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Interakce rostlin a půdní bioty a jejich ovlivnění pěstováním energetických plodin

Interactions between plants and soil biota and effect of energetic crops on these interactions

Disertační práce

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Chapter 1

General introduction

Interactions between plants and soil biota

Soil organisms are closely linked to the plant community whereas aboveground plant biomass provide carbon and other nutrients to the decomposer community but plant roots also play important role as host for many soil organisms, such as herbivores, pathogens, and symbionts (Wardle et al. 2004). The soil biota strongly influence plant communities indirectly by recycling of dead organic matter and thus making nutrients available for plant use, and directly due the interactions of the root-associated organisms which selectively influence the growth of plant species (Wardle et al. 2004). Plant-soil interactions also affect plant productivity and community structure both plant species and soil biota.

Plants may affect soil biota due two types of feedbacks. Positive feedbacks is realized when plant species accumulate soil organisms (mycorrhizal fungi, earthworms, nitrogen fixers etc.) that have positive effects on the plants that cultivate them. Positive feedbacks promote species dominance and lead to a loss of local community diversity (Bever 2002; Bever et al. 1997). Negative feedbacks occur when plant species accumulate pathogenic organisms in their rhizospheres (pathogenic fungi, bacteria, insect herbivores etc.) and these interactions outweigh the benefits received from mutualistic interactions. Negative feedbacks create conditions that are increasingly hostile to the plants that cultivate the pathogens and are thought to promote community diversity (Bever 1994; Eppinga et al. 2006; Klironomos 2002; Vanderputten et al. 1993).

Interaction mediated by litter

Aboveground plant biomass affects soil biota via their litter input. Chemical composition of litter mainly C:N ratio as well as lignin-cellulose content play significant role in biomass and community structure of decomposers community (Bardgett 2005; Eppinga et al. 2011; Hattenschwiler et al. 2005; Wardle et al. 2006). Easily decomposable plant material supports bacterial community while heavily decomposable plant material supports fungal

community (Boddy 1999; Caldwell 2005; Coleman et al. 1983). Aboveground plant biomass also affects community of soil fauna. Kaneda et al., (2012) showed significant increased density of soil mesofauna on field sites mulched by sweet corn residues than non-mulched field sites. Here is important factor besides litter quality also aboveground diversity of plant species (Wardle et al. 2006). Different litter species promote various habitat types for diverse groups of soil fauna. Kaneko and Salamanca (1999) found significant greater diversity of soil micro-arthropods in three litter mixtures than in litter monocultures. This results that dominant vegetation also strongly alters density and composition of decomposers community.

This alternation of soil community may in contrary affect soil condition. Observation of earthworm colonization on the sites where they absent previously such as boreal forest in USA or post mining heaps indicate that they may substantially alter soil conditions (Bohlen et al. 2004; Frouz et al. 2008). By mixing litter in mineral soil they remove litter and fermentation (Oe) soil layer, alter water holding capacity of soil, organic matter content, pH and many other soil properties (Bohlen et al. 2004; Frouz et al. 2009; Frouz et al. 2008; Frouz et al. 2006). These alternations in soil properties may alter plant community (Bohlen et al. 2004; Frouz et al. 2008). Effect of earthworms on the composition of aboveground biomass is well documented (Hopp and Slater, 1948). Scheu (2003) showed that in pot experiments where the *Lolium perenne* benefited more from the presence of earthworms than *Trifolium repens*. Roubickova et al. (2009) also showed that earthworms significantly altered the spoil substrate by producing of excrements and vertical transport of organic matter and thus increased the capacity of spoil to support plant species typical of latter successional stages in the Sokolov mining area. This fact may be explained due patchy distribution of organic matter in soil with earthworms (Scheu 2003) or different rate of uptake ability of released nutrient by plant species (Roubickova et al. 2009).

Very important factor which play significant role in plant-soil interaction is fertility of soil. Fast-growing plant species that growing in fertile soils allocates most of their assimilates to rapid growth and producing easily decomposable litter that is rich in nutrients (Wardle et al., 2004). In contrary, slow-growing plants that dominate in soil with low nutrient availability allocate less assimilates to their growth, producing nutrient-poor litter that contain heavily decomposable compounds such as lignin and phenolics (Hussain et al. 2011; Tang et al. 1995). Fast-growing plants promote bacterial-dominated food webs associated with fast

cycling of nutrients, whereas slow-growing plants promote a fungal-dominated food web (Cornwell et al. 2008; Wardle et al. 2004) and slow cycling of nutrients (Coleman et al. 1983; Moore and Hunt 1988).

Very important role in plant-soil interaction play secondary metabolites. Phenolic compounds provide benefits for plants such as UV protection, herbivore and pathogen defense, and contribution to plant colouration (Bardgett 2005). Polyphenols are divided into two main groups: firstly, low molecular weight compounds, including simple phenols, phenolic acids, and flavonoids; and secondly, oligomers and polymers of relatively high molecular weight such as tannins (McKey et al. 1978; Bardgett 2005; Singleton et al. 1999). Phenolic compounds enter to the soil due two main pathways: firstly as leachates from stem, leaf, and root material; and secondly within leaf, stem, and root litter input (McKey et al. 1978; Muller 1982; Bardgett 2005; Singleton et al. 1999).

Phenolic compounds can alters others co-occurring plant species as well as soil microbial community (Blanco, 2007; Muller, 1982; Tang et al., 1995). Phenolic compounds decreasing population of co-occuring plant species and thereby affect soil biota via litter input of phenols producing plants (Mudrak and Frouz 2012; Muller 1982; Singleton et al. 1999; Williamson and Richardson 1988). Changing of plant community caused by producing of phenolic compounds suppress early successional species and thus play important role in plant succession (Chapin et al. 1994; Mudrak and Frouz, 2012). Polyphenols also strongly reduce microbial community in rhizosphere namely root pathogens (Becker et al. 1997).

For example Zhang et al (2011) found that allelopathical compounds produced by invasive plant *Solidago canadensis* strongly affected activity of fungal plant pathogen *Pythium ultimum* but impact on whole fungal community remains unclear. Secondary metabolites produced by wide range of plant species may be required by some fungal strains. Soil microorganisms are responsible for half of the degradation of secondary metabolites such as m-tyrosine, catechin, ferulic acid, juglone and some flavones in soil and thereby increasing microbial activity in soil (Arunachalam et al. 2003; Kaur et al. 2009; Willis 2000). Producing of allelopathic compounds play also important role in invasion success of introduced plant species (Callaway and Ridenour 2004).

Interaction of plant roots and soil biota

Roots play important role in biological activity of soil via producing of exudates to the rhizosphere. The rhizosphere is the region of soil that surrounds and is influenced by plant roots (Doornbos et al. 2012). A major influence in the rhizosphere is producing of complex organic exudates into the soil from roots (Doornbos et al. 2012; Jackson et al. 2012). The composition of root exudates depends on plant species and cultivars, developmental stage, plant growth substrate as well as stress factors (Doornbos et al. 2012). Microorganisms can utilize these exudates as substrates, and thereby increasing microbial biomass and activity in rhizosphere (Doornbos et al. 2012). Variability in the composition and quantity of root exudates play significant role in composition and density of microbial community in rhizosphere (Jackson et al. 2012; Sanaullah et al. 2011).

Bardgett et al. (1999) showed that individual grassland plant species differ markedly in their impact on compositions and abundances of microbial communities in rhizosphere. Important factor in microbial activity in rhizosphere is also fertility of soil. Innes et al. (2004) found that plant species of grassland showed different effects on the abundance and structure of rhizosphere microbial communities in different soils. Innes et al. (2004) showed that bacteria were positively affected by the growth of various grasses and herbs in fertile grassland soil, but the same plants negatively affected these microbes when grown in non-fertile grassland soil.

Interactions of root exudates and microbial community strongly affect population density of microbial feeders. Wardle et al. (1999) showed that the community composition and population density of soil microbes, microbe-feeding nematodes, and herbivorous nematodes and arthropods were differ in treatment with various plant community structure. These factors play important role in structure of soil community and biological activity of soil.

Interaction of plant, root associated organisms and other soil biota

Interaction of root associated organisms such as mycorhizal interaction and N fixers significantly affect plant community and interactions of plants and soil biota (Bardgett 2005; Wardle et al. 2004). Generally, mycorrhizal interaction is closely related relationship between mycorrhizal fungi (e. g. genus Glomus) and roots or stem of vascular plants. Fungi obtain carbon from plants while fungi providing minerals from soil (Harrison 2005).

Mycorrhizal interactions may affect soil biota directly via interactions between root and fungal feeders or indirectly via changing of plant community (Bardgett 2005). Grime et al. (1987) found that presence of mycorrhizal fungi alter plant community composition. Grime et al. (1987) showed that reducing the dominance of *Festuca ovina* based on presence of arbuscular-mycorrhizal fungi in several subordinate herbs led to shift in plant species community due competitive release interactions.

Direct impact of mycorrhizas is realized due interaction between mycorhizal fungi and root and fungal feeders. For example Hata et al. (2010) showed that arbuscular-mycorrhizal fungi caused large changes in secondary metabolites in roots. Root feeding caused by insect larvae also increased micorrhizal-fungal vesicles and arbusculas in root tissue (Currie et al. 2011; Gange 2001) and thus influence transport of minerals to the leaves as well as producing of secondary metabolites (Hata et al. 2010). Effect of presence of mycorrhizal fungi in roots of several plant species on decreasing diet and development of insect species larvae is well documented (Gange and Ayres 1999; Gange 2001; Gange et al. 1994). Generally, mycorrhizal associated fungi strongly decreasing diet and development of food generalist while effect on food specialist is not so strong (Currie et al. 2011).

Mycorrhizal fungi strongly affect also soil mesofauna. Kaneda and Kaneko (2004) showed negative effect of mycorhizal fungus *Pisolithus tinctorius* associated in root on food preference of *Folsomia candida* while separately cultivated *P. tinctorius* was strongly preferred by *F. candida*. In contrary, Thimm and Larink (1995) showed positive effect of mycorrhizal fungi on population density and development of collembolan species.

Nitrogen fixers are bacteria (e. g. Rhizobium) characterized due forming of nodes in roots. Nitrogen fixers exchange fixed nitrogen for carbon synthetized by photosynthesis (Vanrhijn and Vanderleyden, 1995). Vascular plants with N-fixing symbionts are most

widespread as early colonists in primary succession. Moral (2003) showed beneficial effect of plants with nitrogen fixers in glacial forelands where they are major facilitators of plant succession since. Nitrogen fixers contribute most of the N to developing communities, raising soil N to levels needed to support later successional species (Moral 2003).

The mechanism by which N-fixers increase the availability of soil is realized both by build-up and decomposition of litter of high N content (Chapin et al. 1994; Moral 2003; Bardgett 2005). Elhottova et al. (2009) showed that roots of *Tussilago farfara* with nitrogen fixers cultivated in pots with spoil substrate increased microbial diversity and biomass than control pots. Nitrogen fixers can affect also soil microfauna in rhizosphere. For example Viketoft et al. (2005) showed that legumes supported large populations of certain bacterial-feeders, especially Rhabditis and Panagrolaimus. Bacterial feeders reduce microbial biomass of bacteria and thus alter decomposer system in soil.

Interaction of plant, herbivores and pathogens and other soil biota

Both herbivores as well as plant pathogens strongly influence soil biota mainly due allocation of carbon from aboveground biomass to the plant rhizosphere or via changing of aboveground plant community. Van der Putten et al. (1993) showed that both root pathogens and root-feeding nematodes in the rhizosphere of *Ammophila arenaria* caused complete degradation of this plant and its replacement by *Festuca rubra*.

Klironomos (2002) showed that the rate of pathogen accumulation in the soil affect the distribution and invasibility of plant species in grassland communities. Klironomos suggest (2002) that native species tend to accumulate pathogens that limit their growth, while invasive species accumulate pathogens more slowly. Reinhart et al. (2003) showed that invasibility of *Prunus serotina* in north-western Europe was facilitated by the soil community. In the native areal in USA, soil pathogens near *P. serotina* inhibited the establishment of conspecifics plant species and reduced seedling performance of this tree, whereas in the invaded areal, black cherry established in close proximity to conspecifics plant species, and the soil community enhanced the growth of its seedlings.

There is a wide range of root-feeding fauna in soil, including nematodes, insects, and mites. These organisms are differs in effect on various plant species within communities due

various palatability of root material. Root-feeding fauna strongly influence the dynamics of vegetation cover. According Schadler et al. (2004) insect root herbivores promote succession by reducing the success of early successional plant species in early successional plant communities due facilitated colonization by late successional species. Roubickova et al. (2012) also showed that wireworms negatively affect *Calamagrostis epigejos* and therefore can speed up succession and help establishment of a more diverse plant community on spoil heaps.

Similar effect as root-feeders promotes aboveground plant herbivores. Livestock grazers can strongly change composition of plant community as well as soil properties. For example Yang et al. (2013) showed that livestock grazing changed functional groups of soil microbial community mainly N fixing bacteria in Tibetian alpine grassland soils. Grazers can basically affect soil properties due three factors such as vegetation removal, manure deposition, and trampling (Cingolani et al. 2003; Kohler et al. 2005).

Vegetation removal changes the allocation of carbon and nitrogen between plants and roots, and increase soil extractable carbon in the rhizosphere (Guitian and Bardgett 2000). Manure deposition increase nitrogen cycling by efficiently recycling nutrients through the animal excreta pathway (Kohler et al. 2005). Grazers trampling cause compacts soil and hence decreases air permeability and hydraulic conductivity (Yang et al. 2013).

Effect of alien plant species on soil biota

Plant species can be introduced into new ecosystem by human transport, tourism trade or stowaway (Pysek et al. 2012b; Seastedt and Pysek 2011). These plant species may in some cases strongly affect whole ecosystem including also soil physical, chemical and biological properties respectively (Novoa et al. 2012; Pysek et al. 2012b; Reinhart et al. 2003; Yeates and Williams 2001; Zavaleta 2000). The initial phase of invader establishment is often influenced by stochastic processes and propagule pressure (Eppstein and Molofsky 2007; Pysek et al. 2012b; Tilman 2004). Resource-based niche theory predicts that subsequent growth depends on the amount of limiting resources, such as nutrients and light, that remains

unconsumed by the native plant species (Tilman 2004), this concept is also known as vacant niche theory (Kawata 2002).

Very important trait of new introduced plant species is also producing of phenolic compounds that suppress both other co-occuring species as well as plant pathogens (Jefferson and Pennacchio 2003; Ridenour and Callaway 2001; Weidenhamer and Romeo 2004) also known as a novel weapon hypothesis (Callaway and Ridenour 2004). Exotic plant species alters soil biota indirectly due litter feedback (Eppinga and Molofsky, 2013; Eppinga et al., 2011; Meisner et al., 2012) and directly due producing of allelopathic compounds, shading of other vegetation or competition for nutrients (Callaway and Ridenour 2004; Inderjit et al. 2006; Kawata 2002; Pysek et al. 2012; Quinn et al., 2012; Tilman 2004; Zavaleta 2000). Differences in initial litter chemistry between exotic and native plant species are important agent for soil processes involved in litter decomposition (Meier and Bowman 2008) and are mediated indirectly by the soil decomposer system (Hobbie 1992; Wardle et al., 2004). Effect of plant litter chemistry on soil decomposition and nutrient availability is known also as legacy effect (Eppinga et al. 2011; Eppinga and Molofsky 2013; Meisner et al. 2012).

Lignin contained in plant litter can slow down the phased processes of litter decomposition (Cornwell et al. 2008). This lignin component needs specialized lignolytic fungi for its degradation and can shield the more easily available components such as cellulose from decomposers system during the earliest phases of litter breakdown (Baldrian et al. 2008; Valaskova et al. 2007). High quality litter from exotic plant species may increase soil nutrient mineralization and thereby support growth of itself and native congeneric species (Meisner et al. 2012).

Evolutionary development of exotic plant species introduced in new area may also contribute to the invasion success. As early mentioned litter chemistry strongly influence of soil decomposers (Cornwell et al. 2008; Meier and Bowman 2008) and nutrient availability (Hobbie 1992; Meisner et al. 2012). Changing of genotypes of plants species in both native and invasive range with nutrient rich soils toward C:N rich genotypes in alien range as well as plant-soil feedbacks play important role in invasibility of plant species. Eppinga et al. (2011) presented that evolutionary change of *Phalaris arudinacea* in invasive range in north America showed higher C:N ratios in plant tissue. High C:N ratio in plant tissue may slow down

decomposition (Cornwell et al. 2008) and thus stimulate accumulation of litter. This effect may change the outcome of competition between native and invasive species. Plant-soil feedback and evolutionary change toward more competitive genotypes increase invasive success of exotic plant species (Eppinga et al. 2011; Eppinga and Molofsky 2013). However Meisner et al., (2012) showed that legacy effect of plant litter support both exotic plant species as well as their congeneric species but interaction of exotic plant species and their congenerics remains unclear.

Energy crops

Energy crops are considered as fast growing annual or perennial plants (including body parts e.g., leaves, fruits, seeds etc.) used as addition for biofuels, production of biodiesel or direct combustion (Cozier 2012; Don et al. 2012). Most widespread energy crops on the world are maize, sugarcane, soya been, sweet sorghum, switchgrass, jatropha etc. (Cozier 2012; Youngs and Somerville 2012). In Europe is most widespread energy crop oilseed rape but new introduced energy crops such hybrid sorrel, Miscanthus or hybrid poplar comes to fore (Brant et al. 2011; Lewandowski et al. 2006).

Characteristics of energy crops

There is a separation of biofuels based on their origin into biofuels first, second and third generation (Searchinger et al. 2008). The term first-generation biofuels refers biofuels produced from agricultural crops grown for food and feed (e. g. maize, sugarcane or soya bean), and from new oilseed crops such as jatropha or pongamia. The technologies producing these fuels are well developed and widely used. However these extensive cropping of these biofuel crops may have negative effects on food markets and ecosystem benefits (Cozier 2012; Searchinger et al. 2008).

Biofuels from non-food sources, especially grown as energy crops, are commonly known as second-generation (also referred as cellulosic) biofuels. Technologies for cellulose processing utilize the vast amount of woody biomass including agricultural and forest waste and residues or municipal solid waste. The promise of harvesting these energy crops is attractive since current production pathways cannot utilize the most of plant biomass, which includes cellulose, hemi-cellulose, and lignin (Buckeridge et al. 2012). Lignocellulose can

also be obtained from short-rotation trees and shrubs (e.g., willow, eucalyptus) and short-rotation grasses (e.g., switchgrass and Miscanthus, also known as elephantgrass) (Smith et al. 2013b). Some of these plants produce allelopathic compounds which contribute to the decreasing of soil biota, but generally, effect of large scale cropping of these plants on soil biota and soil functioning is still not well known.

