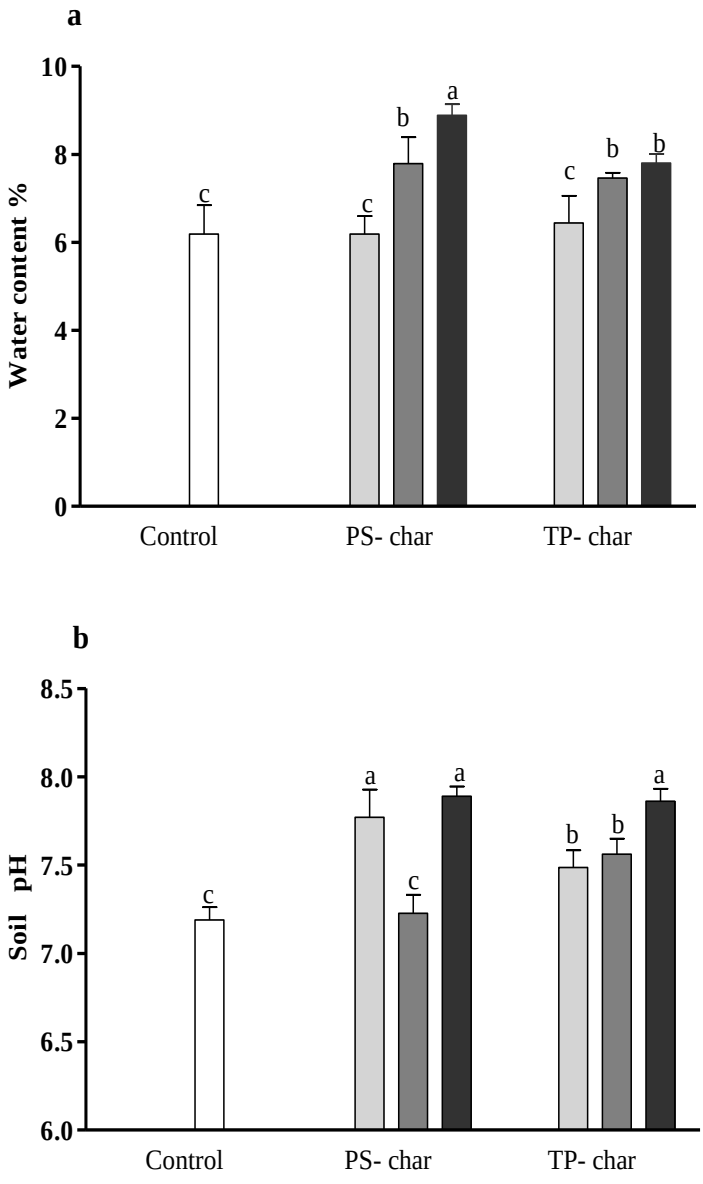
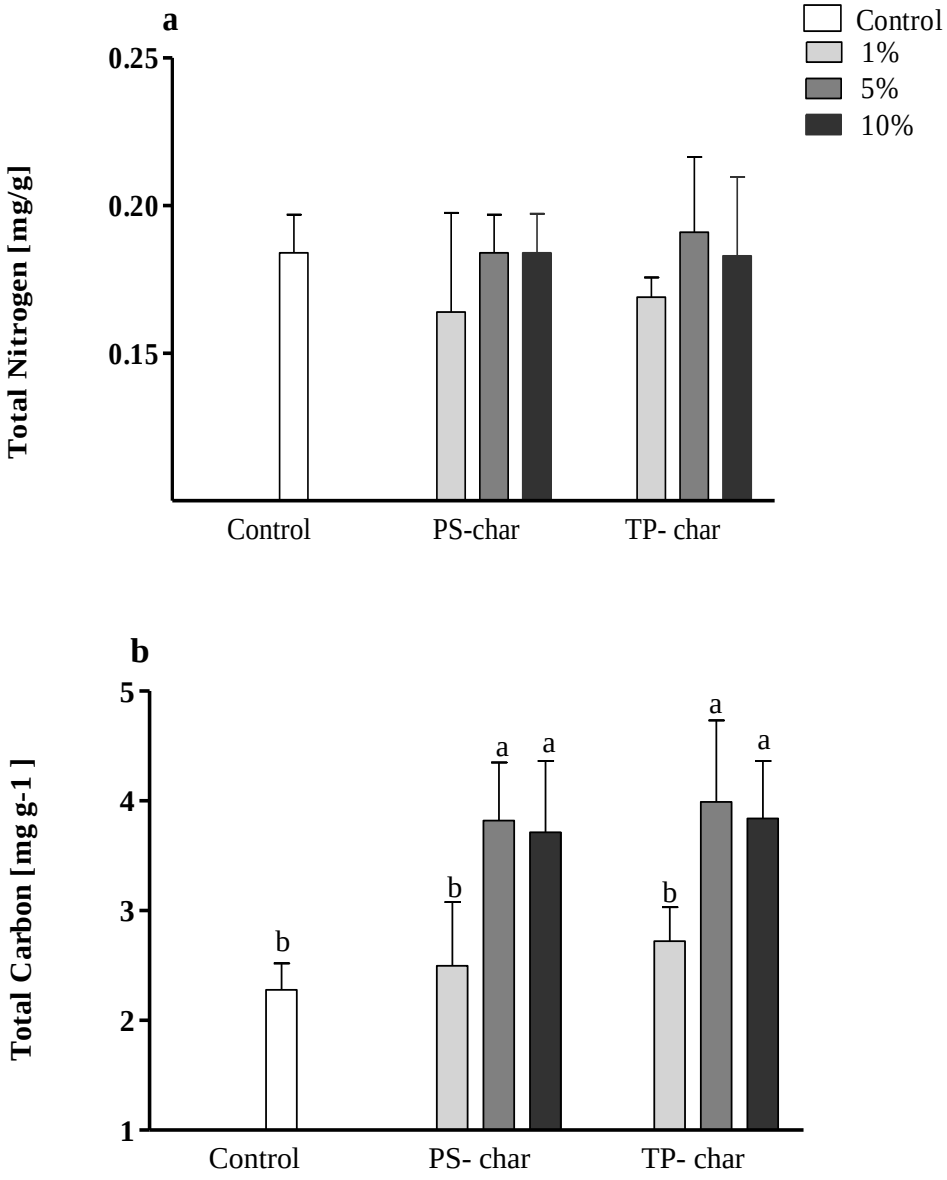


Figure III. 3 Effect of two types of biochar (PS-char &TP-char) and different concentration of biocharon nitrogen (a) and carbon (b) as measured at the end of the experiment. Means and standard deviation are shown (n = 8). Different letters indicate significant differences between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD



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Chapter 4

Effects of biochar amendment on seed viability in the presence and absence of arbuscular mycorrhizal fungi.

