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Effects of woody plants and their residues on crop yield, weeds and soil carbon fractions in selected arable cropping systems

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List of Acronyms

BC	bird cherry
CR	crop rotation
DMY	dry matter yield
EFA	ecological focus area
FD	freeze dried
GW	goat willow
HHV	higher heating values
HV	heating value
OD	oven dried
OR	organic farming
POM	particulate organic matter
RE	renewable energy
SOC	soil organic carbon
SRC	short rotation coppice
UD	undried
WCM	wood chip mulching
WWR	wood to water mixing ratio

1 Introduction

1.1 Definition: woody plants

Woody plants are defined as "plants that produce secondary growth in the form of wood" (Bovey, 2001). "Woody plants" is the counterpart to the concept of "**herbaceous plants**". Woody plants usually have several growth forms such as trees, shrubs, and vines; and they have a high diversity on a global scale: among the total 39,313 species of vascular plants, 45–48% are considered as woody plants which is about 17,690–18,870 species (FitzJohn et al., 2014). Woody plants can appear in almost all terrestrial biomes and environments. Woody plants are always coexisting with herbaceous plants, and globally mixed woody-herbaceous systems were estimated to represent a substantial portion (15–35%) of the terrestrial biosphere (House et al., 2003). They are an essential component for the global ecosystem, and they are an important source for timber, fuel, fiber, food and fodder. The resource potential for woody plants is high, especially their energy potential. In the year 2010, the share of woody biomass was about 10% of the total global energy consumption (Edenhofer et al., 2011). In future decades (2050), its potential share of total global energy consumption could increase to 18% (Lauri et al., 2014).

Generally, there are many differences in ecology between woody and herbaceous plants. For example, woody plants are **perennial** plants, while herbaceous plants can be either annual, biennial or perennial. The stems of woody plants do not contribute much to carbon assimilation, and formation of lignified structures is highly energy-consuming (De Vries et al., 1974). Thus, woody plants are usually considered to have relatively slow growth rates compared to herbaceous plants. Generally, the rooting depth of woody plants is deeper than that of herbaceous plants (Canadell et al., 1996). Woody plants can transfer and store nutrients in stems or roots in autumn and reuse them in spring, and can also recycle nutrients to the soil surface by defoliation and subsequent root uptake. In addition, almost all woody plants use the **C_3** pathway of photosynthesis while herbaceous plants can use either C_3 or C_4 pathways (Pallardy, 2010). All the differences between these two life forms of plants (woody or herbaceous) make them performing different impacts in the same ecosystems (House et al., 2003).

1.2 Woody plants on arable land

Most crops on farmland are herbaceous plants, while some areas – often specific to the region – are grown with woody plants for example fruit trees in orchards, wind break trees or hedgerows, Christmas tree plantations, or bioenergy crops. To describe the combination of herbaceous and woody plants (artificial or semi-natural) on arable land, the concept of **agroforestry** was developed and promoted since the 1980s (El Din, 1980; Nair, 1985). It refers to both land-use systems and their supporting technologies, in which woody perennials (e.g. trees and bamboos) are consciously used together with the crops and/or animals, in a time sequence or in the same agricultural land (FAO, 2017). Agroforestry is a system based on ecological principles, which can manage natural resource efficiently, can sustain and diversify the production, and can serve more social, economic and environmental values for farmers, particularly for smallholder farmers (FAO, 2017).

In Europe, agroforestry systems exist for centuries, for example wood pastures and the *Dehesas* system in Spain (Nerlich et al., 2013). The traditional agroforestry systems in Europe can be differentiated in hedgerows, silvopasture (combination of woody plants and pasture), silvoarable (combination of woody plants with annual crops), and homegardens (Mosquera-Losada et al., 2016). Traditionally, woody plants were simply grown as fruit trees, as border for fields to keep out the wild animals, or as shelter from sun for the livestock. Woody plants can also be supplements for the production of many agricultural products. For instance, in the Mediterranean area, pasture productivity is restricted because of drought in summer. Hence, using woody materials produced from trees (e.g. branches) becomes a necessary and cheapest way to feed animals during the time of gaps in pasture production (Rigueiro-Rodríguez et al., 2009).

There are currently many examples of agroforestry systems all around the world. In the US, silvopastoral systems and alley cropping are the two most widely spread agroforestry systems (USDA, 2017). Silvopastoral systems contain both forestry and grazing, and can produce both forest products and livestock products in the same system. Alley cropping is an intercropping of tree rows with wide spacing and other annual or perennial crops in between the tree rows. In West and Central Africa, cacao (*Theobroma cacao*) as the most important cash crop, is planted under the shade of secondary forest with fruit trees and timber species (Duguma et al., 2001). Globally, the agroforestry is estimated to cover 1,023 million ha of area (Ramachandran Nair et al., 2009). Among these area, the US has about 235 million ha of agroforestry in total (Nair and Nair, 2003), and the EU has about 65 million ha of silvoarable agroforestry (Reisner et al., 2007).

Hedgerows are traditional landscape structures based on small trees and bushes in temperate regions, such as Western Europe, and can be considered as an agroforestry system in general. They include belts of woody plants that separate fields and also the associated organisms in the belts (Forman and Baudry, 1984). Like the other agroforestry systems, hedgerows are beneficial in several ways such as they can increase biodiversity (Baudry et al., 2000), can improve microclimates including wind speed and evaporation (Forman and Baudry, 1984), and can also attract beneficial insects and pollinators and enhance natural pest control (Morandin et al., 2016).

Short rotation coppice (SRC) is another common agroforestry system for fulfilling the large demand of bioenergy feedstocks nowadays. It is relatively new as it uses arable land for the cultivation of non-food crops and relies strongly on heavy machinery for cutting. Over the past 25 years, SRC established in Brazil, New Zealand, and Australia equals to about 50,0 km^2 area; in China, the area of SRC comprises all in all 70,000–100,000 km^2 (Wright, 2006). The most often used tree species for SRC worldwide is the Eucalyptus (*Eucalyptus* spp., 38% of all SRCs; FAO, 2003), and in temperate regions, the predominate tree species are willow (*Salix* spp.), poplar (*Populus* spp.), and black locust (*Robinia pseudoacacia*) which have fast growth rate and good cold hardiness (Weih, 2004). Besides of energy use, SRC is also considered as a potential solution for phytoremediation of contaminated soil and groundwater, because of its fast growth, adaptability to different environments and tolerance to unfavorable soil conditions (Rockwood et al., 2004).

1.3 Ecosystem services of woody plants on arable land

In Europe, about 80% of the arable land is threatened by potential environmental risks for lack of landscape diversity, soil erosion, and/or nitrate leaching (Reisner et al., 2007). Integration of woody plants or agroforestry, respectively, into arable land, could be an option to reduce environmental challenges.

Agroforestry has important functions in sustaining **biological diversity** in various climate and environmental conditions (Jose, 2009). Taking the tropical regions as an example, the agroforestry was found to preserve the biodiversity by: (i) reducing deforestation as it produces both crop and forest products, (ii) providing habitat and resources for forest-dependent species which cannot survive in simple agricultural systems, and (iii) providing greater conservation to the natural vegetation remnants inside it compared to crop land (Schroth, 2004). Similarly, in the temperate regions, agroforestry was also reported to increase insect diversity as well as reduce pest problems by providing greater diversity and complexity in time and in space (Stamps and Linit, 1997). In all, agroforestry can substitute traditional farming systems and at the same time maintain a promising yield and sustainability to the natural environments.

Soil erosion becomes a global and severe environmental problem in the terrestrial ecosystems, particularly for agricultural land which is losing soil by rain and wind at high rates (13–40 Mg ha^{-1} $year^{-1}$; Pimentel and Kounang, 1998). Plant cover on the soil is especially important for erosion control. For instance, woody plants can reduce energy from water droplets, capture the rainfall, increase water infiltration, and fix the soil by roots and litter (Zuazo and Pleguezuelo, 2008). In these ways, soil can be protected by the woody plant cover from erosion. In temperate regions, alley cropping systems with short rotation coppice (SRC) were considered to be able to reduce up to 70% of the soil erosion (Tsonkova et al., 2012). With both, bio-protection and bio-construction on soil from the woody vegetation on arable land, the risk for soil erosion probably can be reduced.