Third-generation biofuels are obtained from algae with better sustainability properties than second generation biofuels. Currently, the most hopeful third generation biofuels comes from microalgae, photosynthetic microorganisms of less than 0.4 mm in diameter that use sunlight, water, and carbon dioxide to produce algal biomass (Chisti 2008; Chisti 2007). Algae can be cultivated in ponds or special photo bioreactors, or in hybrid systems that combine these two approaches, thus avoiding the need to use arable land.

Recent use of energy crops

Biofuels have been at the center of intense interest, discussion, and debate in recent years. The global biofuels boom began in 2004-2005 due the announcement by the United States goverment (US) and the European Union commission (EU) of policies and incentives to support increased use of biofuels (Brant et al. 2011; Lewandowski et al. 2006). Little is known about impact of large scale cultivation of energy crops on functioning of soil ecosystems. There are two views on energy crops cultivation. First allows ecosystem benefits of large scale production of these crops such as carbon sequestration, decreasing of greenhouse gasses, phytoremediation and increasing of water retention capacity etc., (Börjesson 1999; Lewandowski et al. 2006; Makeschin 1994) Second considers large scale production of energy crops as serious threat for diversity of soil fauna which provide important benefits for soil (Blanco-Canqui 2010; Börjesson 1999; Buddenhagen et al. 2009).

Energy crops and their ecosystem benefits

Ecosystem services provided by whole worlds ecosystems were summarized and expressed as $33x10^{12}$ USD per year (Costanza et al. 1997). Although ecosystems also contribute to this amount their role is not yet sufficiently appreciated. Second generation biofuels, like cellulosic ethanol, have potential as important energy sources that can decrease fossil fuel carbon emissions without affecting global food commodity prices.

Perennial short rotation grasses such as switchgrass *Panicum virgatum* belonging to second generation bioenergy crops could provide ligno-cellulosic material for ethanol while increasing belowground carbon storage, sequestering carbon in extensive root structures and accumulating soil organic carbon (Fornara and Tilman 2008; McLaughlin and Kszos 2005). Zeri et al. (2011) also showed perennial grasses promote more carbon sequestration based on eddycovariance measurements of ecosystem-atmosphere CO_2 exchange than annual plants whereas Anderson-Teixeira et al., (2013) showed that perennials also allocated substantially more carbon belowground, resulting in much higher belowground biomass. Crops species and agriculture management contribute to the benefical effect of energy crops (Börjesson 1999; Don et al. 2012). Anderson-Teixeira et al. (2013) found that belowground carbon cycling dynamics were differ between establishing perennial vegetation and the annually tilled corn-soy agroecosystem.

Perennial biofuel crops also strongly influence nitrogen fluxes and cycling. Smith et al., (2013a) demonstrate that environmental nitrogen fluxes from row crop agriculture can be strongly reduced after establishment of perennial biofuel crops. Smith et al. (2013a) showed decreasing of nitrate leaching and N_2O emissions in field sites planted by miscanthus, switchgrass and prairie in comparison to corn and soya-bean. Perennial biofuel crops tend allocate nutrients from aboveground biomass to the root and thereby increased sequestration of carbon a nd nitrogen to the soil (Anderson-Teixeira et al. 2013; Buckeridge et al. 2012; Smith et al. 2013).

Potential effect of energy crops on soil ecosystem

Biofuel crops may have economic benefits, however studies of concomitant environmental risks come to the fore (Buddenhagen et al. 2009; Raghu et al. 2006). The Invasive species belong among the most important direct drivers of biodiversity loss and degradation of ecosystem services (Zavaleta 2000). Effect of invasive plant species is important on islands, where they represent the leading cause of species extinctions (Denslow 2003). Estimation of impact of alien species annually in United States, United Kingdom, Australia, South Africa, India and Brazil has been calculated at over 100 billion USD (Zavaleta 2000) due to reduced productivity of agriculture, forestry and other production systems (McCormick and Howard 2013).

As earlier mentioned shift of genotype of introduced plants in new areas with higher nutrition inputs towards from less C:N content to higher C:N content (Eppinga et al. 2011; Eppinga and Molofsky 2013) may strongly influenced soil decomposers (Meier and Bowman 2008) nutrition cycling (Cornwell et al. 2008) and affect other co-occurring plants as well as soil ecosystem via so called legacy effect of litter (Meisner et al. 2012). Impacts of these invasive plant species on ecosystem are well documented (Murrell et al. 2011; Pritekel et al. 2006; Yeates and Williams 2001; Zavaleta 2000).

For example, *Sorghum halepense* is an introduced plant species that became an alien plant species in 16 of the 48 U.S. states in which it occurs (Raghu et al. 2006). Even the most conservative estimate of competitive losses for cotton and soybean crop production is in excess of 30 million USD annually (Raghu et al. 2006; Wilsey et al. 2011). Several grasses and woody species have been evaluated for biofuel production but these plants are often non-native and they became invasive plants with serious impacts on whole ecosystem (Buddenhagen et al. 2009; Flory et al. 2012). *Arundo donax* introduced from Asia and *Phalaris arundinacea* introduced from temperate Europe and Asia are typical short rotation grasses considered as biofuel species that are invasive in some US regions (Gifford et al. 2002; Raghu et al. 2006).

Soil biota

Soil environment contains a diverse population of edaphic organisms. General discussion of soil organisms commonly considers body size, habitat preference and food web associations (Kampichler 2000; Lavelle 2000; Lavelle et al. 1997). The most widespread categories are based on body size (microflora, microfauna, mesofauna, macrofauna and megafauna) and feeding strategy (microphytophagous, saprophagous, mycophagous, zoophagous) (Faber 1991; Lavelle 2000; Wolters 2000). Microflora, microfauna, mesofauna, macrofauna and megafauna consist of organisms with body size about μm scale, <0.2 mm, 0.2-2 mm, 2 mm-2 cm, >2 cm respectively. Each size and trophic class has its own niche and functions in the ecosystem (Kampichler 2000; Wolters 2000).

There are three main groups of soil biota based on their position in food web (Brussaard et al., 2007; Lavelle et al., 1997; Morris and Blackwood, 2007; Bardgett, 2005; Verstraete and Mertens, 2004). Primary producers are mainly represented by cyanobacteria and green algae. Primary consumers are represented by soil microbes mainly soil bacteria, actinomycetes and fungi (Verstraete and Mertens 2004). Secondary and higher-level consumers are represented by soil microfauna, mesofauna and macrofauna (Brussaard et al. 2007; Faber 1991; Lavelle, 2000; Schroöder 2008).

Chapter 2

Grazing preference and utilization of soil fungi by *Folsomia candida* (Isotomidae:Collembola)

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ABSTRACT:

Soil fungi are important food resources for soil fauna. Here we ask whether the collembolan *Folsomia candida* shows selectivity in grazing between four saprophytic fungi (*Penicillium chrysogenum, Penicillium expansum, Absidia glauca,* and *Cladosporium herbarum*), whether grazing preference corresponds to effects on collembolan reproduction, and whether the effects of fungi on grazing and reproduction depends on the fungal substrate, which included three kinds of litter (*Alnus glutinosa, Salix caprea*, and *Quercus robur*) and one kind of agar (yeast extract). On agar, *Cladosporium herbarum* and *Absidia glauca* were the most preferred fungi and supported the highest collembolan reproduction. On fungal-colonized litter, grazing preference was more affected by litter type than by fungal species whereas collembolan reproduction was affected by both litter type and fungal species. On fungal-colonized litter, the litter type that was most preferred for grazing did not support the highest reproduction, i.e., there was an inconsistency between food preference and suitability. Alder and willow were preferred over oak for grazing, but alder supported the least reproduction.

Key words: Food preference test, Soil microscopic fungi, Reproductive test

Introduction

The study of food biology in soil ecosystems is difficult because the soil fauna ingest and utilize various components of soil organic matter usually associated with soil microflora (Crowther and A'Bear 2012; Jorgensen et al. 2008; Jorgensen et al. 2003). Both springtails (collembolans) and fungi are important members of soil decomposer food webs (Lavelle et al. 1997; Moore et al. 1987; Newell 1984; Nieminen 2008), and soil fungi represent a significant food resource for collembolans and certain other soil invertebrates (Bardgett and Cook 1998; Faber 1991; Frouz and Makarova 2001; Goto 1972; Hanlon 1981).

Fungal grazing by collembolans can alter the composition of the fungal community and thereby alter effects of fungi on litter decomposition and responses of fungi to other stress factors (Berg et al. 2004; Booth and Anderson 1979; Butterfield 1999; Crowther and A'Bear 2012; Dowd 1989; Hanlon 1981; Chen et al. 1995; Kaneda and Kaneko 2004; Kaneda and Kaneko 2002; Shaw 1988). On the other hand, the composition of the fungal community and the interaction between fungi and the substrate affect collembolan food choice and reproductive success (Jorgensen et al. 2008; Jorgensen et al. 2003; VISSER and Whittaker 1977). Soil fauna often prefer some species of fungi over others as food (Butterfield 1999; Hogervorst et al. 2003; Hubert et al. 2004; Kaneko et al. 1998; Klironomos et al. 1999; Lavelle et al. 1997; Lee and Widden 1996; Leonard 1984; Schneider et al. 2004). Fungi preferred by fungivorous fauna often include *Cladosporium cladosporoides*, *Cladosporium herbarum, Alternaria alternata*, and *Ulocladium* sp. (Hurlbert 1984; Klironomos et al. 1999; Klironomos and Hart 2001).

Several authors concluded that the most preferred fungi are also the most suitable for growth and development of fungivorous fauna (Frouz and Makarova 2001; Hubert et al. 2004; Koukol et al. 2009) but Frouz and Nováková (2001) found that some highly preferred fungi did not support fungivore development. In addition, the substrate on which the fungi grown can strongly affect their attractiveness as a food source for fungivores and their suitability for fungivores growth and development (Frouz and Makarova 2001; Hubert et al. 2004). We expect that substrate used for growing fungi used as a food for Folsomia candida may substantially affect fungal preference and suitability. In the current paper, we determined the preference of the collembolan *F. candida* for several species of fungi, whether this preference

corresponds with food suitability, and how both preference and food suitability are affected by the substrate supporting fungal growth.

Materials and Methods

Collembolans, fungi, and litter

Folsomia candida (Isotomidae: Colembolla) was obtained from the Institute of Soil Biology, České Budějovice, and was reared according to a standard protocol (Tordoff et al. 2008; VanStraalen and Verhoef 1997). The following fungi were obtained from the Collection of Micromycete Strain in the Institute of Soil Biology, České Budějovice: *Penicillium expansum, Absidia glauca, Cladosporium herbarum,* and *Penicillium chrysogenum*. The fungi were cultivated by transferring spores to Petri dishes containing 8%yeast extract agar and incubating the cultures at 20–25°C. Litter was collected from alder (*Alnus glutinosa*) and oak (*Quercus robur*) plantations and from willow (*Salix caprea*) trees (spontaneous regrowth) on a post-mining heap near Sokolov (Frouz and Novakova 2005)[13]. Litter was collected by placing nylon bags under these trees; the bag openings (0.5 x 0.5 m) were parallel with the soil surface and about 0.5 m above the soil surface, and the bags were deployed for 14 days during leaf drop in November. The litter was air dried, placed in sealed zip-lock bags, and sterilized with gamma radiation (2.5 MG) before use.

Grazing preference experiments

For the first grazing preference experiment, the four fungi were grown on yeast extract agar for 5 days as described above before fungal-colonized agar disc (1 cm diameter) were cut from the colony. One agar disc of each of the four fungal species was placed in random order around the periphery of an empty, sterile 9-cm-diameter Petri dish (Tordoff et al. 2008). Thirty F. candida were then placed in the middle of the Petri dish, which was then covered and kept at 20°C in the dark. The *F. candida* individuals on each agar disc were counted at the same time of day (14.00 h) during 8 days. The experiment included four replicate Petri

dishes. Cumulative occurrence used to comparison of multiple reading done in one dish was assumed as one event (Hurlbert 1984; Tordoff et al. 2008).

A similar experiment was conducted with pieces of litter that were about 1 cm^2 in surface area and that had been colonized by one of the three fungi. Each of litter pieces (*A. glutinosa, Q. robur, S. caprea*) were colonized by one of three stem of fungi *(A.glauca, C. herbarum, P. chrysogenum*). Nine fungal-colonized pieces of litter (one piece for each combination of fungal species and litter type) were placed on the periphery of an empty 9-cm diameter Petri dish, and 30 *F. candida* were added. The Petri dish was covered and kept at 20°C in the dark. After the F. candida on each piece of litter were counted. The experiment with litter was shorter than that with agar disc because the small litter pieces deteriorated after only a few days. This second experiment also included three replicate Petri dishes.

Reproduction experiments

For the first reproduction experiment, Petri dishes containing yeast extract agar were inoculated with one of the four species of fungi. After the fungus had completely colonized the dish, 10 *F. candida* from a synchronized culture (Tordoff et al. 2008) were added to each dish. There were three replicate dishes, and temperature and light were as described in the grazing experiments. After 30 days, 70% ethanol was added to each dish, and the *F. candida* individuals in each dish were counted. The second reproductive experiment was similar to the first except that each dish contained 2 g of litter (one of three kinds of litter) that had been colonized by one of three species of fungi. In the second reproductive experiment, there were three replicate plates for each combination of litter type (*A. glutinosa, Q. robur, S. caprea*) and fungal species (*A.glauca, C. herbarum, P. chrysogenum*).

Statistical analysis

Data were subjected to a one-way ANOVA (for the first grazing preference experiment and the first reproduction experiment) or a two-way ANOVA (for the second grazing preference experiment and the second reproduction experiment). In case preference tests when several observations were done in one dish, all observations made in one dish was pooled and assumed as one observation for future statistical analysis (R 2005). If an ANOVA was significant, means were compared with the Tukey-Kramer Multiple Comparison Test. The R programme was used for statistical analyses (R 2005).

Results

Grazing preference experiments

In the experiment concerning collembolan preference among fungi growing on discs of yeast extract agar, *F. candida* preferred *C. herbarum* and *A. glauca* over *P. chrysogenum* and *P. expansum*-see Fig. 1 ($F_{3,12}$=28.530, p=0.0004). In the experiment concerning collembolan preference among fungi growing on pieces of litter, the number of F. candida on the litter pieces was significantly affected by litter type (*F. candida* preferred alder and willow litter over oak) but was not significantly affected by which fungus had colonized the litter (Fig. 2). According to a two-way ANOVA, the effect of litter was significant ($F_{2,6}$=6.3, p<0.005) but the effects of fungal species ($F_{2,6}$ 1.1, p=0.346) and the interaction between litter type and fungal species ($F_{8,24}$=1.1, p=0.349) were not significant.

Reproduction experiments

In the first reproduction experiment, in which the fungal-colonized agar discs were offered to the collembolans, F. candida numbers were significant higher ($F_{3,9}$=6.269, p=0.017) with *A. glauca* and *C. herbarum* than with *P. chrysogenum* or *P. expansum* (Fig. 3). In the second reproduction experiment, in which the fungi were grown on different types of litter, *F. candida* numbers were significantly affected by fungal species and litter type but not by their interaction (Fig. 4). As in the second grazing preference experiment, more of the variation

was explained by litter type than by fungal species. *F. candida* numbers were largest on willow litter, intermediate on oak litter, and smallest on alder litter, which differs from the order obtained in the second grazing preference experiment (Fig. 3). According to a two-way ANOVA, the effects of both litter and fungal species were significant ($F_{2,6}$=57.3, $p<0.001$ and $F_{2,6}$=7.1 and $p=0.005$ respectively) but the interaction was not significant ($F_{8,24}$=1.9, $p=0.150$). With respect to fungi in the second reproductive experiment, F. candida numbers were larger with *A. glauca* and *Penicillium chrysogenum* than with *Cladosporium herbarum* (Fig. 4).

Discussion

When offered fungi growing on agar disc in the current study, Folsomia candida preferred some fungal species and also increased to higher number on some species than on others. When offered fungi grown on different litter types (alder, willow, and oak), however, grazing preference and reproduction were more affected by litter type than by fungal species. In agreement with other authors, this indicates that the substrate on which fungi grow is largely responsible for fungal attractiveness and nutritional quality for fungivores (Frouz et al. 2002; Frouz and Novakova 2001; Kaneda and Kaneko 2002). None of previous studies deal with litter which is more field realistic that any growing media, basic novelty of our study is that litter is more important than fungal species itself. This finding also supports the conclusion of Jørgensen et al. (2008) that natural substrates should be used in studies of fungivore–fungus interactions.

In the grazing preference experiment with fungal-colonized agar disks, the two species of Penicillium were the least preferred. In this case, food preference may have been affected by the production of patulin, citrinin, or other mycotoxins. Dowd (1989) reported that patulin and ochratoxin caused arthropod mortality. According to Staaden et al. (2011), olfactory cues affect the food preference of collembolans because volatiles indicate which secondary metabolites are in the food source.

In the reproduction experiment on agar, *F. candida* population growth was greater with *A. glauca* and *C. herbarum* than *P. chrysogenum* and *P. expansum.* These results correspond with those of Hubert et al. (2004), who reported that, when the substrate supporting fungal growth was agar, those oribatid mites that preferred *C. cladosporoides* also had the greatest reproduction on *C. cladosporoides*. Tordoff et al. (2008), who studied the

reproduction of several species of collembolans (*Folsomia candida, Proisotoma minuta, Protaphorura armata*) on four species of Basidiomycetes (*Phanerochaete velutina, Hypholoma fasciculare, Resinicium bicolor*, and *Phallus impudicus*), reported that *P. minuta* survived only on *P. velutina* mycelia. In contrast, *F. candida* was found to be a dietary generalist that was able to increase its abundance on the mycelium of all fourpecies of Basidiomycetes. P. armata could survive but not reproduce well on *P. velutina* mycelium. Frouz and Nováková (2001) showed that the fungi most preferred by the dipteran *Lycoriella ingenua* are most suitable for the growth and development of its larvae. *Folsomia candida* is able to survive and reproduce on more food resources than other collembolans on various substrates (Tordoff et al. 2008).