Abstract

Biochar as a potential soil amendment may have positive effects on seed viability. The potential impact of biochar amendment and arbuscular mycorrhizal (AM) fungi on seed viability and their interactions remain unclear. The goal of the present study was to determine effects of biochar on seed viability under field conditions and to evaluate interactions of biochar and AM fungi on seed viability and plant performance and to identify underlying mechanisms. In a field experiment, we investigated the effect of biochar at different addition rates (control, 1%, 5% and 10% v/v) with or without AM fungi on seed viability of *Taraxacum officinale*, *Dactylis glomerata* and *Centaurea nigra.* We observed a positive interaction between biochar and AM fungi on seed viability in *C. nigra*, added as a single treatment biochar had a positive effect on seed viability of all species, but seed viability in *T. officinale* and *D. glomerata* was no affected by AM fungi. High doses of biochar had negative effects on seed viability in *T. officinale* and *D. glomerata,* but at similar doses had positive impact in *C nigra*. These results contribute to the use of biochar in reducing the negative role of the AM fungi to resist Invasions by exotic plants.

Keywords: AM fungi, Biochar, soil seed bank, seed viability, *Taraxacum officinale, Dactylis glomerata, Centaurea nigra*

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Introduction

The soil seed bank is the viable seed reservoir present in soils (Roberts, 1981; Mitschunas et al., 2006) and as such a critical component of nearly all terrestrial ecosystems. The seed bank is composed of all viable seeds that are in the soil and litter, representing a repository of plant species (Simpson et al., 1989). The soil seed bank represents the regeneration potential of the plant communities and influences their vulnerability to extinction (Williams-Linera, 1993; Willems, 1995).Many factors affect the survival of seeds in the soil, which may include soil nutrients, such as soil nitrogen and carbon (Bekker et al., 1998a; Davis, 2007), soil water content (Mickelson& Grey, 2006; Schafer & Kotanen, 2003), soil moisture and hypoxia (Voesenek & Blom, 1992; Bekker et al., 1998b; Murdoch & Ellis, 2000; Nicol et al., 2003) or soil pH (Chen et al., 2000). Consistently, soil fungi, one of the main agents of seed loss, respond to moisture, plant litter (Blaney & Kotanen, 2001; Schafer & Kotanen, 2003) and interact with other soil microorganisms (Chee-Sanford et al., 2006; Kremer, 1993).

Biochar is a carbon rich product of pyrolysis, whereby organic materials of either plant or animal origin are heated (less than 700°C) in a low or no oxygen environment (Antal & Grønli, 2003). Many countries have recently become interested in the investigation of biochar because of its potential role in climate change mitigation (Laird, 2008; Sohi, 2012), as it is a stable form of C which is thought to remain in soil for 1000 to 10,000 years (Skjemstad et al., 1998; Krull & Skjemstad, 2003; Ascough et al., 2009; Gavin et al 2003; Gouveia et al., 2002). Therefore, biochar as a soil amendment can act as a carbon sink in agricultural soils and can improve soil fertility (Chan et al., 2007; Ogawa et al., 2006). It may also absorb herbicides (Jones et al., 2011) and pesticides, and/or neutralize natural toxins in the decomposing organic materials (Yelverton et al., 1996). Application of biochar at high rates can also increase soil water retention directly due to biochar's high surface area (Lehmann, 2007) and indirectly via subsequent increase in soil carbon content.

Arbuscular mycorrhizal (AM) fungi are one of the key organisms groups in soil, as they are obligate biotroph associating with about 80 % of all vascular plants (Smith & Read, 2008). They are also considered important in the context of modern organic agricultural

practices (Piotrowski & Rillig, 2008), as their mutualistic character can improve plant biomass production (Rillig, 2004).

There are several studies on the impact of biochar on mycorrhiza. Some researchers report root colonization rates to be strongly enhanced by biochar (Ishii & Kadoya, 1994; Blackwell et al., 2010), whereas others present evidence that root colonization decreases (Birk et al., 2009; Warnock et al., 2010). But to our knowledge there are few studies on the effect of AM fungi on the viability of seeds. One earlier study concluded that mycorrhiza has negative effect on survival of seeds in the soil for some plant species (see Chapter 3).

Given the increased interest in the use of biochar as a soil amendment, we aimed to broaden the information base concerning impact of biochar on seed viability. We specifically wished to test effects of biochar concentrations, and also interactions with AM fungi.

Materials and methods

Soil and biochar

The soil used in the experiment was collected from a meadow in Dahlem (Berlin, Germany) at a depth of 10–40 cm below the surface. The soil had the following properties; N = 0.12%; C = 1.87%; C/N ratio 15.58; 74% sand, 18% silt, 8% clay; soil pH = 7.1(Rilliget al., 2010),(analyses conducted by LUFA Rostock Agricultural Analysis and Research Institute, Germany; and on a Euro EA C/N analyzer, HEKA tech GmbH, Wegberg, Germany). The soil was air-dried and sieved (2cm) to remove plant material and stones and to homogenize the soil. The soil pH was assessed at the end of the experiment with a pH-meter (Knick 761 Calimatic) in water (soil: water ratio 1:5w/v). We chose this soil due to its high mycorrhizal inoculum content (Rillig et al., 2010).

The biochar used for the study experiment was obtained from Botanical Garden Berlin. The biochar was prepared from wood chips at 550^{o}C,ground into particles less than 2mm diameter (sieved) before mixing it into the soil. The basic properties of the biochar were: C =73,56% ; O =16,53% ; N= 0,508% ; H= 2.55 % and P= 680 mg g1 (analyses

conducted by Euro EA Elemental Analyzer, HekaTech, Wegberg .Germany). The pH of biochar asmeasured in deionized water (1:5, biochar: water)with a pH electrode was 7.3.

Seed and plants

In all experiments we used three species of seeds, *Taraxacum officinale* G. H. Weber ex Wiggers, *Dactylis glomerata* L., and *Centaurea nigra* L., obtained from the company Albert Treppens & Co Samen GmbH (Berlin, Germany). We selected these species because they have been used previously in similar experiments, and these seeds remain mainly un-germinated when buried in soil (Mitschunas et al., 2006).