Nitrate leaching and **water quality** problems are to a certain extent related to agronomic practices and soil erosion, and are also problematic for today's global environment. Woody plants on arable land are considered to be able to maintain the nutrient status in the crop rooting zone by (i) biological N_2 fixation with N-fixing species (Dommergues, 1995), (ii) reducing erosion and leaching (Nair et al., 1995), and (iii) taking up nutrients from deep soil with the deep rooting system and bringing them back to the topsoil via pruning and litterfall (Beer, 1988). Cultivating woody plants on arable land can be a solution to nutrient losses and water pollution problems. In the tropics, compared to mono-cropping of annual crops with shallow roots, agroforestry with trees and annual crops can enhance nutrient uptake from varying soil depths by both shallow and deep roots, and thus reduce nutrient (especially nitrogen) losses (Hauser and Kang, 1993). In a Mediterranean agroforestry system where chickpea (*Cicer arietinum* L.) was grown in the alleys of walnut trees (*Juglans nigra* x *Juglans regia NG23*), even for the legume crop the environmental conditions and the mineral N availability were improved by the agroforestry (Mahieu et al., 2016). In temperate regions, similar effect of nutrient uptake and water quality purification are also documented for riparian forest buffer zones (Peterjohn and Correll, 1984). Agroforestry of both alley cropping and silvopastoral was found to have the potential to reduce nitrogen loss which might happen in conventional farming in the same site (Nair and Graetz, 2004).

1.4 Organic matter and carbon sequestration in soil under woody plants

Woody vegetation is considered an important contributor to the carbon sink (Churkina et al., 2010). In the background of global warming, increasing air temperature was estimated to have significantly greater stimulation on the biomass response of woody plants (+26.7%) than that of herbaceous plants (+5.2%) (Lin et al., 2010). Additionally, elevated CO_2 level would also increase both the total biomass and net CO_2 assimilation of woody plants (Curtis and Wang, 1998).

Generally, agroforestry is regarded to have **carbon (C) sequestration** potentials by storing C in trees and in soil. The amount of C sequestered in different agroforestry systems can be influenced by several site-specific factors such as climate and environmental type, land use and biological conditions (Ramachandran Nair et al., 2009). For example, in the environments with varying precipitation and evaporation conditions, the C storage in agroforestry was estimated to vary from 9 Mg C ha^{-1} in semiarid regions to 63 Mg C ha^{-1} in temperate regions (Montagnini and Nair, 2004).

In soil, the organic **C stock** is influenced by both input from plant litters and output to microbial decomposition. Agroforestry systems can sequester carbon through several processes: (i) prunings of woody plants returned to the soil (mainly in agroforestry systems of the tropics); (ii) autumnal litterfall of woody plants returned on soil (largest C flux from woody plants in agroforestry in temperate climates); (iii) turnover of coarse and fine root biomass of woody plants; (iv) crop residues; and (v) stabilization of organic carbon in the soil (Oelbermann et al., 2004). Litter of woody plants is more recalcitrant than that of herbaceous plants, thus the resident time in soil can be longer (Hobbie, 1996). In soil, quickly decomposed fresh litter or non-woody material is the main source of the labile C with a residence time of about three to four years; while the woody materials become passive soil C which may persist longer than 1000 years (Parton et al., 1987). If more passive C could enter the stable pools, soil C would slowly and steadily increases in the long run.

In temperate regions, normally prunings are not used on the crops but taken away from area or left in tree rows after being chopped. As prunings are lignin-rich and decompose slowly, therefore, this practice can be a large C input for the soil (Oelbermann et al., 2004). On the contrary, in SRC systems, almost all the aboveground biomass is harvested, while the belowground rooting system is left in soil. The living fine roots is rich of C (average C:N ratio being 41), and globally the carbon stored in fine roots can be higher than 5% of atmospheric C

(Jackson et al., 1997). Short rotation coppice with large rooting systems but without tillage or any other soil disturbance are also likely to build up the soil organic C stock by high input from roots turnover and low loss from decomposition. Such enrichment of soil organic carbon (SOC) in soil under short rotation willow compared to annual herbaceous energy crop (e.g. maize) was found in field study (Lemus and Lal, 2005).

1.5 Other residue effects of woody plants on arable land

There are many kinds of belowground interactions for the woody plants and crops in agroforestry systems. Besides of the effects favorable for crop growth such as the nutrient pump (tree roots can capture and recycle nutrients washed out of the relatively shallow rooting layer of crops), hydraulic lifting (plants with deep roots can take up water from deeper soil layers to shallower soil layers), and biological N_2 fixation and sharing, there also can be unfavorable effects for crop growth such as competition for resources (e.g. light and nutrient) and **allelopathic effects** between woody plants and crops (Jose et al., 2004).

Allelopathy is a widely observed phenomenon in natural and agricultural systems. Allelochemicals can be released into the environment via the **decomposition of residues**, volatilization, and root exudation. In temperate regions, allelopathy phenomena were reported in alley cropping with black walnut (*Juglans nigra* L.) (Jose and Gillespie, 1998). In the semi-arid soils with low leaching and high evaporation potentials, the concentration of allelopathic compounds released by woody species can be relatively high. A study on five common tree species in Botswana also found a variety of phenolic compounds and alkaloids in leaves and suggested a more cautious extension of agroforestry with these species in that region (Nakafeero et al., 2007).

The usually discarded hedgerow prunings can be considered for use on arable land to sequester carbon in the soil. However, their effects on weed control by allelopathy, as well as any potential adverse effect on the crop, should also be taken into consideration. Most of the studies about allelopathy effects in agroforestry were restricted to laboratory bioassay studies and pot culture studies (Bhatt and Todaria, 1990; Lisanework and Michelsen, 1993; Akhtar et al., 2010), and only a few studies were assessing the presence of allelopathic effects under field conditions (Jose and Gillespie, 1998; Masoodi et al., 2013). Thus, any in-situ field study about influence of agroforestry residues using on crops on arable land would improve the understanding of allelopathic effect in agroforestry and the possibility of new usages of woody plants and their residues on arable land.

1.6 Development of woody plants on arable land in Europe

In the last century, the area of agroforestry systems decreased in Europe, mostly attributed to intensification of agriculture, in particular related to mechanization and increase of the working width of machinery. Trees and hedgerows are often considered to be impedimental in a fully mechanized agriculture. For example, the area of *Streuobstwiesen* (orchard meadows), a typical agroforestry system with a long history in Germany, reached its peak in the 1930s and continuously declined in the following decades because of their replacement by intensively managed orchards (Herzog, 1998) or the conversion into arable fields. Similarly, the number and length of hedgerows which were traditionally used as wind break or living fences decreased in whole Europe including the UK (Barr and Gillespie, 2000), Belgium (Deckers et al., 2005), and France (Burel and Baudry, 1990).