As noted earlier, collembolan food preference in the current study was more affected by the substrate on which the fungi grew than on the identity of the fungi. Jørgensen et al. (2003) documented significant differences in food preference when collembolans were offered eight species of soil fungi growing on a natural soil substrate. In agreement with Kaneko et al. (1995), we found that which fungi were preferred by collembolans differed depending on the substrate on which the fungi were growing.

Conclusions

Litter type had an inconsistent effect on *F. candida* grazing preference and F. candida reproduction. Thus, alder was preferred to oak in the grazing preference experiment but oak supported greater numbers than alder in the reproduction experiment. The reason for this inconsistency is not clear but perhaps can be explained by the short duration of the preference test (data were collected after 12 days) in which sterile alder supported better fungal growth than sterile oak litter. In the reproduction test, which lasted for 30 days, addition of collembola undoubtedly resulted in bacterial contamination of the litter, and the bacteria may have reduced fungal growth to a greater degree on alder than on oak, resulting in greater collembolan reproduction on oak than on alder. This result, which is to some extent contrary to that of Jørgensen et al. (2008), indicates that discrepancies between food choice and food suitability may occur even on natural substrates.

Acknowledgements

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Appendices

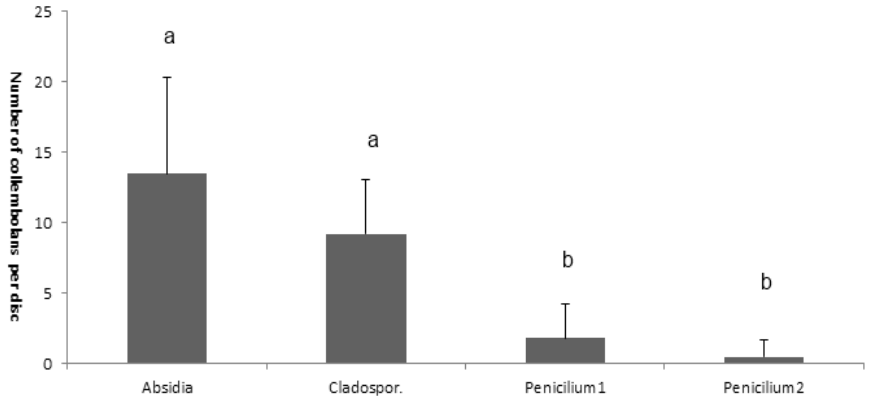

Fig. 1 Numbers of *F. candida* on fungal-colonized agar disks as affected by fungal species; the collembolans were counted 12 days at same time 30 individuals were added per dish. Values are means + SD of all sampling dates. Means with the same letter are not significantly different according to an Tukey-Kramer Multiple Comparison Test ($p>0.05$). Absidia (*Absidia glauca*), Cladospor, (*Cladosporium herbarum*), Penicilium1 (*Penicillium chrysogenum*), Penicilium2 (*Penicillium expansum*).

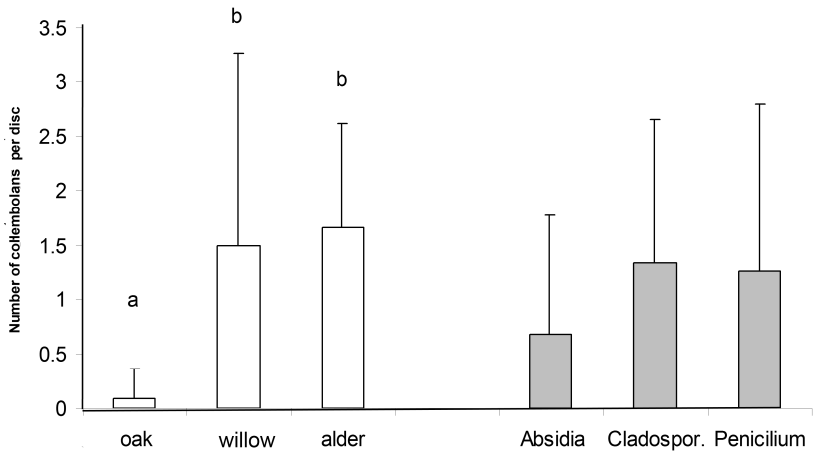

Fig. 2 Numbers of *F. candida* on fungal-colonized litter pieces as affected by species of fungi and litter source; the collembolans were counted 1 and 2 days after 30 were added per dish. Values are means + SD of both sampling dates. Data for litter type were averaged across fungi, and data for fungi were averaged across litter types. Means with the same letter are not significantly different according to an Tukey-Kramer Multiple Comparison Test ($p>0.05$). Absidia (*Absidia glauca*), Cladospor. (*Cladosporium herbarum*), Penicilium (*Penicillium chrysogenum*)

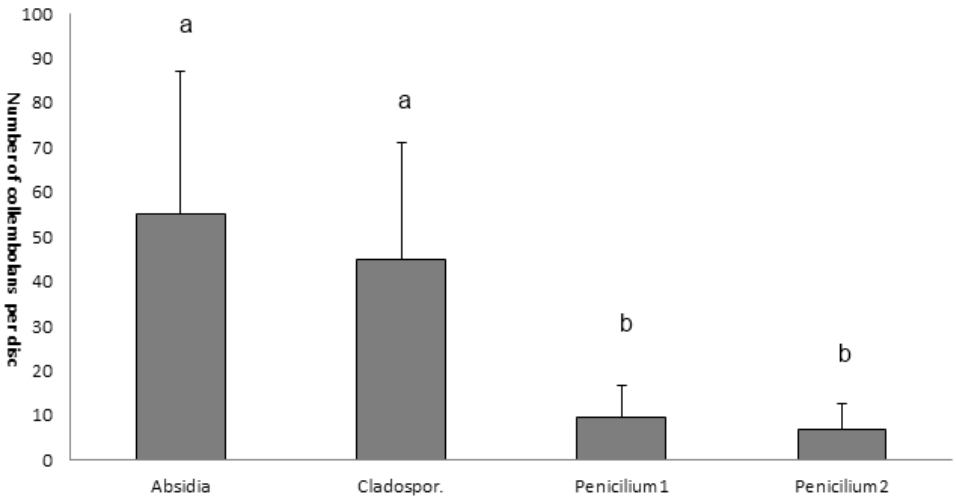

Fig. 3 Numbers of *F. candida* in Petri dishes containing fungi growing on yeast extract agar; the collembolans were counted 30 days after 10 were added per dish. Values are means + SD, and means with the same letter are not significantly different based on an Tukey-Kramer Multiple Comparison Test ($p>0.05$). Cladospor. (*Cladosporium herbarum*), Penicilium1 (*Penicillium chrysogenum*), Penicilium2 (*Penicillium expansum*).

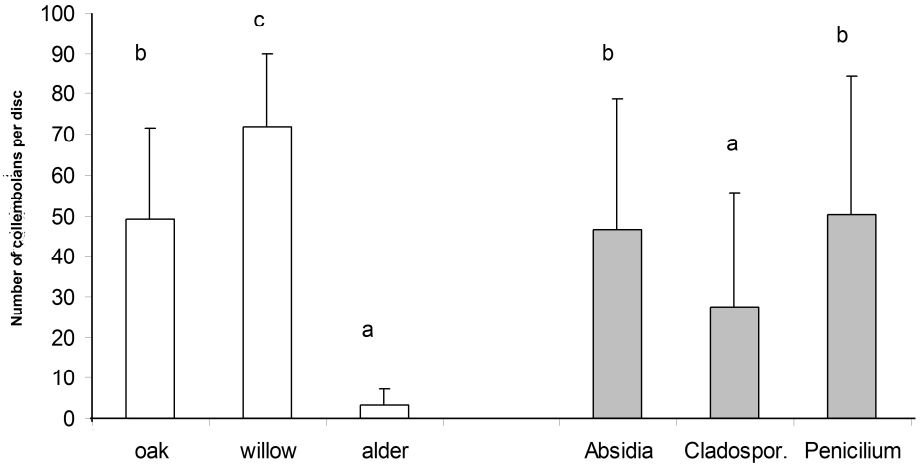

Fig. 4 Numbers of *F. candida* in Petri dishes containing fungal-colonized litter; the collembolans were counted 30 days after 10 were added per dish. Values are means + SD. Data for litter type were averaged across fungi, and data for fungi were averaged across fungal species. Means followed by the same letter are not significantly different based on an Tukey-Kramer Multiple Comparison Test ($p>0.05$). Absidia (*Absidia glauca*), Cladospor. (*Cladosporium herbarum*), Penicilium (*Penicillium chrysogenum*).

Chapter 3

Effect of cropping hybrid sorrel (*Rumex patientia* x *Rumex tianschaniacus*) on soil biota

Submitted manuscript

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ABSTRACT:

Energy crops represent an alternative to fossil fuel. Because energy crops are often introduced species, little is known about how their long-term production affects soil and soil biota. The most important introduced energy crop in the Czech Republic is hybrid sorrel (*Rumex patientia* x *Rumex tianschaniacus*). The current study investigated the effects of long-term production of hybrid sorrel on basal soil respiration, specific microbial respiration, microbial biomass carbon, the composition of the cultivable soil fungal community, and the composition of soil meso- and macrofauna communities. In a split-plot field experiment in Chotýšany near Vlašim (Central Bohemia, Czech Republic), fields with hybrid sorrel (2 and 10 years old), oilseed rape (> 10 years old), and cultural meadow (> 10 years old) were compared. The composition of soil meso- and macrofauna communities differed among fields. Soil meso- and macrofauna diversity and abundance were lowest in oilseed rape fields, and abundance was highest in cultural meadows. Hybrid sorrel fields contained more pathogenic fungi than oilseed rape fields or cultural meadows but the difference was only marginally significant. Basal soil respiration and specific microbial respiration (qCO_2) were highest in oilseed rape fields, and microbial biomass was highest in cultural meadows.

Key words: Composition of soil biota, Basal soil respiration, Specific microbial respiration (qCO_2), Microbial biomass.

Introduction

Energy crops are used to produce biofuels or an additive for biofuels. They grow rapidly, and their biomass is currently an important component of the energy mix in many countries (Blanco-Canqui 2010; Don et al. 2012). According Brant et al. (2011), energy crops will be grown on 500 000 ha^{-1} of arable land in the Czech Republic until 2030. In the Czech Republic, the most common energy crops are oilseed rape (*Brassica napus* spp. *napus*), hybrid sorrel (*Rumex patientia* x *Rumex tianshanicus*), and Miscanthus (*Miscanthus sinensis*) (Lewandowski et al. 2006). While oilseed rape is well established, hybrid sorrel has only recently been cultivated as an energy crop in the Czech Republic, and little is known about the environmental impact of its long-term cultivation.

If fields with energy crops are appropriately located, designed, and managed, they may reduce nutrient leaching and soil erosion and generate additional environmental services such as soil carbon accumulation, improved soil fertility, and the removal of cadmium and other heavy metals from soils or wastes (Börjesson and Berndes 2006; Buckeridge et al. 2012). However, energy crops can have positive or negative effects on biodiversity and soil biota (Börjesson 1999; Buckeridge et al. 2012; Buddenhagen et al. 2009; Hansson and Fogelfors 2000), depending on the intensiveness of the biofuel production, the design of the planting in the landscape, crop management, and land use before conversion (Buckeridge et al. 2012; Makeschin 1994). Some recently introduced energy plants may enhance soil degradation and biological invasion (Buddenhagen et al. 2009; Raghu et al. 2006).

Hybrid sorrel is a perennial plant that can be harvested dry and that can continuously produce high yields for more than 10 years (Ustak and Vana, 1998). In addition, sorrel tolerates a broad range of soils, fertilization regimes, and climates (Ustak and Vana, 1998). Sorrel is harvested in August; the flower stalk with secateurs is cut first, and then the remaining leaves are harvested with a motor scythe (Ustak and Vana 1998).

In the current research, we compared the impact of the long-term production of hybrid sorrel (non-tilled, 2 and 10 years old), oilseed rape (tilled, > 10 years old), and a cultural meadow (non-tilled, > 10 years old) on the biological activity of the soil community. We measured the composition and abundance of various groups of soil fauna, the composition of the cultivable fraction of the fungal community, basal soil respiration, and specific microbial respiration (qCO_2).

Material and Methods:

Study area and sampling

Samples were taken from four kinds of fields located in Chotýšany near Vlašim (°44'38.763 N, 14°48'52.48 E) at 450 m a.s.l. The fields (50x50m) contained hybrid sorrel (2 years old or 10 years old), oilseed rape (>10 years old), or cultural meadow dominated by *Poa annua, Dactilis glomerata*, *Alopecurus pratense*, *Trifolium repens*, and *Plantago major*. Each kind of crop was represented by three replicate fields. The distance between replicates of the same treatments was about the same as distance to replicates of other treatments. The maximal distance between the most distant fields was 300 m. For all fields, the mean annual temperature is 7.8°C, and the mean annual precipitation is 550 mm. The soil was a cambisol with a pH of about 5.8. For determination of soil faunal and soil microbial characteristics, soil cores were collected, and each was 12 cm in diameter and 10 cm deep for soil fauna and 6 cm in diameter and 10 cm deep for microorganisms. Three of each kind of core were collected from each of the three replicate fields in the summer and autumn of 2010 and in the spring of 2011.

Measurement of basal soil respiration and microbial biomass

For estimation of basal soil respiration, soil samples (10 g) were closed in 200-mL air-tight vials and incubated at 20 °C in the laboratory. The carbon dioxide that evolved from the soil was trapped in 3 mL of 0.5 M NaOH during a 7-day incubation and was then quantified by titration with 0.05 M HCl after addition of $BaCl_2$. Respiration was expressed as C-CO_2 $m^{-2} \cdot h^{-1}$. Blanks were used to assess the CO_2 trapped during handling and incubation (Jenkinson and Powlson 1976; Vance et al. 1987). Microbial biomass (C_{mic}) was determined by the chloroform fumigation–respiration method and expressed as g C g^{-1} DW or mg C $m^{-2} \cdot h^{-1}$. After soil samples (10 g) had been fumigated with chloroform vapor for 48 h, respiration was measured as above (Shan-Min et al. 1987). Specific microbial respiration was evaluated as the rate of basal soil respiration per unit of microbial biomass.

Isolation of soil fungi

Soil fungi were isolated by a dilution–plating method (Chesters and Thornton 1956). Samples of soil suspension were placed on Petri dishes containing soil extract agar with rose Bengal, and the cultures were kept at 25 °C. Fungi were identified based on micro- and macro-morphological, physiological, and biochemical features (Frankland et al. 1990).

Extraction and determination of soil meso- and macrofauna

Soil samples (200 g) were placed on a Tullgren extractor for 5 days (Lavelle 2000). Extracted soil invertebrates were fixed in a 0.2% formaldehyde solution and identified to order and family based on their morphological characters.

Statistical analyses

Because the soils and climatic conditions were similar for the fields, we assumed that differences the soil biota resulted from differences in the cropping of hybrid sorrel, oilseed rape, and cultural meadow. Programme R (Simecek and Simeckova 2013) was used for multiple comparisons, and Canoco (Leps and Hadincova 1992) was used for multivariate analysis (CCA, PCA). For comparison of microbial activity among the fields, an ANOVA and the Tukey HSD post hoc test were used. CCA analyses were used to evaluate the multivariate data concerning soil meso- and macrofauna and the data concerning cultivable fungi. Significance was determined at $p < 0.05$.

Results

Microbial activity of soil

Basal soil respiration did not significantly differ between the 10-year-old and 2-year-old hybrid sorrel fields but was significantly higher in oilseed rape fields than in hybrid sorrel fields or in cultural meadows (Fig. 1). Microbial biomass was greater in the cultural meadows than in the hybrid sorrel and oilseed rape fields (Fig. 2). Like basal soil respiration, specific microbial respiration (qCO_2) was higher in the oilseed rape fields than in the hybrid sorrel fields or in the cultural meadows (Fig. 3).

Density and diversity of soil meso- and macrofauna

The total number of soil meso- and macrofauna individuals extracted was highest in the cultural meadows, lowest in the oilseed rape fields, and intermediate in the hybrid sorrel fields (Fig. 4). The composition and abundance of soil meso- and macrofauna differed between the 10- and 2-year-old hybrid sorrel fields. Oilseed rape showed declining abundance of soil meso-and macro-fauna and showed different composition of soil community (Table 1). CCA of the soil meso- and macrofauna community composition divided the data into two groups: one group contained data for the 10-year-old hybrid sorrel fields and cultural meadows and the other group contained data for the 2-year-old hybrid sorrel fields and the oilseed rape fields (Fig. 4). When the identity of the treatments was used as the only environmental variables in CCA model (Monte Carlo permutation test: F-ratio=4.901, p = 0.002) explained significantly 67.4% of data variability, in fauna community composition and all the treatments significantly (p<0.05) contribute to this pattern.

Soil fungi

A total of 36 fungal species were detected on the soil extract agar plates (Table 2). PCA indicated that 10-year-old hybrid sorrel fields tended to be dominated by plant-pathogenic species of *Fusarium* and *Trichoderma* while meadows tended to be dominated by saprophytic species of *Penicillium* and *Clonostachys* but these trends were not statistically significant (Fig. 5). These results indicated that the cultivable part of the soil fungal community was not greatly influenced by crop species and agriculture practices.

Environmental variables used as treatments identity in CCA model (Monte Carlo permutation test: F-ratio=3.305, p = 0.074) explained 21.6% of data variability, in cultivable fraction of soil fungal community.

Discussion

Long-term production of hybrid sorrel affected several soil properties. For example, the soils with 10-year-old hybrid sorrel tended to contain more plant-pathogenic fungi than soils with oilseed rape or cultural meadows. That result is consistent with the finding that invasive plants tend to enhance soil pathogens (Mangla et al. 2008; Mitchell et al. 2010). Some introduced plants, however, produce allelopathic compounds that suppress soil-borne plant-pathogenic fungi (Zhang et al. 2011; Zhang et al. 2009b). Although plant-pathogenic fungi tended to be abundant in the 10-year-old hybrid sorrel fields, the differences in cultivable fungi the hybrid sorrel fields, the oilseed rape fields, and the cultural meadows were not statistically significant.

Soil meso- and macrofauna were most abundant in the cultural meadows and 10-years-old hybrid sorrel fields and were least abundant in the oilseed rape fields. This difference can be explained in part by differences in tillage. Hybrid sorrel is not tilled because it is a perennial crop while oilseed rape is an annual crop that is tilled; cultural meadows are similar to hybrid sorrel fields in that they are not tilled. Tillage typically reduces the abundance and diversity of soil fauna (Bedano et al. 2006; Domínguez et al. 2010; Errouissi et al. 2011; Hulsmann and Wolters 1998).