We used Sudan grass (*Sorghum x drummondii*) as a host plant to provide AM fungal mycelium, which is known as a good AM host (Azcón-Aguilar et al., 1998).

Experiment description and measurements

The experiment was conducted in a meadow, located at Freie Universität, Berlin. The experimental area was 180 x90 cm. Surface layer soil (15 cm) was removed in order to use soil with known characteristics and containing AMF fungi (as described in Rillig et al., 2010).

In a 2 x 4 factorial experiment we tested the impacts of presence/absence of AMF (put together at the same plot), and different doses of biochar (control 0% 1%, 5% and 10% v/v), and interactions between those two factors on seed bank viability. We thus had eight treatment combinations, each treatment was replicated 3 times (with and without AMF in the same plot; see below). The experimental area was divided into 12 plots (45cm length x 30 cm width x15cm depth).

In each plot we placed six cores(3 static and 3 rotated), in total 72 cores. Each core had 15 cm length(32 mm diameter), which had a grid structure with 72 openings per tube of a size of 7 x 8.5 mm. The tubes (cores) were covered with a 38 µm mesh that was attached to the core with silicone glue. This design was to permit growth of fungal hyphae through the mesh, but to exclude roots (root exclusion compartment) (REC).

The soil added to the experimental area was sieved (2 mm) to remove stones and roots, mixed homogeneously with different levels of biochar (1%, 5% and 10% v/v) and soils without biochar (control). (Altland &. Locke 2012).

The cores were filled with the same soil-biochar mixtures as the plot in which they were placed; half of the soil cores were rotated by 1-2 mm for three times a week around their vertical axes in order to sever any hyphae crossing the mesh barrier, while the other half were left static (static compartments then containing AM fungal mycelium)**.** The distance between cores (5cm ± 1)was chosen to enable a connection to the existing mycorrhizal network in the field plot. Before placing the soil core into the plots we added fifty seeds of each species which were enclosed in plastic mesh bags (2×2 cm, mesh pore size 500 µm) to protect them from seed predators and facilitate harvest at the end of the experiment. The mesh bags were placed inside the RECs equidistantly (2±1 mm, distance of mesh bag from side of core; 5±1cm deep from the surface). We selected this depth because it is an appropriate depth for the presence of viable seeds in the soil seed bank and mycorrhizal fungi in soil (Korb et al., 2004).

Seeds of Sudan grass (*Sorghum x drummondii*) purchased from Appels Wilde Samen GmbH (Darmstadt, Germany), were sown on wet paper in plastic containers in a climate chamber at 20° C and 16h light. Ten seedlings were transplanted six days after germination into the experimental plots and three seedlings were planted between the soil cores of each plot. This was done to provide a host for the AM fungal mycelium in this garden experiment.

Analyses

Post-harvest measurements

Sixteen weeks after planting, the seeds were harvested. Shoots were clipped off, dried (60°C) and weighed. Roots were extracted from soil by hand, washed, dried (60°C) and weighed. Root colonization (%) by arbuscular mycorrhizal fungi was determined microscopically (200X) as described in Rillig et al. (1999), but using ink staining

(Vierheilig et al., 1998). Hyphal length was determined from 4.0 g of fresh soil per mesocosm by an aqueous extraction and membrane filter technique modified after Jakobsen et al. (1992). Water content was determined after drying at 70°C for 72 hours.

In order to determine the available phosphorus (P) content in the soil, the calcium-acetate-lactate soluble phosphorus content was determined spectro-photometrically according to the German standard method DIN 3.4.1.30.2a (Blume et al., 2000). Soil pH was assessed at the end of the experiment with a pH-meter (Knick 761 Calimatic) in a 1:5 (w/v) aqueous dilution. The soil pH was measured in 1:2.5 (dry weight) soil: water suspensions.

Post seed-treatment

Seeds were extracted from the soil in pots. Fifty seeds of every species were counted and tested by the modified method of Malone (1967) staining them with a solution of 2, 3,5Triphenyltetrazolium Chloride(TTC; Sigma-Aldrich, St. Louis). The dicotyledonous species, (*C. nigra* and *T. officinale*) and the grass (*D. glomerata*) were exposed to 0.1% and 1% solution of TTC, respectively. After keeping the seeds in darkness for 48 hours at 20ºC and rinsing five times in sterile distilled water, the seeds were agitated between cover slides to remove the test and were then observed using a light microscope. Embryos which were completely pink to red were considered viable, while those embryos which were partially white, yellow or brown were categorized as not viable (Van Waes & Deberg, 1986).

Statistical analysis

Treatment effects were analyzed in R (Version 2.14.1) through mixed-effects generalized linear models (Zuur et al., 2009). In this experiment we used Shapiro-test and Levene's-test to test a normal distribution and the homogeneity of variance, respectively. In the cases where the data were not normal, we used log-transformations. Data of the seed survival of *C. nigra* and *T. officinale*, soil pH, hyphal length and available C and N in soil

were log-transformed. The relationship among water content, viability of seed, soil nutrient concentrations (P, N and C) and soil pH were tested via Pearson correlation coefficients.

Data of the seed viability, soil pH, water content, hyphal length and available C, N and P were analyzed using two-way ANOVA, but shoot and root biomass of *Sorghum x drummondii* was analyzed by single factor ANOVA including all the data.

Block effects were accounted through a random effects factor. In the experiment we assumed each neighboring core pair (rotating and non-rotating cores) to be a different block.

Results

Seed viability

We assessed the impact of biochar and AM fungi on seed viability in soil seed bank. Our results show that biochar had significant effect on seed viability. AM fungi only had an impact on C. *nigra,* while *D. glomerata* and *T. officinale* were not affected (Table 1).

We found that in presence of biochar there was a difference in seed viability between the rotated and static cores. There was an increase of seed viability of *C. nigra* with biochar addition in the absence of AM fungi (68-83%), while in the presence of AM fungi the increase of viability ranged 61-77 % with respect to the control(Figures1a). The seed viability in both species *D. glomerata* and *T. officinale*, was significantly higher with biochar addition (1% and 5%) compared to the control and biochar (10%), while presence or absence of AM fungi had no effect (Figures1b and 1c).

Effect of biochar on hyphal length ,Soil pH and water content

We assessed the impact of biochar on hyphal length of AMF within the RECs. We found that biochar addition had no significant effect on hyphal length (p= 0.168) (Figure 2a).