Nowadays, agroforestry is again attracting interest because of its value for the maintenance of ecosystem services, and modern agroforestry systems are being developed (Smith et al., 2012). For example in Denmark, tree belts with alder (*Alnus rubra*), willow (*Salix* spp.) and hazel (*Corylus* spp.) for bioenergy production are intercropped with crops and pastures as “combined food and energy system” (Porter et al., 2009). Some subjects of the Rural Development Policy of the European Commission (EC) can also be potential reasons for the maintenance and even rebuilding of agroforestry, which were set to make better natural and economical environments, and better life quality in the rural areas (Smith, 2010). In recent years, agroforestry related policies, regulations and grants were introduced into agricultural production to extend agroforestry area and to stimulate farm tree planting, in the EU countries (Lawson et al., 2011). For instance, to follow the “greening rules” and to receive extra payment from the government, maintaining “ecological focus areas” (e.g. hedges and trees) is very important for farmers. Taking the region of Eastern Germany as an example, during the time period of 1964 to 2008, overall tree cover increased 24.8% on an study area of 280 km^2; among all the tree classes used in agriculture, hedgerows had the largest increment (136.5%), while the area of alleys and tree rows (61.5%), isolated trees (63.2%) and tree groups (63.0%) also had significant increase (Plieninger et al., 2012). A research on the plant, soil and their interactions of woody plants grown or used on arable land might improve the understanding of agroforestry systems and also attract wider interest to extend these sustainable production systems.

1.7 Research objectives and hypothesis

The overall objective of this study was to evaluate specific, formerly neglected effects of woody plants on arable land aboveground and belowground (in soil). This thesis specially focuses on the uses and selected effects of hedgerow residues and energy plants. Results from field and laboratory experiments including (i) a long-term field trial of woodchip mulching in organic farm, (ii) a germination test of aqueous extracts of woodchips on different seeds, (iii) a laboratory fractionation of soil organic matter in soil under willow and maize energy cropping systems, and (iv) a long-term field experiment with six representative annual and perennial, herbaceous and woody energy crops were used. The following hypotheses were the basis for each of the independent studies:

- Woody residues (woodchips) from hedgerows can be used on crop field as mulching material to control weeds. Woodchip mulching has an effect on the crops, especially in a continuous long-term application.
- Some common tree species (e.g. *Salix caprea* L. and *Prunus padus* L.) used in hedgerows have an allelopathic potential. When extracted with water (similar to rainfall), the extracts can affect seed germination.
- The contribution of woody energy crops (e.g. willow in short rotation coppice) to soil organic matter (labile and passive) in the topsoil is higher than that of herbaceous energy crops (e.g. mono-cropping maize).
- Willow, as a woody energy crop, can compete with herbaceous energy crops in the studied region, from both perspectives of biomass yield and energy yield. Compared to the annuals, the perennial energy crops can have higher and more stable yield after establishing. Nitrogen fertilization can benefit maintaining a relative high and stable yield level for all energy crops.

2. Publications

The present thesis consists of four scientific articles as reflected by chapter I-IV, which form the body of the dissertation. These articles have been published (Chapter I and IV), submitted to peer reviewed journals (Chapter II), or are in preparation and to be submitted soon (Chapter III).

Publication I

Xu, J., Gauder, M., Zikeli, S., Möhring, J., Gruber, S., & Claupein, W. (2018). Effects of 16-year woodchip mulching on weeds and yield in organic farming. *Agronomy Journal, 110*(1), 359-368.

Publication II

Aqueous extracts of wood chips can suppress seed germination

Jialu Xu, Sabine Zikeli, Martin Gauder, Jens Mohring, Sabine Gruber, and Wilhelm Claupein

Submitted to Organic Agriculture

Publication III

Physical fractionation of soil organic matter in a long-term trial with annual and perennial energy crops and different tillage intensities

Jialu Xu, Sabine Zikeli, Martin Gauder, Sabine Gruber, and Thilo Rennert

In preparation and to be submitted to Science of the Total Environment. Part of the results are included in Chapter III of the dissertation.

Publication IV

Xu, J., Gauder, M., Gruber, S., & Claupein, W. (2017). Yields of annual and perennial energy crops in a 12-year field trial. *Agronomy Journal*, *109*(3), 811-821.

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ORGANIC AGRICULTURE & AGROECOLOGY

Effects of 16-Year Woodchip Mulching on Weeds and Yield in Organic Farming

Jialu Xu,* Martin Gauder, Sabine Zikeli, Jens Möhring, Sabine Gruber, and Wilhelm Claupein

ABSTRACT

To test the possibility of using hedgerow woody waste for weed control in arable organic farming, a 16-yr field trial with a typical organic crop rotation using three rates of woodchip mulching (WCM) was conducted in Southwest Germany. Winter cereals, fodder crops, and legumes representative for this region were included in the crop rotation. The woodchips were produced from cuttings of the hedgerows on the farm and were applied to the field in rates of 0, 80, and 160 m^3 ha^{-1}, respectively (control, WCM80, and WCM160). Weed infestations, including weed density in spring, weed biomass at crop harvest and weed seed bank, were measured in selected years. Crop yields were recorded yearly. Soil temperature and soil mineral N content were measured in selected years. In general, mean weed density was reduced in WCM160 (135 plants m^{-2}) compared to WCM80 and the control (150 and 160 plants m^{-2}, respectively). The relative weed density averaged across the two WCM levels was 91% of the control on average and showed no trend over time. The grain yield of cereals and faba bean (*Vicia faba* L.) did not significantly differ between the mulching treatments. The relative crop yield of plots with WCM compared to the control showed a decreasing trend over time. Soil temperature and diurnal temperature variation were lower in WCM160 compared to the control. Generally, combined with an adapted fertilizer application, WCM in a specific amount could be an efficient tool to control weeds in organic farming without yield loss.

Core Ideas

- Woodchip mulching significantly suppressed weeds under field conditions.
- Sixteen-year woodchip mulching had no impact on grain yield of cereals or faba bean.
- There was a continuous decreasing trend of the relative yield with woodchip mulching.
- There was no impact of woodchip mulching on the weed seed bank after 16 yr.

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Hedgerows (single or mixed species of trees and shrubs) exist on many farms as an important multifunctional component of the landscape: they are substantial and symbolic boundaries; they can control physical, chemical, and biological fluxes such as soil erosion and wind speed; and in particular, they can influence the biodiversity since they are habitats, refuges, corridors, or barriers for many species (Baudry et al., 2000). Although hedgerows are essential for the environment, they scarcely produce economic income for farmers. Moreover, they create an obstacle for mechanization in high-input farming systems. For these reasons, the area of hedgerows has decreased with the intensification of agriculture in the last century. However, the importance of hedgerows in supplying ecosystem services has been recognized in recent years, thus restoration and re-establishment efforts were undertaken (Petit et al., 2003), especially in organic farms (Aude et al., 2004; Boutin et al., 2008; MacNaeidhe et al., 1998).

Hedgerows should be pruned every few years to maintain their functions. Typically stems with a diameter larger than 5 cm can be used as firewood, but the small twigs and branches cannot because of their high bark content and poor combustion quality (Filbakk et al., 2011). As a common practice in agriculture, this material is chopped and left on site without special use.

While woodchips produced by hedgerows are discarded, weeds are causing the highest potential crop losses among all the pests (Oerke, 2005) and chemical compounds for weeding are not permitted in organic farming systems (European Commission, 2008). Mulching is a common and promising method among non-chemical weed control schemes (Bond and Grundy, 2001) and can be conducted with many different natural and synthetic materials (Anzalone et al., 2010; Mohammadi, 2013), thus mulching with woodchips can be a possible tool for weed control on organic farms.

Woodchip mulching (WCM) is often used for decoration purposes in gardening, but it is also found to suppress weeds in orchards, gardens, and vegetables (Treder et al., 2004; Awodoyin et al., 2007; Cregg and Schutzki, 2009). If woodchips were produced from hedgerows on-farm and then spread on farmland as mulch (WCM), reduced transportation distances for WCM could possibly lead to an economically

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Abbreviations: WCM, woodchip mulching.