Tillage, however, cannot explain all the differences because soil fauna were more abundant in the cultural meadows than in the 10-year-old hybrid sorrel fields. Plant species can affect the soil biota by influencing the quantity and quality of food resources that reach the soil (Viketoft et al. 2005; Wardle et al. 1999b). Some introduced plants produce chemicals that are associated with reduced numbers of some groups of soil meso- and macrofauna (Bardgett and Walker 2004; Barrios 2007; Blanco-Canqui 2010; Kappes et al. 2007).

Basal soil respiration and specific microbial respiration were similar among 10-year-old hybrid sorrel fields, 2-year-old hybrid sorrel fields, and cultural meadows. Basal soil respiration and specific microbial respiration was highest in oilseed rape. This can be explained by the tillage of oilseed rape fields, which by disturbing soil aggregates and

increasing soil aeration increases the mineralization of soil organic matter (Kladivko 2001). In contrast, Haney et al. (2010) reported that C mineralization was greater with perennial crops than with annual crops. These contradictory finding might be explained by differences in litter quality and quantity, which greatly affect the rate at which soil organic matter is mineralized (Barrios, 2007; Fioretto et al., 2000; Wedin and Tilman, 1990;Yan et al., 2003).

Perennial biofuel crops tend to generate large quantities of litter that increase microbial biomass C and N (Haney et al. 2010). Cao et al. (2010) reported that microbial biomass and soil organic carbon were greater in older than in younger Eucalyptus plantings. Ma et al. (2000) reported that microbial biomass was greater in soil cultivated with switch grass for 10 years than for 2–3 years. In our study, however, microbial respiration was lower for soil planted with the perennial hybrid sorrel than for soil planted with the annual oilseed rape.

Important role in soil microbial activity could play the production of chemical compounds (Blum et al. 2000; Weidenhamer et al. 1989; Zhang et al. 2009a; Zhang et al. 2011; Zhang et al. 2009b) but in this case we did not focused on allelopathic effect and producing of secondary metabolites. Other important factor which can affect microbial community are agriculture practices and soil quality (Donnison et al. 2000; Frey et al. 1999; Hagn et al. 2003; Kladivko 2001). Fungal community compositions in hay meadows seem to differ from those of arable soils (Donnison et al. 2000). In contrary our results showed fairly stable composition of cultivable fraction of fungal community in soils planted by different energy crops.

Conclusions

Large-scale production of hybrid sorrel may affect the species composition and abundance of soil meso- and macrofauna, i.e., the diversity and abundance of soil fauna in hybrid sorrel fields were less than in cultural meadows but greater than in oilseed rape fields. Although the effect was not statistically significant, the hybrid sorrel soils tended to contain more plant-pathogenic fungi than the cultural meadow or oilseed rape soils. Microbial activity did not significantly differ between young and old hybrid sorrel plantings. That microbial respiration was higher in oilseed rape fields than in hybrid sorrel fields can probably be

explained by the fact that, unlike hybrid sorrel fields and cultural meadows, oilseed rape fields are tilled.

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Appendices

Table 1 Abundance of soil meso- and macrofauna (by taxonomic group) in fields with hybrid sorrel (10 or 2 years old, indicated by Sorrel 10 and Sorrel 2 respectively), oilseed rape, or cultural meadow (indicated by Oilseed and Meadow, respectively). Values are mean numbers of individuals ± SD per m^2.

Taxon	Sorrel 10 ± SD	Sorrel 2 ± SD	Oilseed ± SD	Meadow ± SD
Collembola	1900 ±930	1367 ±518	1633 ±769	933 ±579
Acari	100 ±188	200 ±141	433 ±480	467 ±141
Diplura	0 ±0	0 ±0	33 ±11	33 ±47
Enchytreidae	67 ±47	167 ±170	0 ±0	0 ±0
Lumbricidae	133 ±47	67 ±94	33 ±13	333 ±235
Aranea	0 ±0	33 ±14	33 ±13	133 ±47
Diplopoda	0 ±0	0 ±0	67 ±94	333 ±188
Chilopoda	67 ±90	0 ±0	33 ±13	733 ±546
Isopoda	0 ±0	0 ±0	0 ±0	133 ±249
Diptera	1967 ±1602	1667 ±1744	233 ±170	467 ±171
Coleoptera	0 ±0	267 ±141	133 ±124	233 ±188
Hymenoptera	0 ±0	233 ±330	100 ±141	4300 ±4859
Sternoryncha	0 ±0	0 ±0	0 ±0	367 ±286
Lepidoptera	100 ±47	0 ±0	67 ±47	0 ±0
Total fauna	4333 ±2953	4000 ±3153	2800 ±1877	8466 ±7541

Table 2 List of soil fungi isolated in fields with hybrid sorrel (10 or 2 years old, indicated by Sorrel 10 and Sorrel 2, respectively), oilseed rape, or cultural meadow (indicated by Oilseed and Meadow, respectively).

Sorrel 10	Sorrel 2	Oilseed	Meadow
Cladosporium cladosporoides	*Cladosporium cladosporoides*	*Cladosporium herbarum*	*Cladosporium herbarum*
Cladosporium herbarum	*Cladosporium herbarum*	*Clonostachys rosea* f. *catenulata*	*Clonostachys rosea* f. *catenulata*
Clonostachys rosea	*Clonostachys rosea*	*Humicola grisea*	*Clonostachys rosea* f. *catenulata*
Clonostachys rosea f. *catenulata*	*Clonostachys rosea* f. *catenulata*	*mycelium sterilium*	*Fusarium dimerum*
Fusarium dimerum	*Fusarium culmarium*	*Paecylomyces* sp.	*Humicola grisea*
Fusarium equiseti	*Fusarium ventricosum*	*Penicillium daleae*	*mycelium sterilium*
Fusarium graminareum	*Mucor cincinelloides*	*Penicillium glabrum*	*Paecylomyces sp.*
Fusarium sambucinum	*Mucor circinelloides* f. *circinelloides*	*Penicillium vulpium*	*Penicillium daleae*
Glyocladium viridae	*Mucor circinelloides* f. *griseogamum*	*Rhizomucor* sp.	*Penicillium glabrum*
Mucor hiemalis f. *corticola*	*Mucor hiemalis* f. *hiemalis*	*Rhizopus pusillus*	*Penicillium vulpium*
Mucor hiemalis f. *hiemalis*	*mycelium sterilium*	*Rhizopus stolonifer*	*Rhizomucor* sp.
mycelium sterilium	*Paecylomyces variety*	*Trichoderma* sp.	*Rhizopus pusillus*
Paecylomyces crustacea	*Penicillium spinulosum*		*Rhizopus stolonifer*
Phoma sp.	*Phoma* sp.		*Trichoderma* sp.
Tricoderma koningii	*Trichoderma viridae*		
Trichoderma aureoviridae			
Trichoderma harzianum			

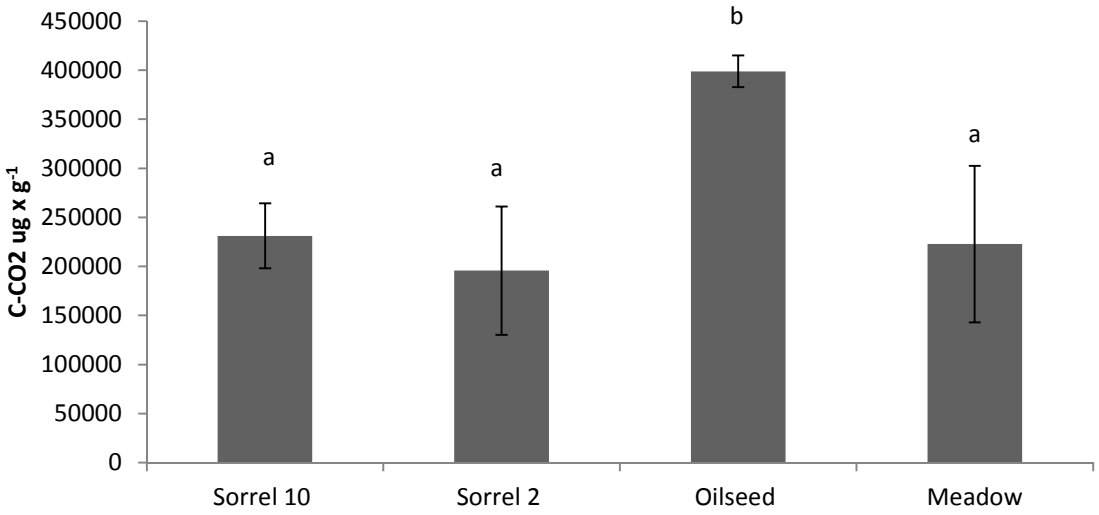

Fig. 1 Basal soil respiration in fields planted with energy crops and in cultural meadows. Sorrel fields were 10 or 2 years old (indicated by Sorrel 10 and Sorrel 2, respectively), and the oilseed rape fields and cultural meadows were > 10 years old (indicated by Oilseed and Meadow, respectively). Values are means $\pm$ SD. Means with different letters are significantly different (ANOVA HSD post hoc test, F=34.19, p <0.001). Same letters indicates statistical homogenous groups (p <0.05).

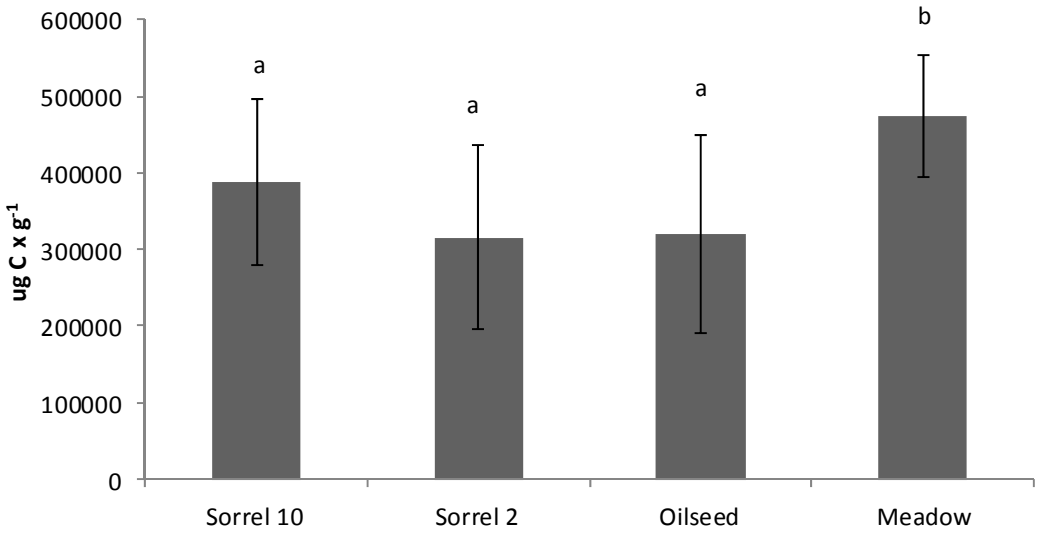

Fig. 2 Microbial biomass carbon in fields planted with energy crops and in cultural meadows. Sorrel fields were 10 or 2 years old (indicated by Sorrel 10 and Sorrel 2, respectively), and the oilseed rape fields and cultural meadows were > 10 years old (indicated by Oilseed and Meadow, respectively). Values are means $\pm$ SD. Means with different letters are significantly different (ANOVA HSD post hoc test, F=5.404, p =0.003). Same letters indicates statistical homogenous groups (p <0.05).

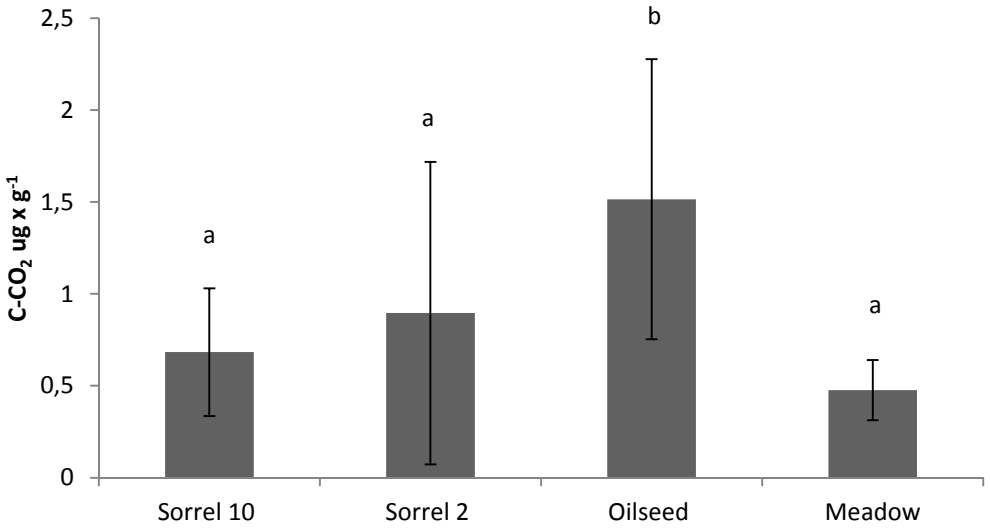

Fig. 3 Specific microbial respiration (qCO_2) in fields planted with energy crops and in cultural meadows. Sorrel fields were 10 or 2 years old (indicated by Sorrel 10 and Sorrel 2, respectively), and the oilseed rape fields and cultural meadows were > 10 years old (indicated by Oilseed and Meadow, respectively). Values are means $\pm$ SD. Means with different letters are significantly different (ANOVA HSD post hoc test, F=6.868, p <0.001). Same letters indicates statistical homogenous groups (p <0.05).

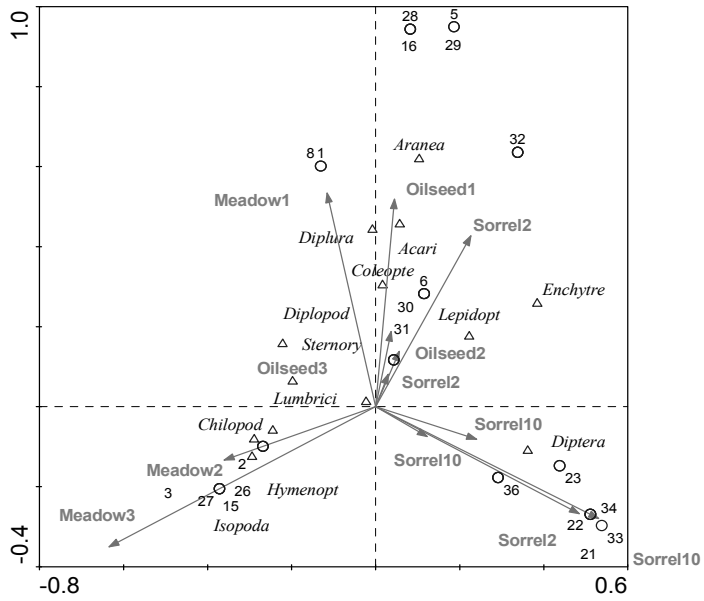

Fig. 4 CCA of data for soil meso- and macrofauna abundance (by taxonomic group) in fields with 10-year-old hybrid sorrel (Sorrel 10, 2-year-old hybrid sorrel (Sorrel 2), oilseed rape (Oilseed), and cultural meadow (Meadow). Monte Carlo permutation test: F ratio=4.901, p = 0.002, explain 67.4% of data variability.

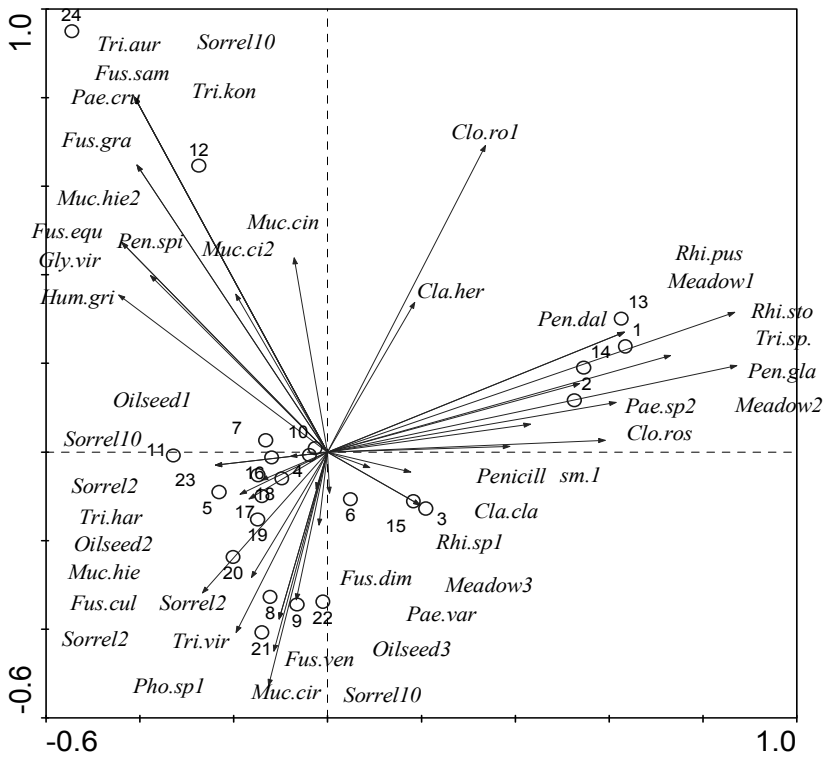

Fig. 5 PCA of data for cultivable fungus abundance (by taxonomic group) in fields with 10-year-old hybrid sorrel (Sorrel 10), 2-year-old hybrid sorrel (Sorrel 2), oilseed rape (Oilseed), and cultural meadow (Meadow). The first and second PCA axis explained 16.7% and 31.1%, respectively, of data variability.