Soil pH was affected by presence AM fungi and biochar or their interactions (Table 1). Soil P(r= 0.654), N(r= 0.604) and C (r= 0.660), correlated positively with soil pH (Table 2). The presence of AM fungi had a significantly positive effect on water content with significant interactions between the factors AM fungi and biochar (Figure 2b, Table 1).

Presence of AM fungi at low biochar doses (1%) did appear to effect negatively the soil water content, but not at higher doses of biochar (Figure 3b).

We also detected a positive correlation between hyphal length and phosphorus concentration in soil (r= 0.272), but hyphal length had negatively correlated with water content (r= - 0.332) (Table 2).

Plant performance and soil nutrients

Plants were harvested after sixteen weeks of growth at ground level and separated into shoots and roots, as described above. We noticed that root biomass was unaffected by concentrations of biochar, while biochar had positive effects, enhancing shoot biomass compared to control (Figure 5).

Plant available soil P and N concentrations were affected by AM fungi and biochar or their interaction (Table 1). This indicates that presence or absence AM fungi of in RECs and biochar levels did appear to affect soil nutrients, but C concentration was not affected by AM fungi or their interaction (Figures 4 a, b and c). Soil P concentration was positively correlated with soil N and C concentration (r=0.366 and r= 0.490, respectively), but C was positively correlated with soil pH(r= 0.660) (Table 2).

Discussion

Although there are studies exploring the effects of carbonized material as biochar on seed germination (Solaiman et al., 2012; Bargmann et al., 2013),there is a dearth of data on the effects of biochar on soil seed bank. We showed here for the first time that biochar could improve seed viability in the soil seed bank at low doses of biochar in the presence or absence of AM fungi under field conditions (see chapter 2)

Effects of treatments on soil seed survival

The seed viability test showed that biochar enhanced seed survival in all species at a moderate level (Figure 1). It is worth to note that biochar at high doses lost this positive effect on seed bank and obtained almost the same rates as the control, especially in *D. glomerata* and, *T. officinale*. *C. nigra* seeds maintained the positive effects of biochar even in high concentrations irrespective of the presence or absence of AM fungi.

Here, higher concentrations of biochar were deleterious to the viability of *D. glomerata* and *T. officinale* seeds. possibility of differences between species could be species – specific attributes such as seed coats which play a key role in the survival of the seed longevity (Abedi et al., 2014; Mohamed-Yasseen et al., 1994).

However, in our study we did not observe positive interactions between biochar and mycorrhiza on seed viability, as it could be expected through the better nutrient availability. This is may be related to changes in soil properties such as soil pH or nutrient availability.

Effects of biochar on AM fungi and plant performance

We assessed the impact of biochar on hyphal length in RECs and plant shoots and root biomass (Figure 3a and 4). The addition of biochar had no effect on hyphal length and root biomass, but it had a significant positive effect on shoot biomass as shown in previous studies(Salem et al., 2013).This can be due to the release of retained nutrients especially nitrogen cations which are adsorbed on active surfaces of biochar particles (Gajic &Koch, 2012). The mechanisms underlying this stimulation may include both the chemical characters of biochar and changes soil physic-chemical parameters, e.g. soil pH, soil water content.

Effects of biochar and AM fungi on soil pH and water content

Biochar increased soil pH sequentially, although it was only slightly higher at 1%, 5%, and 10%, biochar compared to the unamended soil being consistent with previous studies(Lehmann, 2007; Rillig et al., 2010). The underlying mechanisms behind these effects remain unclear, but it is probably due to changes in the nutrient availability like P, K, Ca or Mg (DeLuca et al., 2006; Warnock et al., 2007; Gaskin et al., 2010). So the effects of a pH increase on microbial activity will be minimal. Another possible explanation for the increase in pH is the addition of base cations to soil with the biochar (Warnock et al., 2007; Gaskin et al., 2010). But there was no effect of biochar application son soil water content in the presence and absence of AM fungi.

Conclusions

We showed for the first time effects of biochar and AM fungi in combination on soil seed bank under field conditions. Biochar and AM fungi significantly affected the soil seed bank of *C. nigra* ,but AMF had no effect on seed viability in *D. glomerata* and *T. officinale.* Biochar addition enhanced soil seed bank viability in all species, chemical-physical characteristics and AMF hyphal length in soil. Results suggest that applied biochar may have eliminated detrimental effects of the AM fungi on the soil seed bank.

Our results suggest that biochar could play an important role in the management of the soil seed bank; interactions of AM fungi with these materials should become a future focus of seed bank research.

Table IV.1 ANOVA F values for the effects of biochar (H) and arbuscular mycorrhizal fungi (AMF) and their interaction *(*AMF× H *)*on seeds a viability of *C. nigra* , *D. glomerata* and *T. officinale* and hyphal length ,water Content (%), concentrations of(P , C, N) in soil and pH (* = p<0.05; ** p<0.01; *** = p<0.001)(mean ± SD; n = 8.

Treatment		Mycorrhiza (AMF)	biochar (H)	Interaction (AMF× H)
Viability of seeds%	*C. nigra*	*19.30****	*23.52****	*0.31*
	D. glomerata	*1.60*	*9.95****	*0.45*
	T. officinale	*0.86*	*5.21****	*1.047*
Water Content (%)		*13.15****	*2.38**	*11.69****
P (mg 100 g^{-1} soil)		*88.25****	*78.20****	*10.73****
Soil pH		*11.428****	*284.708****	*3.574**
C (mg 100 g^{-1} soil)		*0.49*	*176.67****	*1.12*
N(mg 100 g^{-1} soil)		*4.494**	*122.8****	*5.797****

Table IV.2 Pearson's correlation coefficients (r) for the effects of biochar (H) and arbuscular mycorrhizal fungi (AMF) and their interaction on all variables (* = p<0.05; **= p<0.01; *** = p <0.001)(n=8).