Aqueous extracts of woodchips can suppress seed germination

Abstract:

To examine the existence of bioactive compounds in woody residues from hedgerows and their potential allelopathic effects, aqueous extracts from woodchips were tested on the germination of selected seeds. Woodchips from two tree species, goat willow (GW, *Salix caprea* L.) and bird cherry (BC, *Prunus padus* L.), which are part of hedgerows in Europe, were used. Germination test was conducted on oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) seeds. To standardize the extraction procedure considering the stability and distribution of potential allelochemicals in woodchips, a systematic testing of woodchips drying methods, milling, wood to water mixing ratio (WWR) and fraction of the material (bark or core wood) was conducted. Extracts of freeze dried and undried (defrosted) wood resulted in the lowest germination rate (<6%) of both crops after two weeks, compared to the relatively higher germination rates (40%, 12% and 53%, respectively) in extracts of 25 °C, 60 °C and 105 °C dried wood. The suppression effect increased with increasing WWR: germination rates were 86% (WWR=1:20), 71% (WWR=1:15) and 35% (WWR=1:10) with milled woodchips. Extracts from the bark resulted in significantly lower germination rates (<4%) than that for the core wood (>88%). Extraction conditions led to highest suppression of seed germination were considered as standardized method. The extraction procedure developed in this study can be used for testing allelopathic effects and potential herbicidal uses of more woody species, or for developing bio-herbicides for organic farming in the future.

Key words: woody material, leachate, *Brassica napus*, *Triticum aestivum*, allelopathy, weeds, bio-herbicides

1. Introduction

In the last decades, the area of organically managed farmland continuously increased (Reganold and Wachter, 2016). However, the yield gap between organic farming (OF) and conventional farming remains a weakness of OF (De Ponti et al., 2012). Weed infestation is one of the main yield-limiting factors, as weeding in OF is mostly restricted to mechanical methods and system approaches such as rotation design. Nowadays there are also a few natural herbicidal products available for OF such as essential oils, organic acids, and crude botanical products (Dayan and Duke, 2010), which can be applied alternatively or in addition to mechanical weeding. However, these natural products are normally non-selective, require high application doses and are currently less economical than conventional methods (Dayan et al., 2009).

Plants can release bioactive secondary metabolites (allelochemicals) into the environment to interfere with the growth of other plants and microorganisms (Rice, 2012), which is called allelopathy. Many groups of secondary metabolites are involved in allelopathic interactions, including complex quinones, simple phenols, phenolic acids, benzoic acids, flavonoids, tannins, terpenoids, alkaloids and others (Seigler, 1996). Allelopathy of plants and plant residues is a feature widely observed in natural and agricultural ecosystems (Zeng et al., 2008), and the possibility of using allelopathic effects in pest management is increasingly recognized (Farooq et al., 2011).

Compared to conventional farms, organic farms often have larger areas of total semi-natural and boundary vegetation including woodlands, field margins, and hedgerows (Gibson et al., 2007). These structural elements of the farms, especially the hedgerows, have few direct economic benefits but provide ecosystem services, such as soil protection, water flux control and biodiversity conservation (Baudry et al., 2000). However, the allelopathic effects, which hedgerow species may contain, are often not included as ecosystem services. Some plant species occurring in hedgerows, such as *Morus alba* L., *Eriobotrya japonica* Lindl, *Citrus reticulata* L. and others, were reported to have allelopathic effects under laboratory conditions (Wu et al., 2012).

Moreover, although little is known about the allelopathic properties of hedgerow species in Central Europe, field studies identified the possibility to use mixed woodchips from hedgerows as mulching material to reduce weed density in OF (Gruber et al., 2008; Wang et al., 2012). This weed suppressing effect of woodchip mulching can be related to shading of the soil, changes in soil temperature, moisture and microorganisms, physical impediment of weed emergence, as well as to the release of allelopathic compounds.

In natural conditions, allelochemicals can be released to the environment as volatile compounds, aqueous leachates, and leaf or root exudates (Rice, 2012). As they need to move with the soil water to reach the target organisms, many allelochemicals are water soluble (Blum, 2006). Thus, using water as solvent can be an efficient and eco-friendly way to extract allelochemicals from plant material. Aqueous extracts of plants, such as sorghum (*Sorghum bicolor* L.), sunflower (*Helianthus annuus* L.) and oilseed rape (*Brassica napus* L.), were already used to partly replace synthetic herbicides in crops (Iqbal et al., 2009; Jamil et al., 2009). Therefore, a simple but effective aqueous extraction might also be a good way to make use of the allelopathic effects of woody plant materials.

The success of the extraction of allelopathic compounds from plant tissues can be related to many factors, such as concentration of the active ingredients in the material, pretreatments and the mixing ratio with the solvent. Since the heat stability and volatility of different allelochemicals can vary (Kohli and Singh, 1991; Iqbal and Fry, 2012), some compounds can be sensitive to the temperature and extraction time. In addition, as bark plays an important role in plant defense mechanisms (Bennett and Wallsgrove, 1994), it might contain more bio-active compounds compared to the core wood. Plants often show a biphasic dose response to allelochemicals, that is the growth being stimulated at low concentrations (hormesis) and being inhibited with increasing concentration (Duke et al., 2006; Belz, 2008). For this reason, the factors in the extraction process that could influence the dose of the effective ingredients in the extracts are especially important for the potential herbicidal use of the extracts.

Thus, the aim of this study was to define and to standardize a method for pre-treatments of an aqueous extraction from woodchips to obtain solutions that prevent germination of seeds at the highest possible rate. A systematic testing of the production of aqueous extracts was set up in the laboratory including the following factors: milling, drying, mixing ratios, and material selection. The following research questions were tested: (i) What is the optimal drying method? (ii) What is the optimal mixing ratio of woodchip material and water for extraction? (iii) Is there an effect of the particle size (milling)? (iv) Is there a difference between the effect of bark and core wood? (v) Are there differences between the species tested in terms of pre-treatments? (vi) Are there interactions of woodchip species and crop species?

2. Material and methods

2.1 Woodchip collection

Hedgerows (single or mixed species of trees and shrubs) were cut before bud break on 28th February 2014 at the experimental station Kleinhohenheim of the University of Hohenheim, in southwest Germany (48°43´N, 9°11´E, and 435m above sea level), and were separated by tree species. The fresh woodchips of bird cherry (BC) and goat willow (GW) from the hedgerows were put into plastic bags and taken to the laboratory. One portion of the material was used for determination of the properties, and the other was deep frozen (-18 °C) until extraction. The average length of the woodchips was 3.8 cm. The water content (105 °C oven-dried) was 54.4% (BC woodchips) and 45.8% (GW woodchips), respectively.

2.2 Aqueous extracts

In general, the extraction was conducted in the following way: (i) The woodchips were dried until a constant weight was reached. (ii) The material was separated from the cambium with a knife and tweezers under a microscope for bark and core, and milled to 4 mm size with a cutting mill (ZM 1 from Retsch). (iii) A specific amount (15, 20, 30 or 60 g) of wood material was filled into a 500 mL plastic bottle according to the wood to water mixing ratio (WWR). (iv) 300 mL of deionized water was added. (v) The bottles were shaken for 96 h (bench top horizontal shaker, 200 rpm). (vi) The extracts were poured through a sieve (plastic, 2 mm mesh size) to remove any coarse parts; (vii) centrifuged in 50 mL portions for 10 min at 4000 r min^{-1}; and (viii) filtered by a medium to fast filter paper (Munktel 1289, core size 8-12 µm, match mn $616^{1/4}$).

To optimize this extraction procedure, four factors were tested in a step-wise approach: (I) the drying method, (II) the pretreatment of milling, (III) the WWR and (IV) the wood fraction (bark or core) (Table 1). When the most suitable level of a certain factor was found, meaning which had the strongest effect of suppressing the germination, it was then used in the next testing approach (Table 1).

Testing approach (I) was to assess the effect of drying method (Table 2). In this step, woodchips were unmilled and used as whole wood (bark and core wood unseparated). Wood to water mixing ratio (WWR) was fixed to a relatively narrow ratio (1:5) to ensure that enough active compounds are left after the drying procedure, since a pre-test indicated a big loss of active compounds with some drying methods.

Approaches (II and III) were to test the effect of milling and WWR (Table 1). In this step, all woodchips were freeze dried and used as whole wood. Woodchips received in total six treatments across the two levels of milling and the three levels of WWR.