Chapter 4

The effect of native and introduced energetic crops on the composition of soil biota communities

Submitted manuscript

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ABSTRACT

Energy crops are an accepted alternative to fossil fuels, but little is known about the ecological impact of their production. The aim of this contribution is to study the effect of native (*Salix viminialis* and *Phalaris arudinacea)* and introduced (*Helianthus tuberosus, Reynoutria sachalinensis* and *Silphium perfoliatum*) energy crop plantations on the soil biota in comparison with cultural meadow vegetation. The study was performed as part of a split plot field experiment of the Crop Research Institute in the town of Chomutov (Czech Republic). The composition of the soil meso- and macrofauna community, composition of the cultivable fraction of the soil fungal community, cellulose decomposition (using litter bags), microbial biomass, basal soil respiration and PLFA composition (incl. F/B ratio) were studied in each site. Some of the introduced energy crops tended to have a high phenol content and high C:N ratio, but are no clear distinctions between native and introduced energy crops. The C:N ratio and content of allelopathic compounds differed among plant species, but these results could not be considered significant between introduced and native plant species. Abundance of the soil meso- and macrofauna was higher in field sites planted with native energy crops than those planted with introduced energy crops. RDA and Monte Carlo Permutation Test showed that the composition of the faunal community differed significantly between native and introduced plants. Significantly different basal soil respiration was found in sites planted with various energy crops; however, this difference was not significant between native and introduced species. Microbial biomass carbon and cellulose decomposition did not exhibit any statistical differences among the energy crops. The largest statistically significant difference we found was in the content of actinobacterial and bacterial (bacteria, G+ bacteria and G- bacteria) PLFA in sites overgrown by native energy crops compared to introduced energy crops. In conclusion, certain parameters significantly differ between native and introduced species of energy crops; however, the functional importance of these differences requires further research.

Keywords: Soil fauna, Energy crops, Composition of soil fungi, Microbial biomass, Basal soil respiration.

Introduction

Energy crops are plants grown for heating or production of biofuels. Their ecological benefits include reduced emissions of greenhouse gasses, carbon sequestration and phytoremediation (Anderson-Teixeira et al. 2013; Buckeridge et al. 2012; Smith et al. 2013a), but their impact on complex soil ecosystems still requires extensive research. The main disadvantage of energy crops is their low economic competitiveness against fossil fuels (Brant et al. 2011; Hellebrand et al. 2008). Growing of energy crops can also cause competition over land with the need to grow food and forage, which may, consequently, compromise ecosystem services which soil provides (Costanza et al. 1997; Lavelle et al. 1997). The supposed economic benefit of these ecosystem services, including ecosystem services provided by soil organisms, for the human society is $33x10^{12}$ USD per year although this is generally not appreciated (Costanza et al. 1997; Lavelle et al. 1997).

Growing of energy crops may also affect the soil biota (Blanco-Canqui 2010). Soil organisms play an important role in soil ecosystems (Barrios 2007; Lavelle 2000; Lavelle et al. 1997). Soil organisms are very important for decomposition of soil organic matter, humus formation and formation of soil microaggregates (Lavelle 2000; Lavelle et al. 1997; Wolters 2000). Increased production of energy crops causes loss of areas available for agricultural crops. Energy crops are often introduced into new environments, which may potentially lead to changes to soil properties in these environments (Buddenhagen et al. 2009; Zavaleta 2000). Gifford et al., (Gifford et al. 2002) and Raghu et al., (Raghu et al. 2006), for example, showed that certain biofuel crops such as *Arundo donax* and *Phalaris arundinacea* imported from temperate Europe and Asia to the USA are typical short-rotation grasses that become invasive in some US states.

Soil biota communities on arable land become depleted and host fewer species and functional groups of the soil biota (Bardgett and Cook 1998; López-Fando and Bello 1995; Wardle et al. 1999b). Compared to the effect of cultural crops, which has been studied intensively (Blanco-Canqui 2010; Searchinger et al. 2008), scant data are available on the impacts of energetic plants. Many energetic plants are perennials, which may be an advantage because, as already mentioned, tillage is the most important disturbance factor in agricultural soils (Errouissi et al. 2011; Kladivko 2001). On the other hand, many energetic plant species are aliens, and some of them have been found to be invasive (Buddenhagen et al. 2009; Pysek et al. 2012a; Raghu et al. 2006). Many invasive plant species may negatively affect entire

ecosystems (Ehrenfeld 2001; Pritekel et al. 2006; Pysek et al. 2012b; Zavaleta 2000). Long-term monocropping cultivation of introduced crops may enhance this effect (Buddenhagen et al. 2009). In this study, we explore the effects of growing various energy crops on the activity and composition of the soil biota. We in particular focus on the question whether there are any differences among native and introduced plant species.

The aim of this study was to test for differences in soil biological characteristics among introduced (*Helianthus tuberosus, Silphium perfoliatum* and *Reynoutria sachalinensis*) and native (*Salix viminialis* and *Phalaris arudinacea*) energy crops in comparison with cultural meadow species. We used the following characteristics: production of phenolic compounds, C:N ratio of plant litter, composition and abundance of various groups of soil fauna, composition and microbial biomass of soil microorganisms, biological activity of soil microbial biomass, basal soil respiration, and microbial biomass of carbon.

Material and methods

Sampling was performed in October 2009 in a split plot field experiment of the Crop Research Institute in the town Chomutov in the Czech Republic (50° 27′ 46" N, 13° 24′ 40" E, 7.86°C mean annual temperature and 550 mm of annual rainfall). Soil samples were collected from field sites planted with five energy crops *(Salix viminialis, Phalaris arudinaceae, Helianthus tuberosus, Reynoutria sachalinensis* and *w*) and a cultural meadow (dominated by *Poa annua*, *Poa pratensis*, *Trifolium repens* and *Plantago major*). Field sites planted with *S. viminialis, P. arudinaceae* and the cultural meadow represented native plant species. Other field sites were overgrown by introduced species (*H. tuberosus R. sachalinensis* and *S. perfoliatum*). For each species, three patches were chosen, and in each one a composite sample was taken consisting of three particular samples. A soil corer 12 cm in diameter was used to sample the soil fauna and 3 cm in diameter to sample the soil microflora, both down to the depth of 5 cm.

Chemical analyses of plant litter

Plant material was dried and then homogenized into small particles smaller than 2 mm. Samples were packed in pewter capsules and then weighed on microscales (Mettler Toledo). The content of carbon and nitrogen was determined in samples of dry crushed soil and leaves using an EA 1108 elemental analyser (Carlo Erba Instruments). Total soluble phenols were extracted by methanol and determined spectrophotometrically using the Folin-Ciocalteu reagent (Singleton et al. 1999).

Analysis of soil meso- and macrofauna

Soil samples were extracted in a Tullgren funnel for 5 days (Lavelle 2000). Extracted soil invertebrates were fixed with a 0.2% formaldehyde solution and determined using morphological characters. Soil invertebrates were classified into orders and families.

Analysis of soil microbial activity

Basal soil respiration of the soil was estimated by the incubation method. Soil (10 g) was enclosed in airtight bottles each equipped with a small container with NaOH and cultivated at 20^{o}C for one week. Carbon dioxide released from the soil was trapped in 3mL of 0.5M NaOH and then quantified by titration with 0.05M HCl after addition of $BaCl_2$ (Jenkinson and Powlson 1976). Its amount was expressed as C-CO_2 m^{-2} x h^{-1}. The same bottles without soil were used to assess CO_2 trapping during incubation (from air closed in vials) and during handling. Microbial biomass (C_{mic}) was determined by the chloroform fumigation–extraction method (Jenkinson and Powlson 1976; Vance et al. 1987) and expressed as mg C g^{-1}. Cellulose decomposition was measured with litterbags (three litterbags per field), which were buried 3 cm under the surface of the soil (October 2009-April 2010). The litter in each litterbag was represented by two sheets of filter paper (2g). The filter paper sheets were weighed and burned at 550 °C for 5 hours. Mass loss of filter paper after burning was used and cellulose weight after decomposition.

Analysis of PLFA

Samples for phospholipid fatty acid (PLFA) analysis were extracted by a chloroform–methanol–phosphate buffer (1:2:0.8; v/v/v). LiChrolut Si-60 solid-phase extraction cartridges (Merck, Whitehouse Station, NJ) were used to separate the extracts, and phospholipid fractions were subjected to mild alkaline methanolysis (Šnajdr et al. 2008). Gas chromatography–mass spectrometry (GC-MS) was used for the analysis of free methyl esters of phospholipid fatty acids (450-GC, 240-MS ion trap detector, Varian, Walnut Creek, CA, USA). The GC instrument was equipped with a split/splitless injector and a DB-5MS column (J&W Scientific, Folstom, CA, 60 m, 0.25 mm i.d., 0.25 μm film thickness) was used for separation.

The temperature programme started at 60 °C and was held for 1 min in splitless mode. Then the splitter was opened and the oven heated to 160 °C at a rate of 25 °C min-1. The second temperature ramp was up to 280 °C at a rate of 2.5 °C min-1, this temperature being maintained for 10 min. The solvent delay time was set to 8 min. The transfer line temperature was set to 280 °C. Mass spectra were recorded under electron impact at 70 eV, mass range 50–350 amu. Methylated fatty acids were identified according to their mass spectra and quantified using their individual chemical standards obtained from Sigma–Aldrich, Prague, Czech Republic and Matreya LLC, Pleasant Gap, PA, USA.

Fungal (eukaryotic) biomass was quantified based on 18:2ω6,9 content; bacterial biomass was quantified as the sum of i14:0, i15:0, a15:0, 15:0, i16:0, 16:1ω7, 16:1ω9, 16:1 ω5, 10Me-16:0, i17:0, a17:0, cy17:0, 17:0, 10Me-17:0, 18:1ω7, 18:1ω9, 10Me-18:0 and cy19:0. Biomass of Gram+ and Gram – bacteria was estimated using concentrations of i14:0, i15:0, a15:0, 15:0, i16:0 and 16:1ω7, 18:1ω7, 16:1 ω5, cy19:0 cy17:0, respectively (Oravecz et al. 2004; Šnajdr et al. 2008). The fungal-bacterial ratio was estimated as the sum of fungal PLFA divided by the sum of bacterial PLFA (Šnajdr et al. 2008).

Isolation of cultivable soil fungi

From each soil sample, one gram of soil was added to a plastic tube with 10 ml of sterile distilled water. The plastic tubes, each inoculated with a soil solution of each soil sample planted with a different energy crop, were diluted to $1^{-4}ml^{-1}$. Isolations of fungi were performed following the suspension-plating method (Chesters and Thornton 1956). Samples of the soil suspension were plated onto Petri dishes with soil extract agar and incubated at a constant temperature of 25 °C. Fungi were determined on the basis of micro- and macromorphological, physiological and biochemical features (Frankland et al. 1990).

Statistical analysis

Soil respiration, soil microbial biomass and cellulose decomposition, and results of PLFAs among different groups of soil microbes were compared by a one-way ANOVA followed by a Tukey-Kramer Multiple Comparison Test (ANOVA HSD post hoc test) in R (R 2005; Simecek and Simeckova 2013). The effects of energy crops on the composition of the soil fauna, PLFA assay and composition of the cultivable fungal community were visualized by a PCA analysis. The significance of the effect of native and introduced plants on the fauna, and microbial (PLFA) and microfungal community structure was tested by a redundancy analysis (RDA) and a Monte Carlo Permutation Test (with 499 permutation) where “native” or “introduced” was used as the sole explanatory variable in Canoco application (Leps and Hadincova 1992).

Results

Chemical composition of litter

The C:N ratio was differed significantly among litter collected from individual crops (ANOVA HSD post hoc test, $F_{5,15}$=33.388, p<0.0001) (Table 1). The highest C:N ratio was recorded in litter from *S. perfoliatum* and *P. arudinacea. Helianthus tuberosus, R. sachalinensis* and the cultural meadow exhibited an intermediate C:N ratio, and *S. viminialis* had the lowest C:N ratio. The content of phenols isolated from litter collected from individual plants significantly differed as well (ANOVA HSD post hoc test, $F_{5,15}$=47.873, p<0.0001) (Table 1). The highest content of phenols was found in litter collected from *R. sachalinensis*, while the other litter types showed a significantly lower content of phenols. Neither the C:N ratio nor the content of phenols revealed any clear distinction between native and introduced energy crops although some of the introduced crops contained more phenols and showed a higher C:N ratio.

Soil meso- and macrofauna

The soil fauna in all sites was dominated by collembolan species (Table 2). The density of all fauna groups combined was higher in sites planted with native energetic crops than those planted with introduced energetic crops. The highest number of soil animals was recorded in field sites planted with *S. viminialis* and *P. arudinaceae*, and in the cultural meadow. The least individuals of soil fauna was observed in field sites planted by *R. sachalinensis, S. perfoliatum* and *H. tuberosus* (Tab. 2).

The PCA ordination diagram (Fig. 1) shows that the field sites with native energy crops hosted a higher abundance and taxonomic diversity of soil organisms than field sites planted with introduced energy crops. Native crops were also more similar to the meadow than introduced crops. According to the RDA and Monte Carlo Permutation Test, this difference between native and introduced plants appears to be highly significant (p=0.002) and explains 28.6% of data variability.

Soil microbial activity

Significantly different basal soil respiration (HSD ANOVA post hoc test, $F_{5,15}$=8.701, p =0.001) was found in sites planted by various energy crops; however, there was no clear difference between native and introduced plants. The highest rate of microbial respiration was recorded in sites planted with *S. viminialis, S. perfoliatum* and *R. sachalinensis* (Fig. 2), while the lowest soil respiration was measured in sites planted with *H. tuberosus* and the cultural meadow. The highest microbial biomass was measured in the meadow. The lowest microbial biomass was recorded in sites planted with *H. tuberosum*, but the difference was not statistically significant (HSD ANOVA post hoc test, $F_{5,15}$=0.714, p =0.624) (Fig. 3). The highest rate of cellulose decomposition was recorded in the field site planted with *S. perfoliatum* and the meadow (Fig. 4), but differences among the sites were not significant (HSD ANOVA post hoc test, $F_{5,15}$=0.743, p =0.667) among the crops.

Composition of soil microbial community recorded by PLFA

The concentration of whole PLFA in soil samples did not show a clear pattern between native and introduced plants and not even among all plant species (Fig. 5). The highest concentration of PLFA was recorded in field sites planted with *P. arudinaceae*, but differences between sites were not statistically significant (HSD ANOVA post hoc test, $F_{5,15}$=0.719, p =0.621) (Table 3). PCA ordination (Fig. 6) based on the concentration of PLFA of specific taxonomical groups of microorganisms showed significant effects of native and introduced crops (RDA, Monte-Carlo Permutation Test, p=0.002 explaining 58.6% data variability).

Concentrations of fungal PLFA (Table 3) were not significantly different among treatments (HSD ANOVA post hoc test, $F_{5,15}$=0.719, p=0.621). Concentrations of bacterial PLFA (Tab. 2) were statistically significantly affected by different crops (HSD ANOVA post hoc test, $F_{5,15}$=4.28, p=0.01). The highest concentration of bacterial PLFA was measured for the site planted with *P. arudinaceae*. Sites planted with *R. sachalinensis*, *S. perfoliatum*, meadow species, *H. tuberosum* and *S. viminialis* showed significantly lower of bacterial PLFA.

The concentration of actinobacterial PLFA (Table 3) reached the significantly highest level in sites planted with *P. arudinaceae.* The other field sites showed lower actinobacterial

PLFA concentrations. Concentrations of G+bacterial PLFA (Table 3) were statistically significantly affected by different crops (HSD ANOVA post hoc test, $F_{5,15}$= 6.606, p = 0.004). Concentration of bacterial PLFA was highest on sites planted with *P. arudinaceae*.

Other sites (*R. sachalinensis*, *S. perfoliatum*, meadow species, *H. tuberosum* and *S. viminialis*) showed a statistically significantly lower concentration of G+bacterial PLFA. The highest G-bacterial biomass PLFAs were recorded in sites planted with *P. arudinaceae* whereas *R. sachalinensis*, *S. perfoliatum*, meadow species, *H. tuberosum* and *S. viminialis* showed a lower concentration of G-bacterial PLFA. The concentration of total microbial biomass PLFA as well as the F/B ratio did not differ significantly among the various energy crops (Table 3).

Cultivable soil microscopic fungi in soil planted with introduced energy crops

A total of 22 species of soil microscopic fungi were found at the sites investigated (Table 4). PCA based on the presence of fungal species (Fig. 7) indicated that different energy crops affected the presence of different species of soil fungi, but this difference was only marginally significant (RDA, Monte Carlo Permutation Test, p=0.07 explain 11.3% of data variability). Native energy crops showed a higher number of fungal strains than introduced energy crops. All sites were dominated by the genera *Cladosporium* and *Penicillium.*

Discussion

The results of our study revealed significant differences between native and introduced energy crops in the community structure of the soil fauna, the microbial community recorded by PLFA and marginally also in the cultivable fraction of the community of soil microscopic fungi. Introduced energy crops more reduced the abundance and number of groups of soil animals. The reduction in the density and diversity of the soil fauna observed in our study was similar to the influence of extensive crop production (Crossley Jr. et al. 1992; Edwards 1989; Chan 2001; Wardle et al. 1999b) or consequences of tillage when comparing tillage and non-tillage management (Reynolds et al. 2007), which has received a lot of attention in the literature (Blanchart et al. 2006; House and Parmelee 1985; Kladivko 2001; Marasas et al. 2001; Stinner et al. 1988). Similar differences between native and introduced crops have also been reported in the literature (Raghu et al. 2006; Yeates and Williams 2001). Gremmen et al. (1998) showed a statistically significant impact of the introduced grass *Agrostis stolonifera* on vegetation and soil fauna communities at Marion Island (sub-Antarctic) but a negative impact of *A. stolonifera* on the density of the soil fauna. Pritekel et al. (2006) recorded higher numbers of soil microarthropods in soil from non-invasive sites compared to soil from the invasive range in the Rocky Mountain National Park, USA.

The changes in the soil biota community observed in our study may have several reasons. The lower number of microarthropods in invasive plots could have resulted from the increase in bare ground between plants and lower plant cover found in plots withinvasive plants, causinga decrease in food availability (Eisenhauer and Reich 2012) In our study, plants reached a high cover in all treatments, but there was a substantial difference between native and introduced species, as introduced species did not produce much litter, so there was mostly bare soil between plants. Under native plants, by contrast, the soil was covered with litter. Bare soil in non-native energy crops in our case was likely caused by the fact that our species are tall herbs with limited litter fall between harvests, which may not apply to all introduced plant.

Impact of allelopathic compounds of invasive and introduced plants in the host environment has been discussed in the literature (Jefferson and Pennacchio 2003; Novoa et al. 2012; Sera 2012; Vrchotova and Sera 2008). Although allelopathy was not considered in this study, it could have contributed to the reduction of soil fauna as well. Some of the introduced energy crops showed a higher content of phenols, but there was no clear trend that would

differentiate between native and introduced energy crops. Plant polyphenols may contribute to plant defense against herbivores and pathogens (Tang et al. 1995; Zhang et al. 2009b) and, in some cases, may partly enhance differences caused by the structure of the litter layer (Bardgett et al. 1998; Wardle et al. 2006) described above and thus contribute to differences observed in this study.