	Soil pH	*Hyphal length*	*Phosphorus*	*Water content*	*Nitrogen*	*Carbon*	*C. nigra*	*T. officinale*	*D. glomerata*
Soil pH	-	**0.251***	**0.654*****	NS	**0.604*****	**0.660*****	**0.560*****	NS	0.0611
Hyphal length		-	**0.272***	**-0.332*****	NS	NS	NS	**-0.0229**	**-0.187**
Phosphorus(mg100g^{1}soil)			-	NS	**0.366*****	**0.490*****	**0.452*****	NS	NS
Water Content				-	NS	NS	NS	NS	NS
Nitrogen(mg100g^{-1}soil)					-	**0.870*****	**0.509*****	**0.331*****	**0.418*****
Carbon(mg100 g^{-1} soil)						-	**0.534*****	**0.244****	**0.509*****
C.nigra							-	**0.463*****	**0.291***
T. officinale								-	**0.249***
D. glomerata									-

Figures

Figure IV.1 Effect of biochar and four different concentration from each type (0%, 1%, 5%and 10%) and in the presence and absence of AM fungi on viability of *C. nigra* (1a), *D. glomerata* (1b) and *T. officinale* (1c) seeds. Means and standard deviation are shown (n=8). Different letters indicate significant differences between the treatments at $p< 0.05$ according to the Tukey-Kramer HSD test.

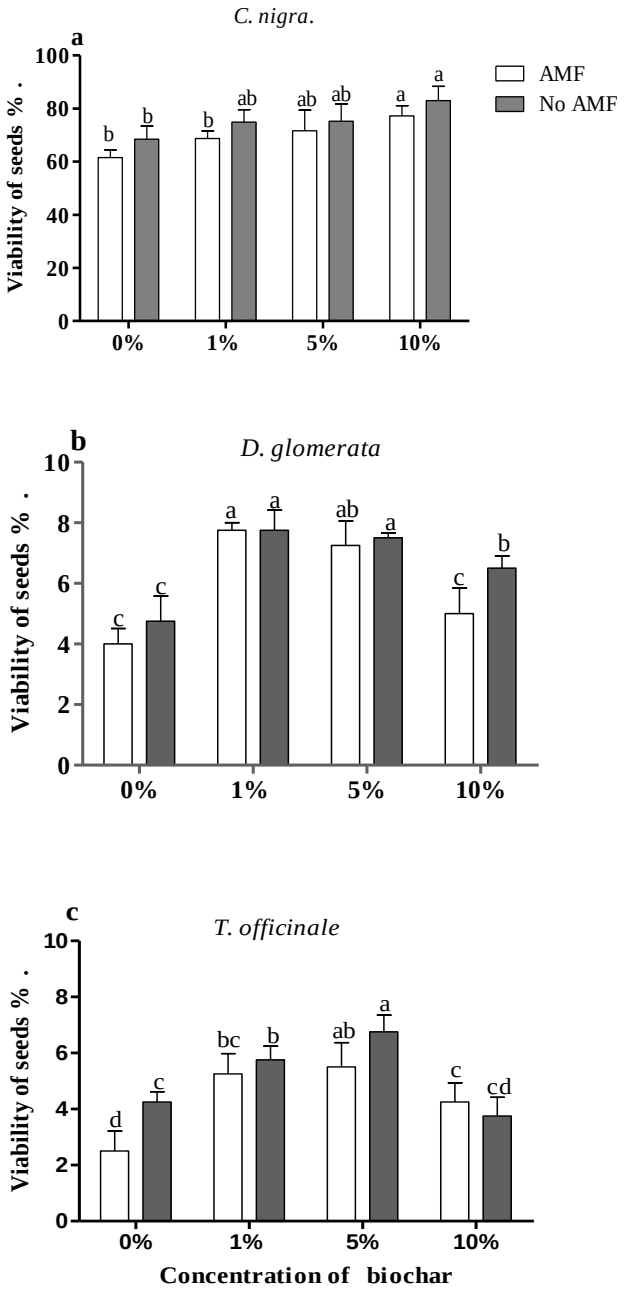


Figure IV.2 Effect of biochar and four different concentration from each type (0%, 1%, 5% and 10%) and in the presence and absence of AM fungi on total C % (2a), N% (2b) available P mg per 100 g soil (2c),as measured at the end of the experiment. Means and standard deviation are shown (n = 8). Different letters indicate significant differences between the treatments at $p< 0.05$ according to the Tukey-Kramer HSD test.