Testing approach (IV) was to reveal effects of the wood fraction (Table 1). In this step, woodchips were freeze dried, bark and core wood were separated and both were milled and mixed with water by 1:10 of WWR.

Table 1. Testing approaches to develop a standardized aqueous extraction procedure of wood chips from hedgerows

Approaches	Testing conditions				Testing species	
	Drying	Milling	WWR[1]	Fraction	Seed	Wood
(I) Drying	All[2]	unmilled	1:5	whole wood		
(II+III) Milling x WWR	FD[3]	Milled[4] unmilled	1:10 1:15 1:20	whole wood	OSR[5] Wheat	GW BC[6]
(IV) Fraction	FD	milled	1:10	bark core wood		

[1] Wood to water mixing ratio (g:mL);

[2] All drying methods: undried (defrosted), freeze dried, and oven dried at 25, 60 or 105°C;

[3] FD=freeze dried under -50°C;

[4] Milled to pass through 4 mm sieve;

[5] OSR=Oilseed rape;

[6] GW=goat willow, BC=bird cherry.

Table 2. The testing conditions of the drying methods used in the first step to develop a standardized aqueous extraction procedure of wood chips from hedgerows.

Abbreviation	Equipment	Temperature (°C)	Time (day)[3]
UD[1]	None	/	/
FD	Freeze dryer[2]	-50	3
OD25	Oven	25	4
OD60	Oven	60	2
OD105	Oven	105	1

[1] Undried (defrosted) wood chips;

[2] Laboratory freeze dryer, VaCo 5 from Zirbus Technology;

[3] Time was set to ensure that material is completely dried;

2.3 Laboratory germination test

The aqueous extracts of woodchips were tested for their effects on seed germination of oilseed rape (*Brassica napus* L., var. Vitara) and wheat (*Triticum aestivum* L., var. Montana). The germination tests were carried out based on the method of Weber et al. (2010), but modified in some steps. One hundred oilseed rape seeds, or 50 wheat seeds, were homogenously distributed in a petri dish (9 cm) inlaid with two layers of filter paper (Munktel 3hw, Ø 90 mm). Six mL of the extract was carefully pipetted onto the filter paper. Deionized water (6 mL) was used as control (CTR) in all three experiments. Bubbles under and in-between the filter papers were eliminated to ensure that all seeds have sufficient contact with the fluid.

The treatments were tested in four replicates each (four petri dishes) in an incubator at 20 °C and under continuous light for 14 days. Seeds were rated as germinated when the radicle was longer than 2 mm. The seed germination was recorded on a daily basis and the experiment was continued for two weeks. After the two weeks, almost all seeds were germinated or dead (rotten). Every day, germinated or dead seeds were removed from the petri dishes. For wheat, after one week another 6 mL of extract or water was pipetted into the petri dish to supplement the loss of liquid caused by the germinated seeds.

3. Statistical analysis

In the section of drying method or wood selection, germination rate response to the treatments was analyzed according to the experimental design using procedure PROC MIXED (SAS version 9.2, SAS Institute Inc., Cary, NC, USA) with the following model:

$$y_{ijkrl} = \mu + \gamma_k + \tau_{ik} + \varphi_{jk} + (\tau\varphi)_{ijk} + \chi_r + (\gamma\chi)_{kr} + (\tau\chi)_{ikr} + (\varphi\chi)_{jkr} + (\varphi\tau\chi)_{ijkr} + e_{ijkrl},$$

(1)

with

y_{ijkrl} = germination rate of the *j*th seed species in the aqueous extract of *r*th tree species with *i*th treatment (either for drying method or for wood fraction) as the *k*th extract

μ = general effect

γ_k = main effect of *k*th extracts

τ_{ik} = main effect of the *i*th treatment

φ_{jk} = main effect of the *j*th seed species

χ_r = main effect of the *r*th tree species

$(\tau\varphi)_{ijk}$, $(\gamma\chi)_{kr}$, $(\tau\chi)_{ikr}$, $(\varphi\chi)_{jkr}$, and $(\varphi\tau\chi)_{ijkr}$ are the interactions of the factors, e_{ijkrl} is the residual error effect associated with y_{ijkrl}.

In the section of milling and WWR, data were analyzed by the replacement of the treatment factor in (1) with WWR, milling and their interaction. The model is as follows:

$$y_{ijknrl}=\mu + \gamma_k + \eta_{ik} + \lambda_{nk} + (\eta\lambda)_{ink} + \varphi_{jk} + (\eta\varphi)_{ijk} + (\lambda\varphi)_{njk} + (\eta\lambda\varphi)_{ijkn} + \chi_r + (\gamma\chi)_{kr} + (\eta\chi)_{irk} + (\lambda\chi)_{nrk} + (\eta\lambda\chi)_{inrk} + (\varphi\chi)_{jkr} + (\eta\chi\varphi)_{ijkr} + (\eta\lambda\varphi)_{njkr} + (\eta\chi\lambda\varphi)_{ijnkr} + e_{ijknrl}, \quad (2)$$

with

y_{ijknrl} = germination rate of the *j*th seed species in the aqueous extract of *r*th tree species with *i*th WWR and *n*th milling level as the *k*th extract,

μ = general effect,

γ_k = main effect of the *k*th extracts

η_{ik} = main effect of the *i*th WWR

λ_{nk} = main effect of *n*th milling level

φ_{jk} is the main effect of the jth seed species

χ_r is the main effect of the rth tree species

$(\eta\lambda)_{ink}$, $(\eta\varphi)_{ijk}$, $(\lambda\varphi)_{njk}$, $(\eta\lambda\varphi)_{ijkn}$, $(\gamma\chi)_{kr}$, $(\eta\chi)_{irk}$, $(\lambda\chi)_{nrk}$, $(\eta\lambda\chi)_{inrk}$, $(\varphi\chi)_{jkr}$, $(\eta\chi\varphi)_{ijkr}$, $(\eta\lambda\varphi)_{njkr}$, and $(\eta\chi\lambda\varphi)_{ijnkr}$ are the interactions of the certain factors, e_{ijknrl} is the residual error effect associated with y_{ijknrl}.

To achieve a normal distribution of the data, all germination rates were transformed using the following equation (McCullagh, 1984):

$y'=\sin^{-1}(((y+0.375)/(100+0.75))^{0.5})$

where y' is the transformed data and y is the original data (germination rate).

Multiple comparisons were done based on LSD only in case of significant F-tests. Means and contrasts between the treatments were compared at $\alpha < 0.05$.

4. Results

4.1 Effect of woodchips drying methods on germination (testing approach I)

In this approach, germination was strongly and significantly affected by the factors of drying method (freeze drying at -50 °C and oven drying by 25, 60 and 105 °C), seed species, wood species, and all their interactions except for that between seed species and CTR-T (Table 3).

In the GW extracts from whole (bark and core wood unseparated) woodchips (WWR=1:5) dried with different methods, germination of oilseed rape seeds was completely inhibited except for the woodchips dried at 105 °C which allowed 7.5% germination (Fig. 1 A). The germination of oilseed rape seeds was suppressed in all BC extracts (WWR=1:5). OD60, FD and UD showed germination rates below 8%, while in OD25 and OD105 germination rates were 81% and 61%, respectively (Fig. 1 B).

Table 3. Statistical evaluation (ANOVA) for the germination rate of oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.) seeds in petri dishes (after two weeks) soaked in aqueous extracts of wood chips of goat willow (*Salix caprea*) and bird cherry (*Prunus padus*). Wood chips were dried with different methods including freeze-drying at -50 °C and oven drying by 25, 60 and 105 °C.