Although the mechanisms of how introduced energetic plant influence the soil biota will probably become subject of future research, we expect that this effect is at least partly caused by a combination of litter chemistry (Bardgett et al. 1998) and the way that litter reaches the soil (Bardgett et al. 1999). Litter not only represents a food source but also a habitat for soil organisms (Kaneda et al. 2012; Kaneko and Salamanca 1999). It therefore strongly affect the formation of the soil biota community (Tian et al. 1992). If the litter input regime continues over longer periods, which is the case in perennial plants, litter substantially affects the conditions in the soil surface horizon (so-called humus forms), which affects the soil biota community and its functioning even further (Ponge 2013).

Our results show that microbial biomass based on PLFA differed significantly between native and introduced crops in some groups of the microbial community such as bacteria, G+ bacteria, G- bacteria and actinobacteria although there were no differences for total PLFA. Litter quality may be an important factor here, as mentioned earlier. Short rotation plants which produce easily decomposable litter promote bacterial-dominated food webs associated with fast cycling of nutrients, whereas slow-growing plants promote a fungal-dominated food web and slow cycling of nutrients (Coleman et al. 1983; Moore and Hunt 1988). Evolution change in genotype composition towards high C:N ratio genotypes may explain the invasive success of some introduced plant species in their invaded range (Eppinga et al. 2011). Eppinga et al. (2011) showed increased an C:N ratio in *Phalaris arudinace* in newly invaded habitats compared to its native range. This hypothesis is partly consistent with our results because we recorded the highest C:N ratio in plant biomass from *S. perfoliatum,* which is an introduced energy crop in the Czech Republic (Pysek et al. 2012b). Higher fungal biomass based on PLFA was recorded in plants with higher C:N ratios (*S. perfoliatium* and *P. arurinacea*), but our results were only marginally significant. This is in agreement with literature data, which associated higher fungal biomass with higher C:N ratios (Bardgett et al. 1998; Meier and Bowman 2008; Wardle et al. 2004; Yang and Chen 2009). We also found distinct differences in the composition of the cultivable fraction of the fungal

community between native and introduced energy crops, but our results were, again, only marginally significant. Similar changes have already been described in the literature, however (Culman et al. 2010; Liang et al. 2012; Wang et al. 2010). Changes in litter quality may combine with the amount of litter on the soil surface, as the litter layer also affects the habitat for the soil microflora. Moreover, in natural systems, litter with a high C:N ratio usually decomposes on soil surface (Ponge 2013).

Microbial respiration differed among crops, but no consistent difference was found between native and introduced crops. This fact may be explained by a complex combination of biotic and abiotic factors such as temperature and soil water content, chemical composition of litter and nutrition conditions in the soil, and production of root exudates (Bardgett 2005; Bardgett et al. 1998; Wardle et al. 2006). We did not record any statistically significant difference in cellulose decomposition in field sites planted with native and introduced energy crops. This may be caused by the fact that cellulose decomposition is a very complex factor. Decomposition is realized by a wide spectrum of microorganisms and affected by many environmental factors (Valaskova et al. 2007).

Conclusion

We found significant differences among individual plant species in several parameters. A significant difference between native species and exotic energetic crops indicates that introduced crops support a less diverse soil biota community than native ones. Our results, however, do not show any clear pattern in some of the parameters such as microbial respiration, microbial biomass and cellulose decomposition, which is possibly attributable to the high degree of redundancy in these parameters. More research is needed to explore the question whether changes in the soil community may contribute to the competitive advantage of exotic plants in local floras. Various exotic plants apparently take different strategies.

Acknowledgements

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Appendices

Table 1 Chemical analyses of litter collected from native and introduced energy crops. Cultural meadow was used as a control. The C:N ratio and content of phenols are expressed as means ± SD of three replicates (mg/g). Same letters indicate statistically homogenous groups ($p<0.05$).

	C:N ratio ±SD	Phenols ±SD
HEL	37.423 ±1.527a	2.526 ±0.281a
SIL	57.375 ±5.964b	2.220 ±1.397a
RE	35.633 ±1.875a	29.223 ±5.163b
PHA	41.474 ±5.650a	11.230 ±1.605c
SAV	21.256 ±0.890c	5.052 ±3.321ac
MEA	30.204 ±2.159a	9.251 ±1.329c

Table 2 Mean abundance of individuals of soil fauna components in different field sites planted with various energy crops (PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis*, SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis)* per m^2. Values show means ±SD of three replicates of each field site.

	Introduced energy crops						Native energy crops					
	HEL	±SD	SIL	±SD	RE	±SD	PHA	±SD	SAV	±SD	MEA	±SD
Collembola	812	±50	108	±21	2220	±490	175	±331	2811	±310	3212	±540
Other Acari	-	-	-	-	-	-	413	±91	-	-	600	±160
Oribatida	-	-	-	-	611	±166	612	±52	-	-	-	-
Diplura	-	-	-	-	-	-	-	-	-	-	200	±90
Symphylla	-	-	-	-	-	±50	-	-	100	±50	-	-
Enchytreidae	-	-	-	-	-	-	301	±50	-	-	-	-
Lumbricidae	-	-	-	-	-	-	300	±20	-	-	100	±50
Aranea	-	-	103	±50	-	-	-	-	-	-	100	±50
Diplopoda	-	-	205	±50	-	-	211	±50	100	±51	-	-
Chilopoda	224	±50	103	±50	-	-	1811	±46	1300	±260	1011	±291
Isopoda	-	-	102	±50	-	-	-		-	-	-	-
Diptera	-	-	202	±91	-		100	±111	-	-	-	
Coleoptera	210	±50	103	±53	205	±91	408	±50	100	±50	701	±120
Hymenoptera	-	-	100	±50	-	-	-	-	-	-	602	±161
Total	1246	±150	1026	±415	3036	±797	4331	±801	4411	±721	6526	±1462

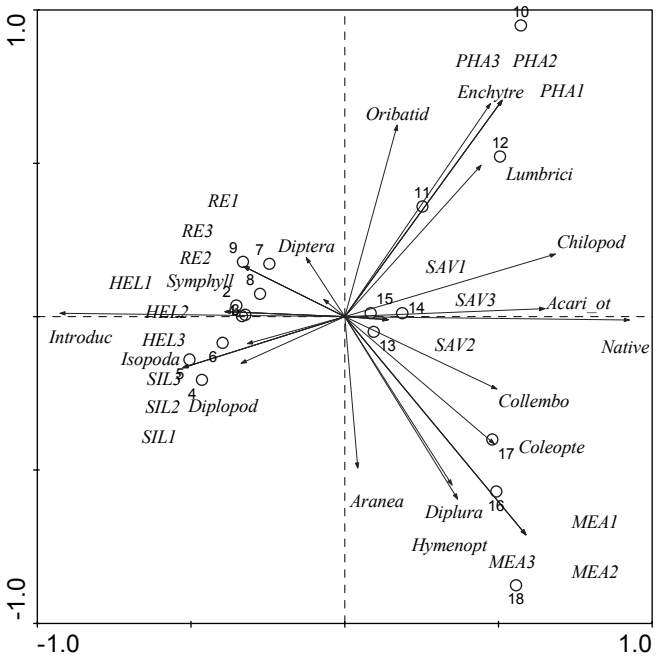

Fig. 1 Effect of energy crops on the composition of the soil fauna (PCA) based on abundance of different groups of soil animals. PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis*, SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis.* The first and second PCA axes explain 22.2% and 38.4% of data variability, respectively.

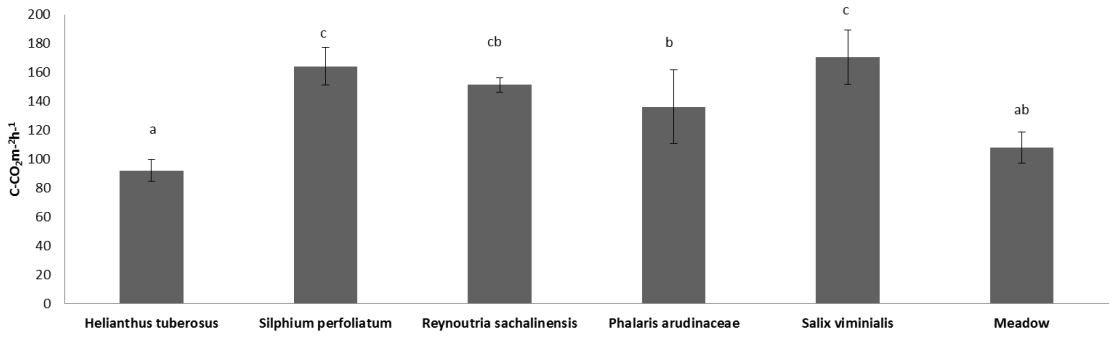

Fig. 2 Effect of energy crops on basal soil respiration. Error bars represent SD. Different letters indicate statistically homogenous groups ($p<0.05$). Tukey-Kramer Multiple Comparison Test (ANOVA HSD post hoc test, $F_{5,15}=8,701$, $p=0.001$).

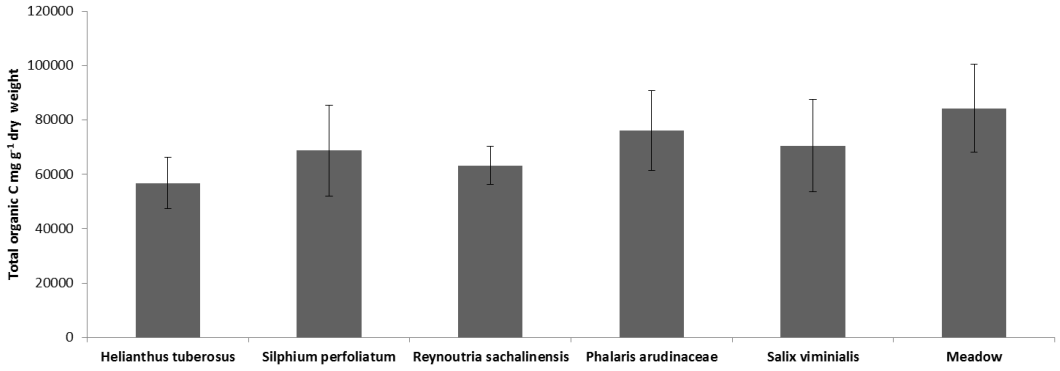

Fig. 3 Effect of energy crops on microbial biomass. Error bars represent SD. Tukey-Kramer Multiple Comparison Test (ANOVA HSD post hoc test, $F_{5,15}=0.714$, $p=0.624$).

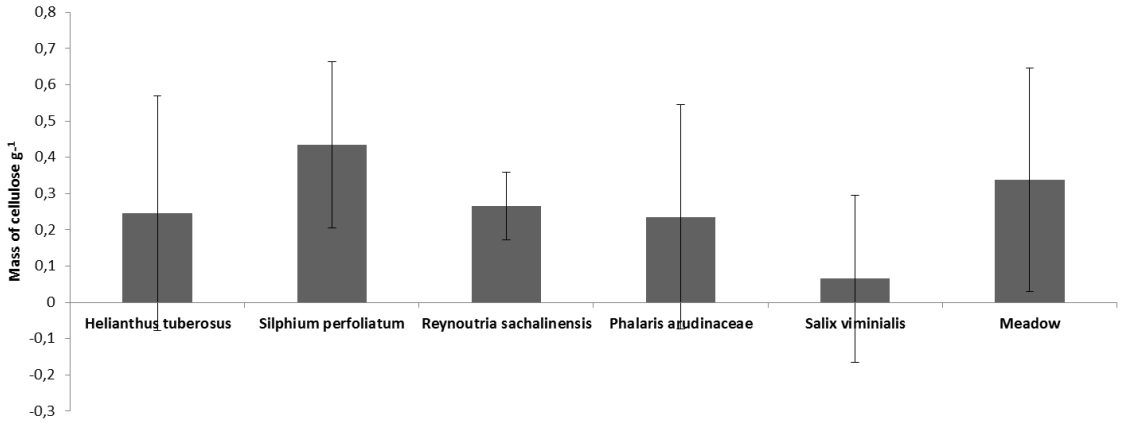

Fig. 4 Effect of energy crops on decomposition of cellulose. Error bars represent SD. Tukey-Kramer Multiple Comparison Test (ANOVA HSD post hoc test, $F_{5,15}$=0.743, p=0.667).

Table 3 Concentration of FAME (ng/g) in different field sites PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis*, SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis*. Values show means ±SD of three replicates of each field site.

FAME concentrations ng/g in soil samples

	Introduced energy crops						Native energy crops					
PLFA	**HEL**	**±SD**	**SIL**	**±SD**	**RE**	**±SD**	**PHA**	**±SD**	**SAV**	**±SD**	**MEA**	**±SD**
i14:0	9.05	±3.98	5.83	±7.21	6.02	±2.50	42.43	±28.87	2.68	±2.67	0.80	±1.73
i15:0	141.34	±31.87	132.67	±63.68	182.20	±87.68	583.94	±249.84	69.66	±56.96	91.09	±22.04
a15:0	138.03	±28.56	138.25	±60.32	141.02	±43.64	590.62	±192.57	73,36	±59.64	98.01	±26.85
15.0	14.86	±3.57	15.01	±7.16	16.05	±6.64	48.49	±33.38	7.94	±5.42	9.31	±4.31
i16:0	56.75	±12.91	58.03	±29.53	60.42	±23.06	207,74	±90.92	32.11	±23.57	37.88	±12.10
16:1w9	187.99	±38.33	238.49	±135.94	98.62	±53.69	562,07	±350.79	99.97	±82.76	125.75	±72.03
16:1w7	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00	0.00	±0.00
16:1w5	256.57	±48.73	325.01	±155.64	131.84	±82.20	1125.98	±795.02	187.54	±145.71	175.57	±94.37
16:0	658.98	±116.48	728.59	±171.82	689.04	±294.68	1701.08	±960.36	428.67	±260.48	522.73	±221.53
10Me-16:0	110.04	±17.72	115.90	±18.60	99.05	±27.35	289.89	±55.98	68.40	±42.10	126.05	±16.46
i17:0	47.33	±5.33	50.11	±13.17	45.71	±18.14	159.71	±57.12	34.49	±19.85	45.32	±11.76
a17:0	43.86	±6.31	47.68	±14.77	34.60	±11.81	136.25	±49.68	19.24	±6.92	41.61	±10.92
cy17:0	188.96	±33.95	158.31	±34.13	150.96	±56.99	396.30	±129.71	91.96	±80.09	147.17	±67.69
17:0	38.08	±4.88	40.01	±13.95	40.90	±2.93	90.92	±21.64	20.12	±11.40	34.07	±3.96
10Me-17:0	0.00	±0.00	24.30	±34.37	0.00	±0.00	25.20	±35.64	0.00	±0.00	0.00	±0.00
18:2w6,9	209.26	±156.63	360.20	±329.3	226.00	±242.51	827.70	±974.12	176.00	±148.44	96.78	±173.89
18:1w7	688,25	±232.65	886.29	±365.44	356.53	±190.41	1829.04	±947.42	418.25	±292.78	555.27	±312.18
10Me-18:0	89.77	±10.77	68.74	±40.39	43.69	±15.30	135.74	±53.07	38.44	±29.29	38.35	±27.55
cy19:0	197.18	±50.39	231.40	±19.54	204.04	±68.75	726.69	±148.80	127.98	±61.60	305.03	±49.99

FAME concentration of soil microbial biomass ng/g in soil samples

	Introduced energy crops						Native energy crops							
	HEL	±SD	SIL	±SD	RE	±SD	PHA	±SD	SAV	±SD	MEA	±SD	F value	p value
fungi	209.26	±156.63	337.21	±329.43	226.00	±242.51	827.70	±974.12	176.00	±148.44	96.78	±51.86	0.72	0.62
bacteria	2208.05	±369.52a	2315.84	±929.04a	1611.67	±670.75a	6951.01	±3111.68b	1292.15	±909.71a	1831.29	±746.21a	4.29	0.01
actinobacteria	199.81	±20.37a	172.83	±39.47a	142.74	±41.35a	450.83	±134.73b	106.85	±71.36a	164.40	±54.41a	6.15	0.004
G+	436.35	±79.55a	399.68	±188.21a	469.97	±184.68a	1720.70	±666.36b	231.54	±163.66a	314.71	±137.30a	6.61	0.004
G-	1330.96	±353.85a	1448.45	±555.18a	843.38	±392.10a	4078.01	±1907.41b	825.73	±579.41a	1183.04	±496.49a	3.78	0,03
total biomass	3076.29	±626.30	3290.39	±1402.50	2526.71	±1086.98	9479.80	±5018.23	1896.83	±1318.10	2450.80	±1082.52	4.78	0,89
F/B	0.08	±0.10	0.12	±0.17	0.12	±0.10	0.05	±0.01	0.09	±0.06	0.11	±0.04	5.32	0,98

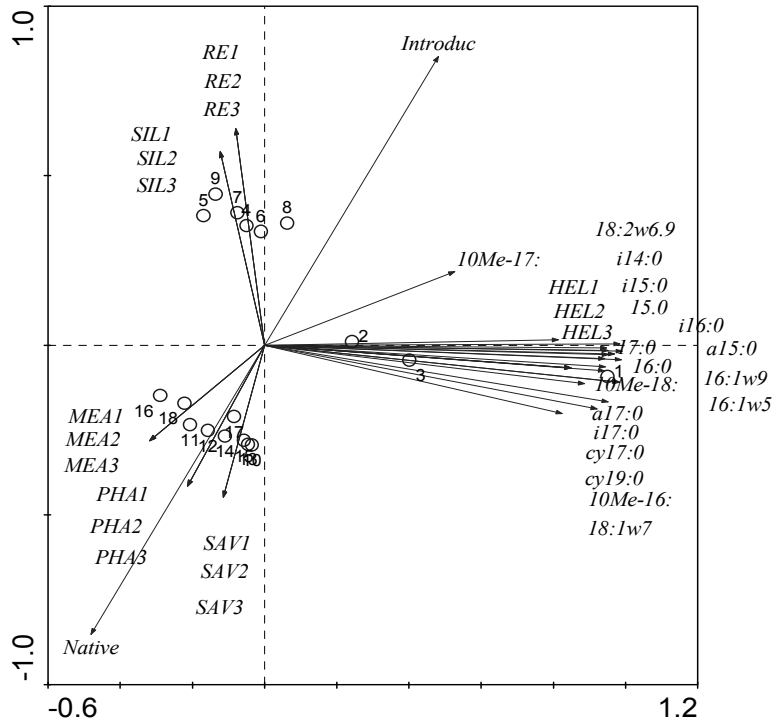

Fig. 5 PCA of PLFA assay based on FAME concentrations (ng/g) of soil samples. PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis*, SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis*. The first and second PCA axes explain 14.7% and 37.4% of the variability in the data, respectively.