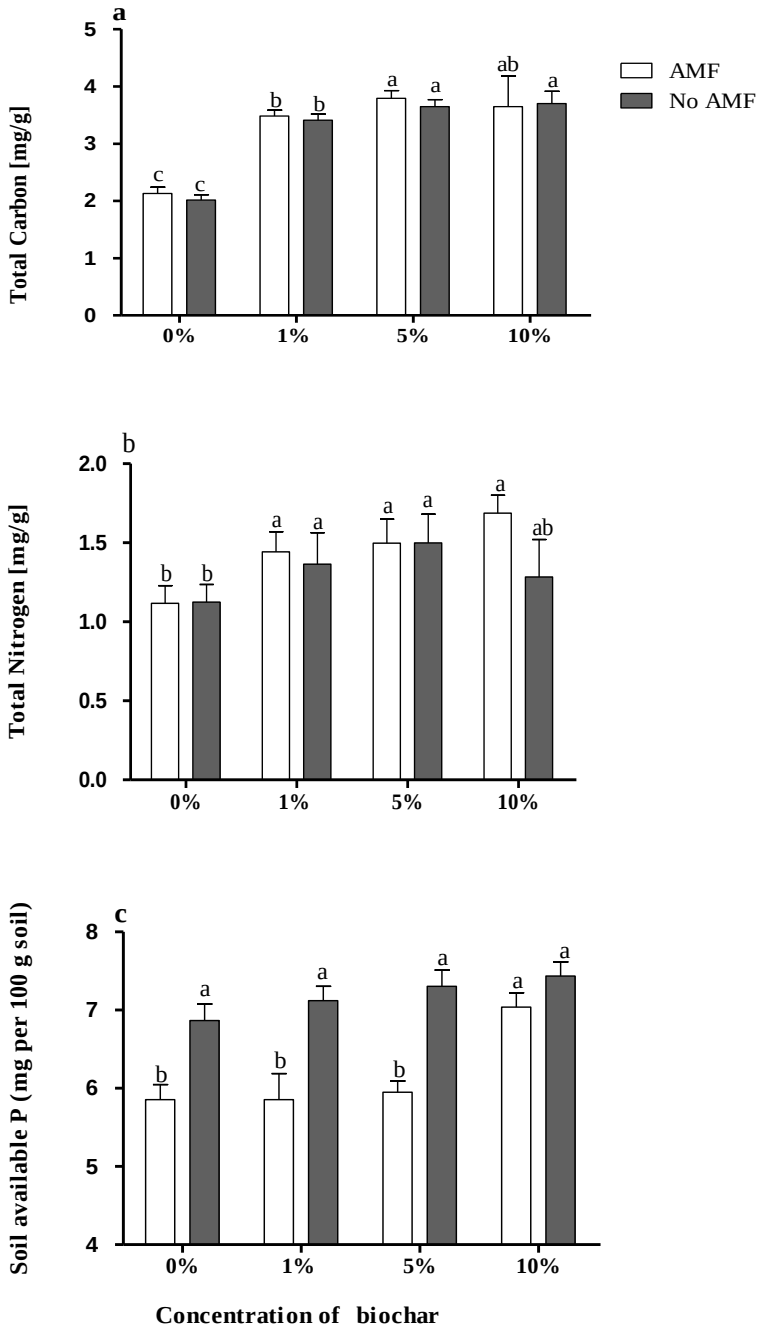


Figure IV.3 Effect of biochar and four different concentration from each type (0%,1%,5%and 10%) and in the presence and absence of AM fungi on Hyphal length m g-1 soil (3a), water content %(3b), and soil pH (3c), as measured at the end of the experiments. Means and standard deviation are shown (n = 8). Different letters indicate significant differences between the treatments at $p< 0.05$ according to the Tukey-Kramer HSD test.

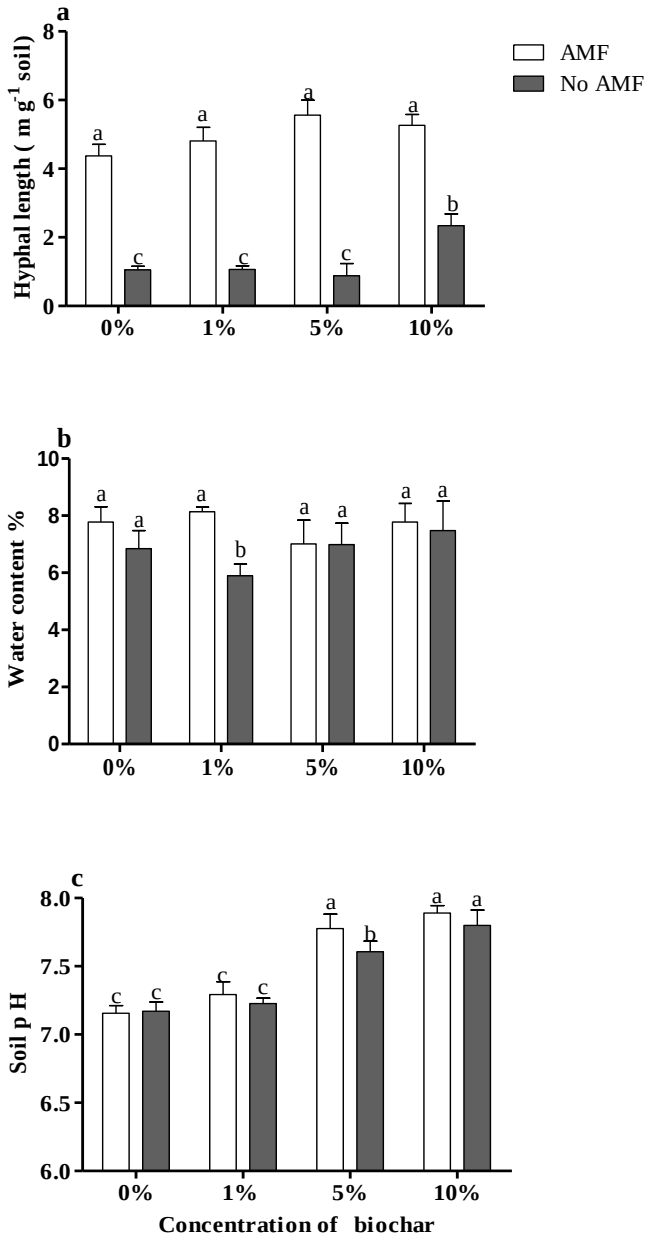
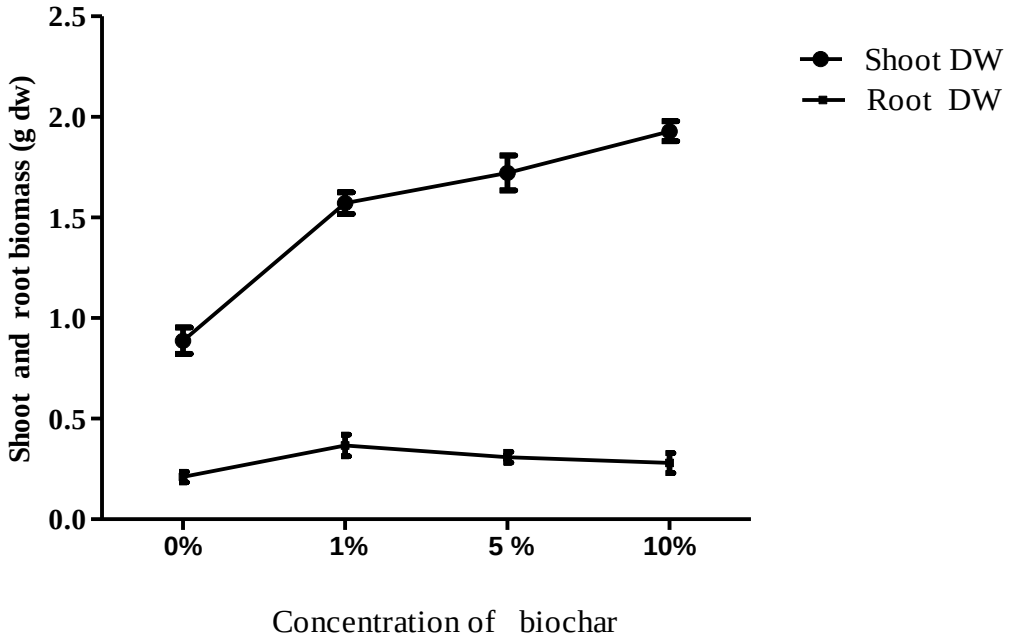


Figure IV.4 Effect of biochar and four different concentration (0%, 1%, 5% and 10%) and in the presence and absence of AM fungi on *Sorghum x drummondii* shoot and root biomass (g/dw), as measured at the end of the experiments. Means and standard deviation are shown (n = 8). Different letters indicate significant differences between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.