Effect	Num DF	F Value	Pr > F
CTR-T[1]	1	731.37	<.0001
Drying (CTR-T)	4	149.71	<.0001
Seed spec.[2]	1	105.69	<.0001
Wood spec.[3] (CTR-T)	1	194.43	<.0001
Seed spec. x wood spec. (CTR-T)	1	74.25	<.0001
Seed spec. x CTR-T	1	0.76	0.3865
Seed spec. x drying (CTR-T)	4	10.17	<.0001
Wood spec. x drying (CTR-T)	4	44.84	<.0001
Seed spec. x wood spec. x drying (CTR-T)	4	10.13	<.0001

[1] Comparison of the extracts with deionized water (control)

[2] Seed spec. is the effect of the two seed species (oilseed rape or wheat) in this approach;

[3] Wood spec. is the effect of the two wood species (goat willow or bird cherry) in this approach.

Except in the control with deionized water, the germination rates of wheat seeds were always the first and second highest in the extracts of OD105 and OD25 (Fig. 1 C, D). However, even in GW extracts (WWR=1:5) of OD105, the germination of wheat seeds was strongly inhibited, as 48% seeds were not germinated (Fig. 1 C).

Generally, extracts of UD and FD material had the strongest suppression of germination of both oilseed rape and wheat seeds. The germination rate decreased for oilseed rape in the following order: CTR> OD105> OD25, OD60, UD, FD in the GW extracts (Fig. 1 A); and in the order: CTR> OD25> OD105> OD60, UD, FD in the BC extracts (Fig.

1 B). The germination rate decreased for wheat seeds in the order: CTR> OD105 > OD25, OD60> UD, FD in the GW extracts (Fig. 1 C); and in the order: CTR, OD105> OD25> OD60, UD, FD in the BC extracts (Fig. 1 D).

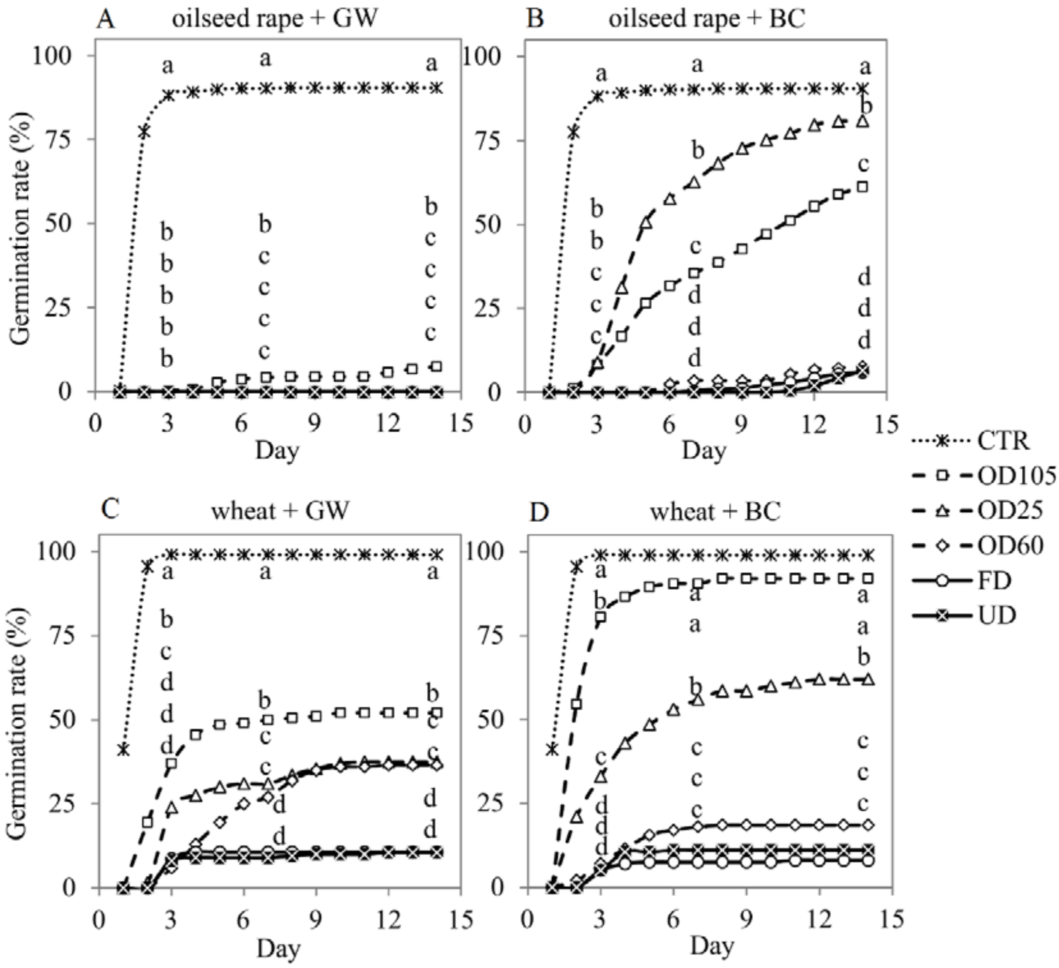

Fig. 1 A-D. Seed germination of oilseed rape (A, B) and wheat (C, D) in petri dishes soaked in aqueous extracts of woodchips from goat willow (GW, *Salix caprea*) and bird cherry (BC, *Prunus padus*) treated with different drying methods. Extracts were derived from unmilled whole woodchips (3.8 cm), wood to water mixing ratio was 1:5 (w:v). CTR: control with deionized water; OD25: oven dried at 25 °C; OD60: oven dried at 60 °C; OD105: oven dried at 105 °C; FD: freeze dried at -50 °C; UD: undried (defrosted). Lower case letters indicate significant differences among the drying methods for $P < 0.05$.

4.2 Effect of woodchips milling and wood to water mixing ratio (WWR) on germination (testing approach II+III)

Generally, when woodchips were freeze dried, the factors milling, WWR, seed species and wood species, as well as all their interactions except seed*wood*WWR and wood*milling, had significant effects on seed germination in this testing approach (Table 4).

Table 4. Statistical evaluation (ANOVA) for the germination rates of oilseed rape and wheat seeds in petri dishes (after two weeks) soaked in aqueous extracts of wood chips of goat willow (*Salix caprea*) and bird cherry (*Prunus padus*) with different pretreatments (milling and wood to water mixing ratio).

Effect	Num DF	F Value	Pr > F
CTR-T[1]	1	128.33	<.0001
WWR[2] (CTR-T)	2	183.51	<.0001
Milling[3] (CTR-T)	1	14.79	0.0002
WWR x milling (CTR-T)	2	6.22	0.0031
Seed spec.[4]	1	25.29	<.0001
Wood spec.[5] (CTR-T)	1	218.87	<.0001
Seed spec. x wood spec. (CTR-T)	1	6.8	0.0109
Seed spec. x WWR (CTR-T)	2	7.31	0.0012
Wood spec. x WWR (CTR-T)	2	66.69	<.0001
Seed spec. x wood spec. x WWR (CTR-T)	2	0.98	0.3806
Seed spec. x milling (CTR-T)	1	5.44	0.0223
Wood spec. x milling (CTR-T)	1	2.35	0.1291
Seed spec. x wood spec. x milling (CTR-T)	1	8.96	0.0037
Seed spec. x WWR x milling (CTR-T)	2	4.3	0.0169

Wood spec. x WWR x milling (CTR-T)	2	14.16	<.0001
Seed spec. x wood spec. x WWR x milling (CTR-T)	2	6.09	0.0035

[1] Comparison of the extracts with deionized water (control).

[2] WWR: wood to water ratio (weight to volume).

[3] Milling effect including two treatments (with and without milling).

[4] Seed spec. is the effect of the two seed species (oilseed rape or wheat) in this approach.

[5] Wood spec. is the effect of the two wood species (goat willow or bird cherry) in this approach.

Milled woodchips were slightly more effective in suppressing seed germination than unmilled woodchips. This effect seemed stronger with woodchips from BC (Fig. 2), as the germination rate with milled BC woodchips (oilseed rape 26% and wheat 1%) was significantly lower compared to the use of unmilled BC woodchips (oilseed rape 49% and wheat 19%).