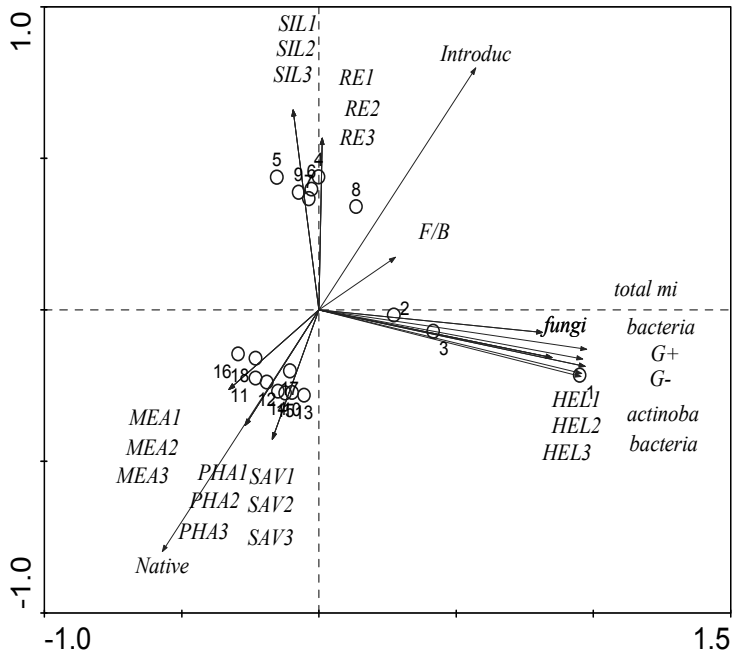

Fig. 6 PCA of PLFA assay based on PLFA concentrations of taxonomical groups of the soil microbial community (ng/g) of soil samples. PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis*, SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis.* The first and second PCA axes explain 87.5% and of 98.2% of the variability in the data, respectively.

Table 4 List of fungi isolated from all field sites (PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis*, SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis*.

Fungi	**Abbreviation**	**Field sites**
Absidia cylindrospora	Abs.cyl	SIL
Absidia glauca	Abs.gla	HEL, MEA
Cladosporium cladosporoides	Cla	PHA, HEL, MEA, SAV, SIL
Cladosporium herbarum	Cla.her	PHA, HEL, MEA
Clonostachys rosea	Clo.ros	RE
Fusarium cf. *dimerum*	Fus.dim	HEL
Gongronella butleri	Gon.but	MEA, SIL
Mucor hiemalis	Muc.hye	SIL
Oidiodendron sp	Oid.sp	SIL
Paecilomyces cf. *byssochlamydoides*	Pae.byss	PHA
Penicillium arenicola	Pen.are	SIL
Penicillium citrinum	Pen.citr	HEL
Penicillium crustaceum	Pen.cru	SAV
Penicillium commune	Pen.com	RE
Penicillium chrysogenum	Pen.chry	PHA, MEA, SAV
Penicillium griseofulvum	Pen.gri	SIL
Penicillium janczewskii	Pen.jan	PHA
Penicillium miczynskii	Pen.micz	MEA
Penicillium purpurogenum	Pen.pur	HEL, SIL, RE
Trichoderma harzianum	Tri.har	PHA, HEL, MEA
Trichoderma minutisporum	Tri.min	PHA
Trichoderma viride	Tri.vir	PHA, HEL, MEA

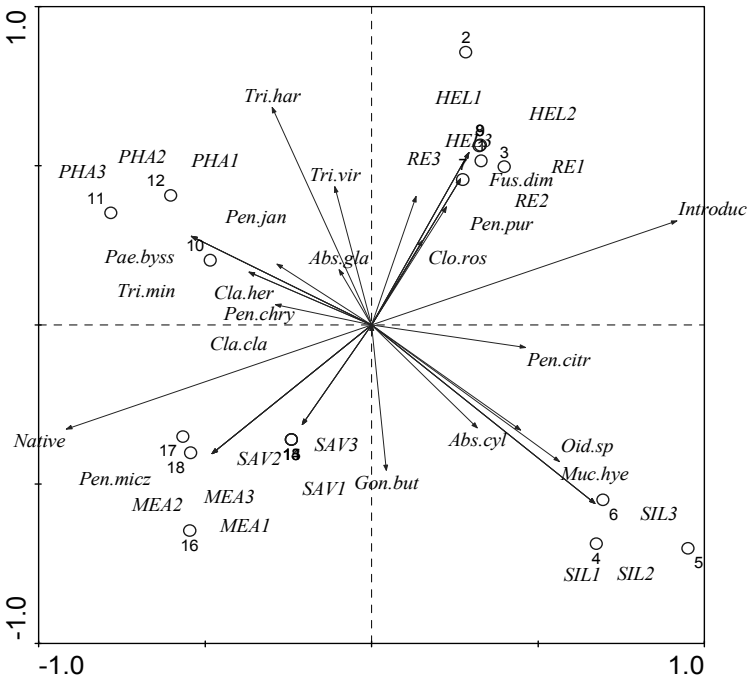

Fig. 7 PCA based on the presence of fungi species on Petri dishes enriched by soil samples. PHA-*Phalaris arudinaceae*, RE-*Reynoutria sachalinensis,* SIL-*Silphium perfoliatum*, MEA-Meadow, HEL-*Helianthus tuberosum*, SAV-*Salix viminialis*. The first and second PCA axes explain 19.3% and 36.3% of the variability in the data, respectively.

Chapter 5

Allelopathic effect of introduced energy crops on the soil biota: A comparative study

Submitted manuscript

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ABSTRACT

Energy crops as an alternative to fossil fuels are a component of the energy mix in many countries. Many of them are introduced plants, so they pose a serious threat of biological invasions. Production of allelopathic compounds can increase invasion success by limiting co-occurring species in the invaded environment (novel weapons hypothesis). In this study, we focused on plant chemistry and production of allelopathic compounds by energy crops (hybrid sorrel *Rumex tianschanicus* x *Rumex patientia* and miscanthus *Miscanthus sinensis*) in comparison with invasive knotweed (*Fallopia sachalinensis*). First, we tested the impact of leachates isolated from hybrid sorrel, miscanthus and knotweed compared to deionized water, used as a control, on seed germination of mustard (*Sinapis arvensis*) and wheat (*Triticum aestivum*) cultivated on sand and soil. Secondly, we studied the effect of leachates on the growth of soil fungal pathogens *Fusarium culmorum*, *Rhizoctonia solani*, *Sclerotonia solani* and *Cochliobolus sativus*. Finally, we tested the effect of litter of hybrid sorrel, miscanthus, knotweed and cultural meadow litter (as a control) mixed with soil on population growth of *Enchytraeus crypticus* and *Folsomia candida*. Miscanthus and knotweed litter had a higher C:N ratio than the control meadow and hybrid sorrel litter. Miscanthus and hybrid sorrel litter had a higher content of phenols than knotweed and cultural meadow litter. Leachates from hybrid sorrel, miscanthus and knotweed litter significantly decreased seed germination of wheat and mustard in both substrates. Soil fungal pathogens grew less vigorously on agar enriched by leachates from both energy crops than on agar enriched by knotweed and control leachates. Litter from hybrid sorrel, miscanthus and knotweed significantly altered (both ways) the population growth of the soil mesofauna.

Key words: Energy crops, Invasive plant species, Plant biomass chemistry, Seedling germination, Allelopathic effect, Soil fungal pathogens, Soil mesofauna

Introduction

Energy crops are plants cultivated for their fast production of biomass and used for heating, generation electricity or production of bioethanol (Brant et al. 2011; Buckeridge et al. 2012; Lewandowski et al. 2006). They have become part of the energetic mix in many countries despite their low economic competitiveness against fossil fuels (Brant et al. 2011; Cozier 2012; Don et al. 2012; Lewandowski et al. 2006). Extensive cultivation of energy crops can also cause competition over land with the need to produce food and forage, which may consequently compromise ecosystem services that soil provides (Costanza et al. 1997; Lavelle et al. 1997).

If energy crop plantations are well situated, designed and managed, they may reduce nutrient leaching, soil erosion and provide additional environmental services such as soil carbon sequestration, improved soil fertility or the removal of pollutants from contaminated soils (Anderson-Teixeira et al. 2013; Buckeridge et al. 2012; Smith et al. 2013a; Ust'ak and Vana 1998). But there are also risks. Besides competition over land with the need to produce food, the risk of biological invasions is among the most important threats posed by newly introduced energy crops (Buddenhagen et al. 2009; Raghu et al. 2006). The most widespread energy crop in the Czech Republic is oilseed rape, which is grown to produce biofuel or additives to bio-ethanol fuel. The most widespread newly introduced energy crops are hybrid sorrel (*R. tianschanicus x R. patientia*), miscanthus (*M. sinesnsis*) and hybrid poplar (*Populus nigra* x *Populus maximowiczii*) (Brant et al. 2011; Lewandowski et al. 2006; Ust'ak and Vana 1998).

Hybrid sorrel was bred from a hybrid of *R. tianschanicus* x *R. patientia* in the 1980s in the former Soviet Union (Ust'ak and Vana 1998). It is a fast growing. herbaceous perennial producing vast amounts of seeds and large volumes of biomass. Hybrid sorrel is an established energy crop in the Ukraine and other countries of the former Soviet Union. There are well known case studies of biological invasions focused on the negative effect on the host environment caused by other sorrel species, e.g. *Rumex obtusifolius* (Pysek et al. 2012a). Hybrids of the genus *Fallopia* are significant invaders, too (Bailey 2013). *Miscanthus sinensis* is native to eastern Asia(most of China, Japan, Taiwan and Korea) and has newly been introduced as an energy crop to the Czech Republic (Pysek et al. 2012a; Quinn et al. 2012). It is a perennial grass growing up to 0.8–2 m tall (modern cultivars up to 4 m), which forms

dense clumps from an underground rhizome. Miscanthus escapes from ornamental plantings and forms extensive growths in disturbed areas, displacing native vegetation (Quinn et al. 2012).

Some newly introduced energy crops have turned invasive and have had serious economic and environmental impacts by strongly suppressing biodiversity in natural habitats (Buddenhagen et al. 2009; Kalusova et al. 2013; Pysek et al. 2012a; Pysek et al. 2012b; Raghu et al. 2006; Seastedt and Pysek 2011; Zavaleta 2000). Plants produce certain chemical compounds to suppress co-occurring plant species. According to the novel weapons hyphothesis, invasive plants excrete substances which are new to invaded communities and therefore have a stronger allelopathic effect on native competitors and the microbial community (Callaway and Ridenour 2004; Inderjit et al. 2006). Knotweed (*F. sachalinensis*) is a well known invasive species with a strong allelopathic effect in the Central European region (Bailey 2013; Kappes et al. 2007; Murrell et al. 2011; Pysek et al. 2012a; Vrchotova and Sera 2008). The aim of this contribution is to explore the allelopathic effect of newly introduced energy crops on seed germination, the microbial community and the soil fauna. We also examine whether plant chemistry corresponds with the effect of allelopathy.

Material and methods

Plant biomass sampling and chemical analysis

We studied two introduced energy crops, miscanthus (*M. sinensis*) and hybrid sorrel (*R. tianschanicus* x *R, patientia*), and one invasive plant, knotweed (*F. sachalinensis*). Senescent plant biomass was harvested at the end of August at experimental field sites of the Crop Research Institute in Chomutov (50° 27′ 46" N, 13° 24′ 40" E, 7.86°C mean annual temperature and 550 mm of annual rainfall). For control we used senescent aboveground biomass collected in a cultural meadow (dominated by *Poa annua*, *Poa pratensis*, *Trifolium repens* and *Plantago major*) in the same location. Soil from the meadow was also used in a germination experiment. For chemical analysis, plant material was dried and homogenized into particles smaller than 0.2 mm. The content of carbon and nitrogen was determined using an EA 1108 elemental analyser (Carlo Erba Instruments). Total soluble phenols were extracted by methanol and determined spectrophotometrically using the Folin-Ciocalteu reagent (Singleton et al. 1999).

Design of seedlings experiment

Senescent aboveground plant material (*R. tianschanicus* x *R. patientia*, *M. sinensis* and *F. sachalinensis*) was dried and then leached into deionized water for 48 hour (10 g of dried biomass per 100mL of deionized water) (Mudrak and Frouz 2012), the pH of all leachate types was about 6.3. Two types of non-sterile substrates were used: sand and soil (distric chernozem, loam, pH 6.9, C content 4.2%). Both soil types were sieved through a sieve with a mesh size of 2 mm before use. Ten grams of both substrates were separately added to 6 cm diameter Petri dishes. Into each Petri dish, 25 seeds of wheat (*T. aestivum*) or mustard (*S. arvensis*) were sown. The seeds were obtained from the Crop Research Institute in Prague. The Petri dishes containing the substrate and seeds were kept in a climatic chamber at 21°C with 12 hours light and 12 hours dark (Mudrak and Frouz 2012) and watered by 6 mL of a leachate every second day. Each leachate treatment comprised four replicates. Distilled and

deionized water was used as a control. The number of seedlings in each Petri dish treated with a different leachate type was counted after 2 weeks.

Design of fungal cultivation and growth measurement

Four fungal strains (*Fusarium culmorum, Rhizoctonia solani, Sclerotonia solani* and *Cochliobolus sativus*) were obtained from the Department of Mycology of the Crop Research Institute in Prague. Malt extract agar (Frankland et al. 1990; Chesters and Thornton 1956) was prepared on Petri dishes and enriched by leachates isolated from senescent aboveground biomass (10g of dried plant biomass per 100 mL distilled and deionized water; pH adjusted at 6.3) of two energy crops (*R tianschanicus* x *R. patientia*, *M. sinensis*) and one invasive species (*F. sachalinensis*). Petri dishes with pure malt extract agar were used for control. Each Petri dish was inoculated by one of four fungal strains in one spot in the middle of the dish. Each plant extract agar and control had six replicates of each strain. After one week, we measured the diameter of the colony of each fungal strain (*F. culmorum, R. solani, S. solani* and *C. sativus*).

Design of microcosm experiment

Two soil mesofauna species, springtails *F. candida* and pot-worms *E. crypticus*, were used in a microcosm experiment. Soil animals (*F. candida* and *E. crypticus)* were obtained from a culture collection of the Institute of Soil Biology in České Budějovice. For this experiment, we used synchronized cultures of animals (Franchini and Ottaviani 2008; Tordoff et al. 2008)**.** The animals were kept in plastic pots 4 cm in diameter and 5 cm high. To the each pot, 20 g of deep-frozen soil (-70°C, distric chernozem) was added to kill all other soil fauna supplemented with 10 g of autochtonous senescent plant biomass (*R. tianschanicus* x *R. patientia*, *M. sinensis*, *F. sachalinensis*) separately as above mentioned. As a control, we used a mixture of autochtonous plant biomass collected from a cultural meadow of the Crop Research institute in Chomutov. Into each microcosm with the given litter type, we placed 10 individuals of *F. candida* or *E. crypticus.* Each litter and fauna combination had four

replicates. All microcosms were placed into a dark climatic chamber at 21°C. After 30 days, we fixed the microcosms with 70% ethanol and counted all animals.

Statistical analysis

Differences between individual treatments in all collected data were analysed by a one-way ANOVA followed by a Tukey´s-Kramer Multiple comparison test (ANOVA HSD post hoc test) in R (R 2005; Simecek and Simeckova 2013).

Results

Chemical analysis of litter

Miscanthus (*M. sinensis*) exhibited the highest C:N ratio whereas hybrid sorrel (*R. tianschanicus* x *R. patientia*) showed the lowest. Knotweed (*F. sachalinensis*) and the control (cultural meadow) showed a similar C:N ratio. These differences were statistically significant (ANOVA HSD post hoc test, $F=68.260$, $p<0.0001$) (Table 1). The content of phenols differed significantly among the various kinds of plant biomass (ANOVA HSD post hoc test, $F=25.361$, $p=0.0002$). Miscanthus (*M. sisensis*) had the highest content of phenols followed by hybrid sorrel (*R. tianschanicus* x *R. patientia*),while knotweed (*F. sachalinensis*) had an intermediate phenol content, the lowest being in the control (cultural meadow) (Table 1).

Impact of leachate of energy crops on seed germination

Germination of wheat (*T. aestivum*) seeds was most suppressed by leachates isolated from hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*) and knotweed (*F. sachalinensis*) (Fig. 1) on the sand substrate. On the soil substrate, a strongly negative effect on germination was recorded in knotweed (Fig. 1). This negative effect was recorded for leachates extracted from both energy crops (hybrid sorrel and miscanthus; Fig. 1). Germination of mustard (*S. arvensis*) seeds was suppressed most by all three types of leachates (hybrid sorrel, miscanthus and knotweed) on the soil substrate (Fig. 2). On the sand substrate, hybrid sorrel and knotweed significantly decreased germination, but miscanthus did not show any significant effect on seed germination (Fig. 2).

Impact of leachates of energy crops on the growth of certain strains of pathogenic fungi

Our results show that three out of four strains of fungal pathogens were sensitive to the different leachates isolated from the energy crops (*R. tianschanicus* x *R. patientia, M. siensis*) as well as the invasive species (*F. sachalinensis*). The growth of *F. culmorum* was strongly suppressed by the extract of miscanthus (*M. sinensis*), but the extract isolated from hybrid

sorrel (*R. tianschanicus* x *R. patientia*) and knotweed (*F. sachalinensis*) did not show any significant difference (Table 2). Growth of *R. solani* was strongly suppressed by the leachate extracted from hybrid sorrel, but the other leachates did not show any suppressive effect on the growth of *R. solani*. The leachate of hybrid sorrel as well as the control malt extract showed a strong inhibitory effect on the growth of *C. sativus*, while leachates isolated from miscanthus and knotweed did not show any significant impact on the growth of *C. sativus* colonies (Table 2). We found that *S. solani* did not respond by inhibited growth to different leachates of any of the plants included in the study (Table 2).