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Chapter5

Summary

Soil seed banks are considered essential constituents of plant communities, since they contribute significantly to ecological processes. The seed bank is composed of all viable seeds that are in the soil and litter. Seeds of species forming seed banks must be viable for long periods of time. This requires extended periods of dormancy, thus ensuring viability and persistence in the seed bank until conditions are favorable for germination. There are a lot of factors that affect the survival of the seeds in the soil including type of seeds, chemical and physical soil characteristics and other soil characteristics like soil microorganisms, including arbuscular mycorrhizal fungi.

Biochar can be used a soil amendment to improve soil properties, fertility and to foster long-term carbon storage in soil. However, applied at high concentrations, biochar in soil can have uncertain consequences, especially on the survival of seeds in the seed bank.

The main objective of this thesis was therefore to i) assess how AM fungi influence seed viability in the soil seed bank; ii) determine impacts of biochar on viability of seeds; iii) test different types of feedstock of biochar and application rates;iv) study the joint effect of both AM fungi and biochar on the survival of the seeds in soil seed bank.

For these objectives, we carried out a series of experiments:

For objective i)

We conducted greenhouseand field experiments to examine the seeds of three herbaceous plant species (*Taraxacum officinale, Dactylis glomerata,* and *Centaurea nigra*) under mesocosm (experiment 1 and 2) and field conditions (experiment 3). To allow only hyphae to grow in and to inhibit root penetration, paired Root Exclusion Compartments (RECs) were used in experiments 2 and 3, which were either rotated (interrupted mycelium connection) or kept static (mycorrhizal connection). After harvesting, seeds viability, water content,available phosphorous, soilpH and hyphal length in soil was measured. We have found a significant relationship between AM fungi and viability of seeds of different species,was observed in experiments 1 and 3, but not in experiment 2.

All three experiments showed that water content, soil pH and AMF extraradical hyphal lengths were increased in presence of AM fungi, but available P were decreased significantly.

Therefore, the results suggest that viability of seeds in soil seeds bank correlated negatively with water content, soil pH, AMF extra radical hyphal lengths and soil P availability.

For objective ii) We carried out another greenhouse experiment,in this experiment we studied the effect of two types of biochar (from peanut shell pellets and plant twigs) at different addition rates (control, 1%, 5% and 10%, v/v) on the viability of three types of plant seeds (*T. officinale, D. glomerata* and *C. nigra*). We observed a significant increase of the viability of *D. glomerata* and *T.officinale* seeds at 1% and 5% biochar addition compared to the control and 10% biochar. This may be due to the difference in seed coat thickness in the seed species that were studied. Applied at high doses, biochar may have detrimental effects on viability of *C. nigra* seeds. Our results indicate that low doses of biochar may have positive impacts on seed viability in the soil, while the reverse may be true for high doses. These results may have important implications for restoration effects employing biochar.

For objective iii) In this part we built on the results of the first experiment and second objective, to study the combined effect of biochar and AM fungi on seed viability, in the field. The goal of the study was to determine effects of biochar on seed viability and to evaluate interactions of biochar and AM fungi on seed viability and plant performance and to identify underlying mechanisms. In a field experiment, we investigated the effect of biochar at different addition rates (control, 1%, 5% and 10% v/v) with or without AM fungi on seed viability of *T. officinale*, *D. glomerata* and *C. nigra.* We observed a positive interaction between biochar and AM fungi on seed viability in *C nigra*, added as a single treatment biochar had a positive effect on seed viability in all species, but seed viability in *T. officinale* and *D. glomerata* was not affected by AM fungi. High doses of biochar had a negative effect on seed viability in *T. officinale* and *D. glomerata,* but at similar doses had a positive impact in *C. nigra*

We demonstrated that AM fungi had a negative impact on soil seed viability,and this is caused probably through indirect effects i.e. by changing soil physicochemical properties through the absorption of nutrients such as phosphorus.

The results of these experiments indicate that the presence of biochar may reduce seed mortality in some species but not in others. Interactions AM fungi with these materials should become a future focus of biochar research.

Future perspectives:

We suggest further testing while considering the direct effect of AM fungi on seed viability under field conditions. Studying the direct relationship between AM fungi and the soil seed bank will help to better understand effects of AM fungi on plants overall, with previous work having focused mostly on plant growth effects.

In order to apply knowledge of this work in a restoration context, more work needs to be carried out on the appropriate concentrations of biochar in field studies; and interactions between AM fungi and these materials should become a future focus of biochar research.

Zusammenfassung

Bodensamenbanken sind wesentliche Bestandteile von Pflanzengemeinschaften, da sie einen wichtigen Beitrag zu ökologischen Prozessen liefern. Die Samenbank ist die Summe aller lebensfähigen Samen, die im Boden und in der Streu vorkommen.Diese Samen müssen für längere Zeit lebensfähig bleiben. Diese Samen persisitieren in der Samenbank bis günstige Bedingungen für die Keimung vorhanden sind. Es gibt eine Menge von Faktoren, die das Überleben der Samen im Boden beeinflussen: dies schließt ein die Art des Saatguts, chemische und physikalische Bodeneigenschaften und andere Bodeneigenschaften, wie beispielsweise Bodenmikroorganisme, inklusive arbuskulärer Mykorrhizapilze.

Die Biokohle ist bekannt als Produkt für die Bodenverbesserung. Bodeneigenschaften, Fruchtbarkeit und langfristige Speicherung von Kohlenstoff im Boden können verbessert werden bie Biokohle-Zugabe. Allerdings könntenhohe Konzentration von Biokohle im Boden auch negative Auswirkungen haben.

Das Hauptziel dieser Arbeit war es daher, i) zu beurteilen, wie AM-Pilze die Samenlebensfähigkeit in der Bodensamenbank beeinflussen; ii) zu bestimmen wie sich Biokohle auf die Lebensfähigkeit von Samen auswirkt; iii) Effekte von Biokohlenmenge und –art zu untersuchen; iv) die gemeinsame Auskwirkung von AM Pilzen und Pflanzenkohle auf das Überleben von den Samen in Samenbank zu untersuchen.