The ratio between the woodchips and the solvent water showed significant effects in seed germination (Table 4), the highest concentration of woodchips (WWR 1:10) always resulting in the lowest germination rates. This effect seems to be higher for BC (Fig. 3).

The effect of milled GW on oilseed rape germination did not differ significantly depending on WWR (Fig. 3 A). On the contrary, for BC extracts, the lower WWR resulted in a lower suppression. The germination rate of oilseed rape was in the following order: CTR, 1:20 > 1:15 > 1:10 in the BC extracts (Fig. 3 B). Wheat germination was more susceptible to the effects of the different WWRs of both wood species. Increased WWR resulted in decreased germination rates of the following orders: CTR > 1:20, 1:15 > 1:10 in the GW extracts (Fig. 3 C); CTR > 1:20 > 1:15 > 1:10 in the BC extracts (Fig. 3 D).

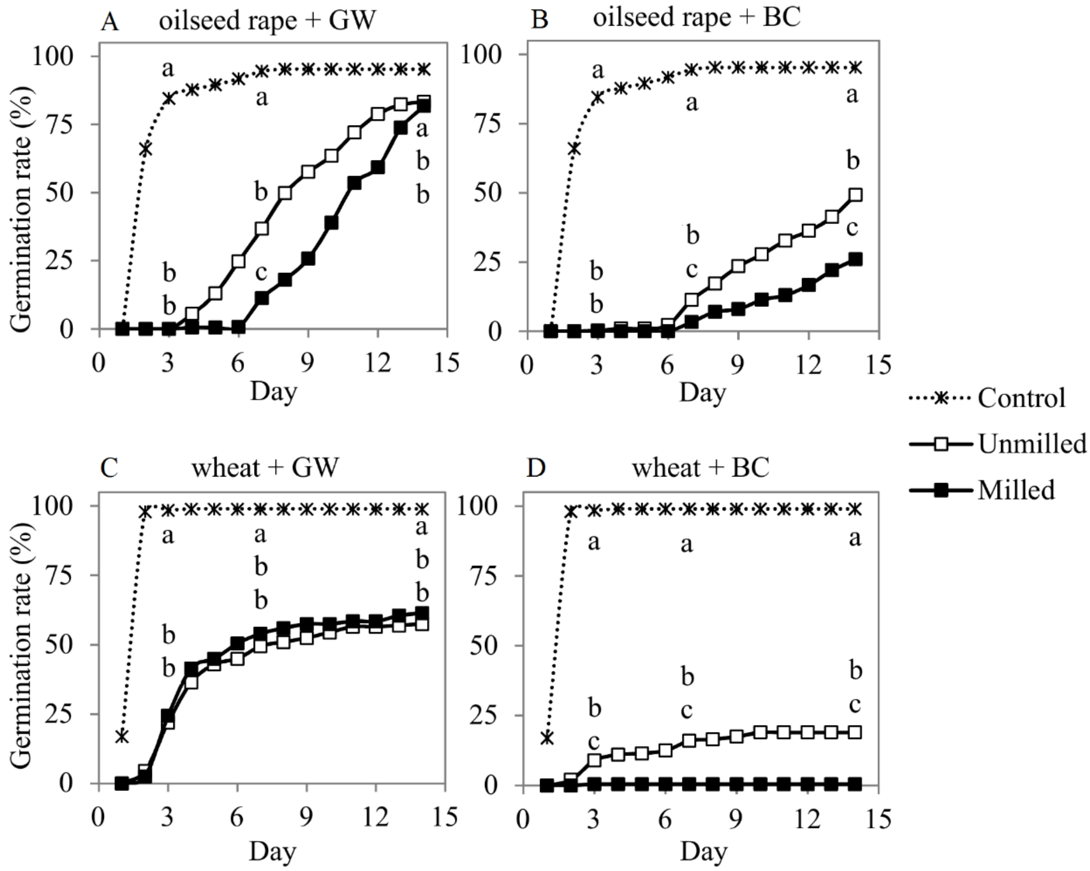

Fig. 2 A-D. Seed germination of oilseed rape (A, B) and wheat (C, D) in petri dishes soaked in aqueous extracts of milled and unmilled woodchips from goat willow (GW, *Salix caprea*) and bird cherry (BC, *Prunus padus*), wood to water ratio=1:10; significant differences among the treatments are indicated by lower case letters at $P < 0.05$.

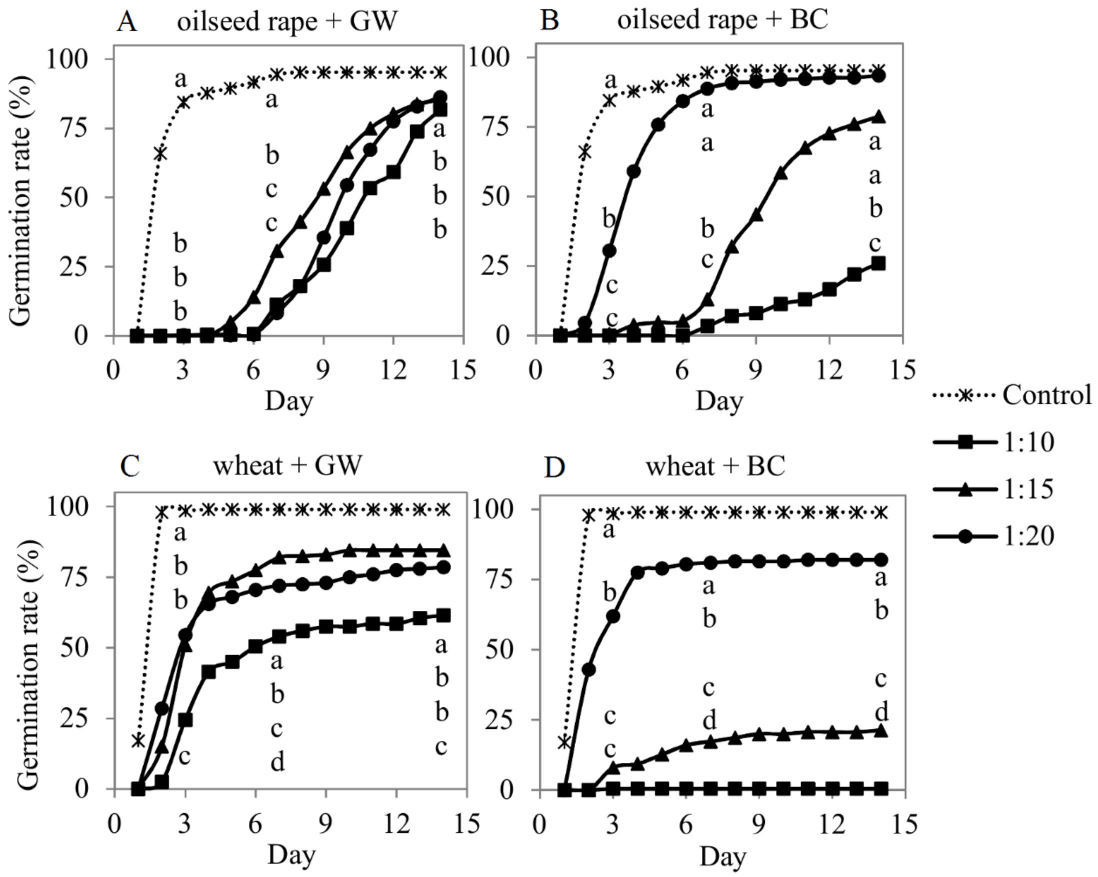

Fig. 3 A-D. Seed germination of oilseed rape (A, B) and wheat (C, D) in petri dishes soaked in aqueous extracts of milled woodchips from goat willow (GW, *Salix caprea*) and bird cherry (BC, *Prunus padus*) with different wood to water ratio (1:10, 1:15 and 1:20); significant differences among the treatments are indicated by lower case letters at $P < 0.05$.