Impact of different litter types of energy crops on population of soil fauna

We found a significant effect of different types of litter on population growth and size of both mesofauna species *F. candida* and *E. crypticus* (Fig. 3–4). *Enchytraeus crypticus* was more sensitive to knotweed and meadow litter than litter of hybrid sorrel and miscanthus. Miscanthus showed the most positive effect on population growth and development of *E. crypticus* (Fig. 3.). We found that population density of *F. candida* was most suppressed by knotweed and miscanthus litter whereas hybrid sorrel and cultural meadow litter allowed significantly higher population growth of *F. candida* (Fig. 4).

Discussion

Both the energy crops as well as the invasive plant tested had a significant allelopathic effect on the other plant species. A similar phytotoxic effect has repeatedly been found for plants growing in agricultural ecosystems (Muller 1982; Weidenhamer et al. 1989; Weih et al. 2008; Williamson and Richardson 1988). Moreover, certain invasive plants producing allelopathic compounds are known to strongly reduce the density of native plants (Inderjit et al. 2006; Kalusova et al. 2013; Novoa et al. 2012; Pritekel et al. 2006; Pysek et al. 2012b; Ridenour and Callaway 2001; Zhang et al. 2009a). Our results show that both wheat and mustard differ in their sensitivity to different leachates in the substrate. We used seeds from plants growing in an agricultural field, which could be more sensitive to allelopathic compounds than seeds from plants growing in a natural ecosystem (Weih et al. 2008). This may be caused by monocropping cultivation, which can strongly reduce interspecific competition of agricultural crops. Petri dishes with soil substrates usually showed a stronger reduction of seed germination than sand substrates. This may be caused by higher water retention of soil compared to sand (An et al. 2002). The reduction in the number of seedlings may have been caused by different chemical compounds produced by various plant species (Williamson and Richardson 1988), as mentioned previously, although substrate type may play a significant role in the effect of allelopathy (Tang et al. 1995). Many exotic species tend to produce allelopathic compounds (Inderjit et al. 2006; Mangla et al. 2008; Ridenour and Callaway 2001), but the relationship between native and introduced species remains unclear.

In the present study, we used only leachates, but direct contact with litter can cause a stronger allelopathic effect during continual leaching than the mere presence of leachates from plants (Mudrak and Frouz 2012). We used a semi-sterilized substrate, which may including fungal and bacterial strains that can decompose allelopathic substances and thus provide facilitation for co-occurring plants (Arunachalam et al. 2003; Kaur et al. 2009; Willis 2000). The success of an alien plant species also depends on other factors such as plant height, growth strategy, propagule pressure, residence time, growth rate, plant-pathogen interaction, plant-herbivore interaction, plant-pollinator interaction etc. (Eppinga et al. 2006; Kalusova et al. 2013; Mitchell et al. 2010; Murrell et al. 2011; Pysek et al. 2012a; Pysek et al. 2012b; Seastedt and Pysek 2011).

Miscanthus sinensis showed a higher content of phenols than our invasive plant (*F. sachalinensis*), hybrid sorrel (*R. tianschanicus* x *R. patientia*) and cultural meadow biomass. This fact may contribute to the allelopathic effect and, consequently, also to the invasive success of this energy crop (Callaway and Ridenour 2004). Production of polyphenols is caused mainly by stress factors such as lack of nutrients in soil, herbivores or pathogens (Tang et al. 1995). Callaway and Ridenour (2004) found increased production of allelopathic compounds in invaded ranges compared to native ranges. Although the effect of allelopathic compounds such as polyphenols on invasion success is well documented (Inderjit et al. 2006; Inderjit and Weiner 2001), they are clearly not the only factor causing the allelopathic effect.

The highest C:N ratio was found in *M. sinensis* and *F. sachalinensis* whereas control sites and hybrid sorrel *R. tianschanicus* x *R. patientia* showed a lower C:N ratio. This fact may be explained both by fertility of the soil and evolutionary traits in the genome related to C:N content (Bardgett 2005; Eppinga et al. 2011; Eppinga and Molofsky 2013; Wardle et al. 2004). Miscanthus, hybrid sorrel and knotweed showed a higher content of phenolic compounds than plant material collected from a cultural meadow. High nutrient input contributes to the allocation of assimilates towards rapid growth and production of easily decomposable litter (Bardgett 2005; Wardle et al. 2004). By contrast, slow-growing plants that dominate in soil with low nutrient availability allocate less assimilates to their growth, producing nutrient-poor litter that contains heavily decomposable compounds such as lignin and phenolics (Hussain et al. 2011; Tang et al. 1995). This is inconsistent with our results because short rotation plants contained more phenolic compounds and lignin, whose decay requires specialized decomposers such as lignolytic fungi (Valaskova et al. 2007). This fact may be explained by evolutionary genotype changes. Evolutionary trends in exotic plant species introduced to new areas contribute to their invasion success due to a shift towards genotypes thriving in nutrient-rich soils with a high C:N ratio in the invaded range (Eppinga et al. 2011; Eppinga and Molofsky 2013).

We found that some introduced or invasive plants may reduce the growth rate of certain fungal pathogens. Complex allelopathic compounds produced by plants may strongly affect the growth and development of fungal strains in the soil (Becker et al. 1997). We studied four strains of common fungal plant and soil pathogens occurring in Central Europe. According the novel weapons hypothesis (Callaway and Ridenour 2004), local pathogens are

strongly suppressed by chemical compounds excreted by newly introduced invasive plants (Inderjit et al. 2006). Our results support this hypothesis because our introduced and invasive plants showed a significant effect on colony growth almost in all cases.

Consistently with our results, invasive plants are known to produce a wide range of chemical compounds that can affect soil pathogens (Jefferson and Pennacchio 2003; Zhang et al. 2009a; Zhang et al. 2011). Zhang et al (2011), for example, found that allelopathic compounds produced by the invasive plant *Solidago canadensis* strongly decreased the activity of the fungal soil and plant pathogen *Pythium ultimum*. The impact on the whole fungal community is little known, however. Secondary metabolites in the soil are often influenced by abiotic factors (e.g., soil texture, soil pH, soil moisture, plant litter and soil organic matter) (Blanco 2007; Inderjit et al. 2006; Inderjit and Weiner 2001; Meiners and Kong 2012) and biotic factors (e.g. activity of the soil microbial community and soil fauna) (Arunachalam et al. 2003; Glinwood et al. 2011; Kaur et al. 2009; Palmer et al. 2004).

Secondary metabolites produced by a wide range of native as well as introduced plants may be decomposed by certain fungal strains. Soil microorganisms are responsible for half of the degradation of secondary metabolites such as m-tyrosine, catechin, ferulic acid, juglone and some flavones in soil (Arunachalam et al. 2003; Kaur et al. 2009; Willis 2000). We recorded a positive effect of leachates from *F. sachalinensis* and *M. sinensis* present in agar on certain soil and plant fungal pathogens. These results correspond with other studies showing that invasive plants can accumulate local generalist pathogens that have a more negative effect on native plant species than the invasive species themselves, thus potentially resulting in exclusion of native plant species (Eppinga et al. 2006; Mangla et al. 2008; Seastedt and Pysek 2011).

We found a negative effect of knotweed on the abundance of *E. crypticus*. Miscantus and hybrid sorrel, on the other hand, support higher population growth than control meadow biomass. The abundance of *F. candida* was most reduced in the microcosm containing litter from knotweed and miscanthus. Very important is direct contact with litter, as mentioned above (Mudrak and Frouz 2012). Light decomposable litter supports growth of the microbial community, which might be required by the soil fauna or at least support its development and reproduction (Heděnec et al. 2013; Kaneda and Kaneko 2002). The diversity of plant biomass

is an important factor affecting population density and diversity of the soil fauna. Kaneko and Salamanca (Kaneko and Salamanca 1999) showed significant greater diversity of soil microarthropods in three litter mixtures than in litter monocultures. This is partly consistent with our results although our results suggest that the population of *F. candida* significantly increased in the control microcosms with mixed litter, more so than in the microcosm with *M. sinensis* and *F. sachalinensis*.

We found a strong effect of knotweed on population growth of the soil fauna. It is known that the invasion of knotweed profoundly alters ecosystem structure and functioning, with negative effects cascading up through the food chain (Kappes et al. 2007). This may explain the reduced abundance of certain species of the soil mesofauna, mainly soil fungivores, but our study gave us the opportunity to study a microcosm with one species only. In general, long-term cultivation of introduced plants may affect other soil organisms depending on soil microbes in the food web (Bardgett and Walker 2004; Barrios 2007; Blanco-Canqui 2010). Pritekel et al. (2006) showed that the invasive plants *Euphorbia esula* and *Cirsium arvense* strongly reduce the density of soil micro-arthropods in mountain meadows. Declining microarthropod density may affect the density of the soil macrofauna, which depends on microarthropods in the food web (Barrios 2007; Kappes et al. 2007).

Conclusions

Our experiments show that both tested energy crops as well as the tested invasive plant have a strong allelopathic effect on a wide spectrum of organisms. This indicates that these plants may potentially alter ecosystem functioning in places where they are introduced or which they invade, which may substantially increase their invasive potential. Still, more research is needed to explore what changes in the soil community may contribute to the competitive advantage of exotic plant species and what strategies different exotic plants take to establish in local floras.

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Appendices

Table 1 Chemical analysis of litter collected from two energy crops and invasive plant species. Cultural meadow biomass was used as a control. The C:N ratio and content of phenols is the mean of three replicates (mg/g) ± SD. Same letters indicate statistically homogenous groups ($p<0.05$). Hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*), knotweed (*F. sachalinensis*), control (Cultural meadow).

Treatment	C:N ratio ± SD	Polyphenols ± SD
Hybrid sorrel	16.3 ± 1.4a	39.3 ± 4.2a
Miscanthus	57.1 ± 6.3b	44.9 ± 8.4a
Knotweed	35.6 ± 1.9c	29.2 ± 5.2b
Control	30.2 ± 2.2c	9.2 ± 1.3c

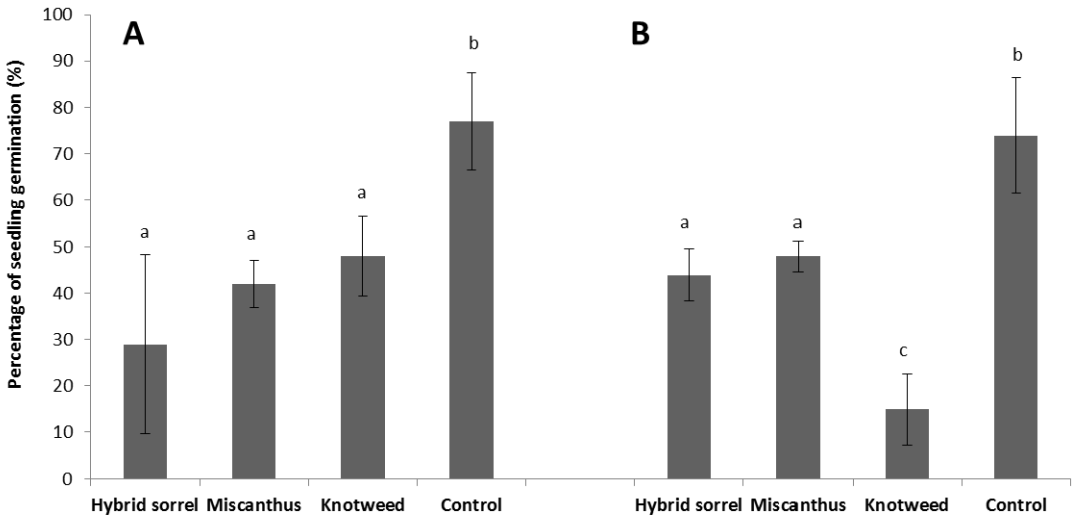

Fig.1 Impact of energy crop leachates on germination of wheat (*Triticum* spp.) seedlings on sand (A) and soil (B) substrates. Error bars represent SD. Same letters indicate statistically homogenous groups ($p<0.05$). Tukey´s test (post hoc HSD ANOVA, $F=11.169$, $p=0.0009$). Hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*), knotweed (*F. sachalinensis*), control (deionized water).

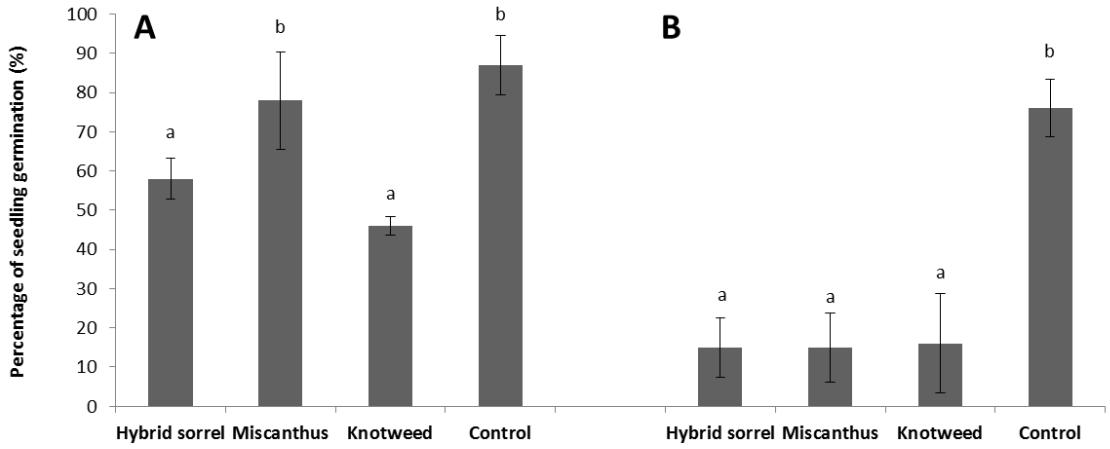

Fig. 2 Impact of energy crop leachates on germination of mustard (*S. arvensis*) seedlings on sand (A) and soil (B) substrates. Error bars represent SD. Same letters indicate statistically homogenous groups ($p<0.05$). Tukey´s test (post hoc HSD ANOVA, $F=22.792$, $p<0.0001$). Hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*), knotweed (*F. sachalinensis*), control (Deionized water).

Table 2 Impact of energy crop leachates on growth of several strains of soil fungal pathogens (*F. culmorum*, *R. solani*, *S. solani* and *C. sativus*). Values represent the diameter of fungal colonies on Petri dishes. The ± symbol indicates SD. Same letters indicate statistically homogenous groups. Hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*), Knotweed (*F. sachalinensis*), Control (Pure malt extract).

Plant extract	*Fusarium culmorum* ± SD	*Rhizoctonia solani* ± SD	*Sclerotonia solani* ± SD	*Cochliobolus sativus* ± SD
Hybrid sorrel	8.50 ± 0.0a	4.47 ± 1.3a	7.08 ± 3.4	3.12 ± 1.4a
Miscanthus	7.70 ± 0.4b	8.50 ± 0.0b	8.5 ± 0.0	7.17 ± 0.5b
Knotweed	8.50 ± 0.0a	7.17 ± 2.0b	8.5 ± 0.0	7.52 ± 1.3b
Control	8.50 ± 0.0a	8.50 ± 0.0b	7.75 ± 1.8	5.22 ± 1.7a
Tukey´s test				
F-value	22.326	14.286	0.724	14.604
***P*-value**	<0.0001	<0.0001	0.549	<0.0001

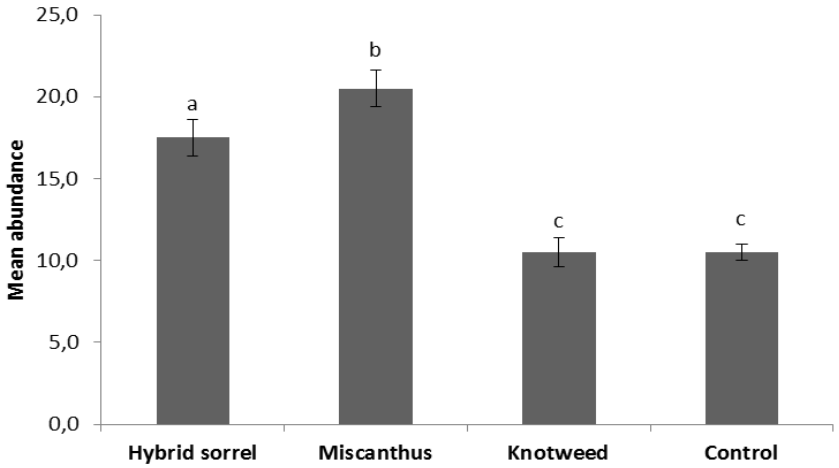

Fig. 3 Impact of different litter of energy crops on the population of *Enchytraeus crypticus.* Error bars represents SD. Same letters indicate statistically homogenous groups. Tukey´s test (post hoc HSD ANOVA, F=87.714, p<0.0001). Hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*), knotweed (*F. sachalinensis*), control (cultural meadow).

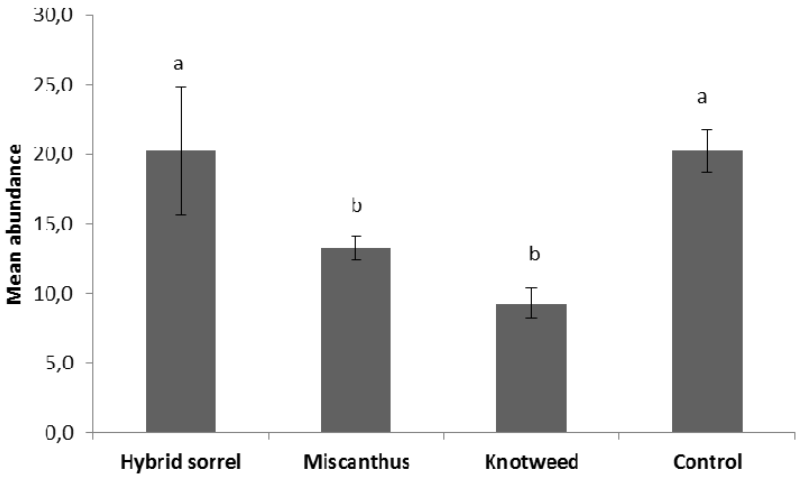

Fig. 4 Impact of different litter types of energy crops on the population of *Folsomia candida*. Error bars represent SD. Same letters indicate statistically homogenous groups. Tukey´s test (post hoc HSD ANOVA, F=14.099, p=0.0003). Hybrid sorrel (*R. tianschanicus* x *R. patientia*), miscanthus (*M. sinensis*), knotweed (*F. sachalinensis*), control (cultural meadow).

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