Für diese Ziele führten wir eine Reihe von Experimenten durch, sowhl im Feld als auch im Gewächshaus. Zum Einsatz kamen drei krautigen Pflanzenarten (*T. officinale, D. glomerata, und C. nigra*). Es konnte wiederholt ein negativer Effekt von AM Pilzen auf die Lebensfähigkeit von Samen im Boden beobachtet werden. Hierzu wurden auch korrelativ einige Variablen gemessen. Die Ergebnisse legen nahe, dass die Lebensfähigkeit der Samen in der Bodensamenbank negativ mit Wassergehalt, Boden-pH, AMF extraradikalen Hyphenlängen und Verfügbarkeit von Boden-P korreliert ist.

Wir führten einen weiteren Gewächshausversuch duch mit zwei Arten von Pflanzenkohle (aus Erdnussschale Pellets und Pflanzenzweige) bei verschiedenen Zugabemengen (Kontrolle, 1%, 5% und 10%, v / v) durch, ebenfalls mit den gleichen drei Arten von

Pflanzensamen (*T. officinale, D. glomerata und C. nigra*). Wir beobachteten eine signifikante Steigerung der Lebensfähigkeit von *D. glomerata* und *T. officinale* Samen bei 1% und 5% Biokohle im Vergleich zur Kontrolle und zu 10% Pflanzenkohle. Bei hohen Dosierungen kam es zu einer nachteiligen Auswirkung auf die Lebensfähigkeit von *C. nigra* Samen. Unsere Ergebnisse zeigen, dass niedrige Dosen von Biokohle positive Auswirkungen auf die Samenlebensfähigkeit im Boden haben, während höhere Dosen schädlich sein können. Diese Ergebnisse können wichtige Implikationen für denEinsatz von Biokohle haben.

Im letzten Teil Teil haben wir die kombinierte Wirkung von Biokohle und AM Pilzen im Feld untersucht, und imWesentlichen Ergebnisse erzielt, die die der vorigen Studien bestätigten.

Zukunftsperspektiven:

Wir schlagen vor, weitere Tests zur unmittelbaren Wirkung von AM-Pilzen auf Samenlebensfähigkeit unter Feldbedingungen durchzuführen. Solche Messungen sind hilfreich um die Effekte von AM Pilzen auf Pflanzen umfassender zu verstehen.

Um Biokohle-Einsätze zu optimieren, sollten weitere Tests im Feld durchgeführt werden

Contribution to Chapters

Chapter 2: Effects of hyphal severance upon cultivation on soil seed bank viability.

Own contributions: Design of work (together with Prof. MC Rillig), collection of materials, performation of the experiments in the laboratory, and statistical analyses. Writing the manuscript (together with Dr Salem, Dr. Kohler, J).

Chapter 3: Effects of biochar and arbuscular mycorrhizal fungi on the soil seed bank.

Own contributions: Design of work (together with Prof. MC Rillig), collection of materials, performation of the experiments in the laboratory, and statistical analyses. Writing the manuscript (together with Dr Salem, Dr. Kohler, J).

Chapter 4: Impacts of biochar on seed viability in the presence and absence of AM fungi.

Own contributions: Design of work (together with Prof. MC Rillig), collection of materials, performation of the experiments in the laboratory, and statistical analyses. Writing the manuscript (together with Dr Salem, Dr. Kohler, J).

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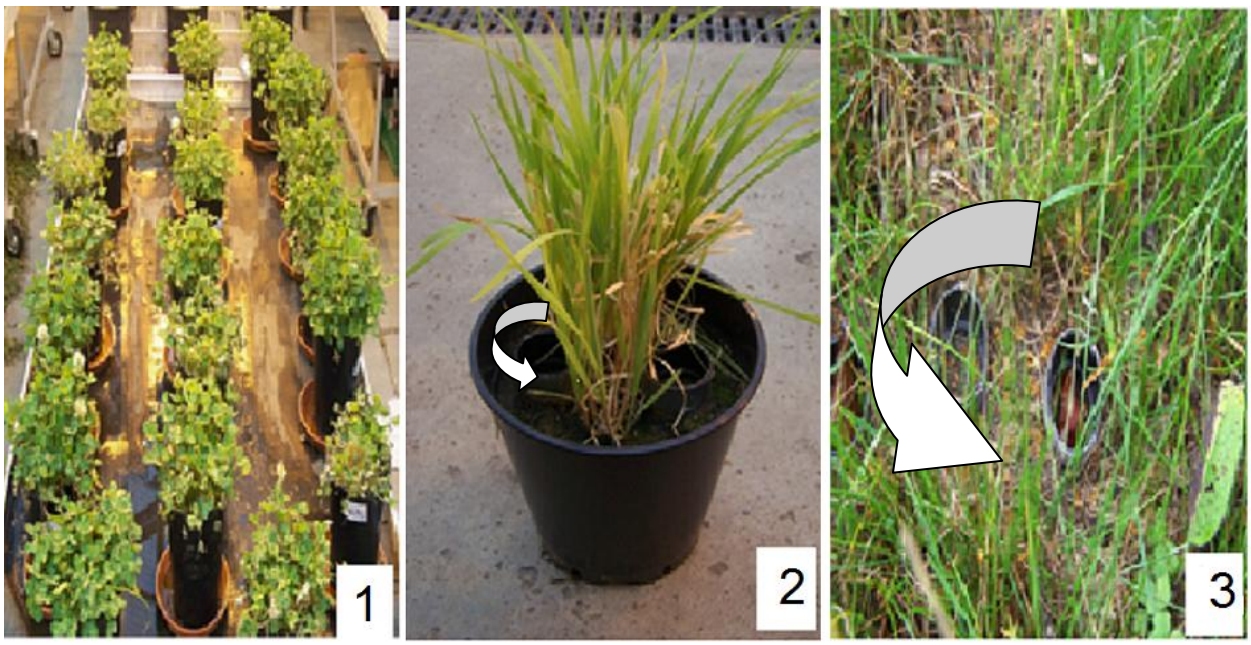
APPENDIX

Supplemental Figures S1 and S2 to Chapter 2

Supplemental Figure IV.S1-Experiences Greenhouses inoculation-based study (1) (n=10), Greenhouse using experimental unit of a modified in-growth core design (2). Root exclusion compartments (RECs). Placement of 2 RECs each in the pot (n=10), were moved three times per week to cut off any hyphal connections across the mesh. (3) Field study using rotated RECs, Placement of 2 RECs each beside each other's (n=10), were moved three times per week to cut off any hyphal connections across the mesh



Supplemental Figure V.S 2-The seeds of *C. Nigeria* after extracted from the soil and staining with Triphenyltetrazolium solution(1), Decomposed Seeds(2) and Fresh Seeds(3).