4.3 Effect of woodchips fraction (bark and core wood) on germination (testing approach IV)

The wood fraction, seed species, wood species, and the interactions of seed*wood had significant effects on the germination rate (Table 5). Generally, extracts of bark resulted in lower germination rates (1%) for both crop seeds and thus stronger suppression of germination than extracts of the core wood with germination rates of 91%. Both treatments resulted in significantly lower germination rates than the control treatment with deionized water (96%).

Table 5. Statistical evaluation (ANOVA) for the germination rate of oilseed rape and wheat seeds in petri dishes (after two weeks) soaked in aqueous extracts of with different parts of wood chips (core or bark) of goat willow (*Salix caprea*) and bird cherry (*Prunus padus*).

Effect	Num DF	F Value	Pr > F
CTR-T[1]	1	678.7	<.0001
Part[2] (CTR-T)	1	2172.76	<.0001
Seed spec.[3]	1	13.28	0.001
Wood spec.[4] (CTR-T)	1	0.35	0.5571
Seed spec. x wood spec. (CTR-T)	1	6.43	0.0166
Seed spec. x fraction (CTR-T)	1	1.44	0.2396
Wood spec. x fraction (CTR-T)	1	3.75	0.0622
Seed spec. x wood spec. x fraction (CTR-T)	1	0	0.9959

[1] Comparison of the extracts with deionized water (control);

[2] Wood fraction (bark or core);

[3] Seed spec. is the effect of the two seed species (oilseed rape or wheat) in this approach;

[4] Wood spec. is the effect of the two wood species (goat willow or bird cherry) in this approach.

In the extracts of core wood, the seeds always had a period of fast germination in the first few days to reach the maximum germination rate, except for the oilseed rape in the extract from GW-core wood (Fig. 4). The seed germination in the extracts of bark was completely (100%) inhibited except for a few germinating wheat seeds in the extract of GW-bark (96% inhibition).

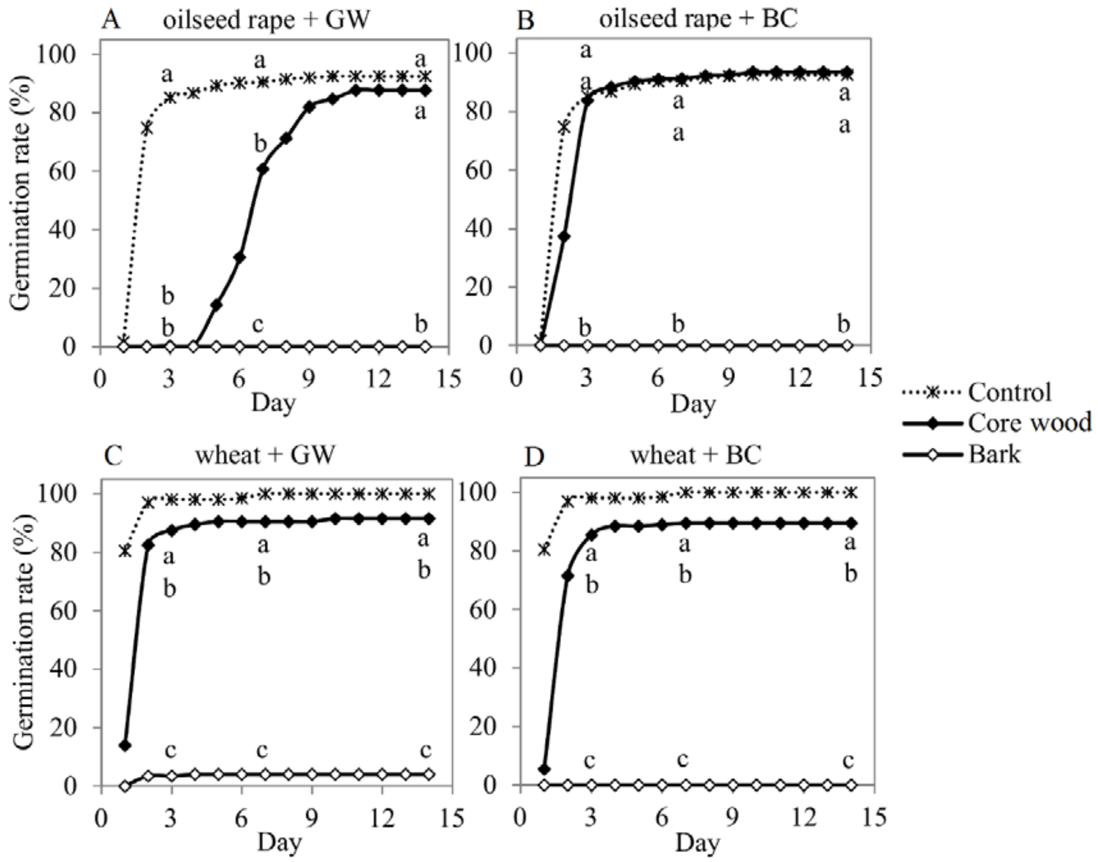

Fig. 4 A-D. Seed germination rate of oilseed rape (A, B) and wheat (C, D) in petri dishes soaked in aqueous extracts of different part of woodchips (core wood, bark) from goat willow (GW, *Salix caprea*) and bird cherry (BC, *Prunus padus*); significant differences among the treatments are indicated by lower case letters at $P < 0.05$.

4.4 General effect of the extracts, wood species and seed species

In all, the woodchip extracts always had a significant effect on the germination rate compared to the deionized water (control) in all testing approaches (Tables 3, 4 and 5). In the control treatment, most seeds germinated in the first days, while in the treatments with woodchip extracts, germination was either delayed or inhibited to a certain extent, in some cases even a complete inhibition was observed (Fig. 1, 2, 3 and 4).

The woodchips species used for extraction had a significant effect on the germination rate in testing approaches (I) and (II)+(III) (Tables 3 and 4). In testing approach I with a narrow WWR (1:5), the average germination rate in GW (10%) was significantly lower than in BC (32%); while in the second approach with a wide WWR (1:10, 1:15 and 1:20), the average germination rate in GW (82%) was significantly higher than in BC (54%). The two crop species (oilseed rape and wheat) also had significantly different germination rates in all testing approaches (Tables 3, 4 and 5).

5. Discussion

5.1 Woodchip species extracts

Woody parts of plants can contain several bioactive secondary metabolites to resist herbivores (vertebrates or invertebrates) and pathogens e.g. fungi or bacteria causing diseases (Bennett and Wallsgrove, 1994; Obst, 1998). Among these, some phenolic compounds, terpenoids and other constituents are considered as allelochemicals that can influence the growth of other plants (Williams and Hoagland, 1982; Langenheim, 1994). All of the germination tests in this study showed that the aqueous extracts of GW and BC could suppress the germination of oilseed rape and wheat seeds. Possibly, the suppression effect was related to allelopathic effects.

GW is a fast-growing and wide-spread tree species in Europe, and it is mainly used for hedges, gardening or biomass production (Enescu et al., 2016). It was found to contain many kinds of bioactive constituents, especially a high content of tannins in the bark (Ahmed et al., 2011). Aqueous leachates obtained from GW leaves can suppress the root growth of spruce seedlings (Schütt and Blaschke, 1980). Leachates of fresh GW litters can reduce germination and suppress shoot growth of other plants such as *Plantago lanceolata* and *Lotus corniculatus* (Mudrák and Frouz, 2012). These authors document the allelopathic effects of GW on other plants, which imply for our study that the germination suppression effect of GW extracts can be an allelopathic effect.

BC is the most widely distributed species of the *Prunus* genus in Europe, and it is used as wind-break or sound-break because of its rapid growth to form thickets (Durrant and

Caudullo, 2016). Irrigating aqueous extract from *Prunus cerasoides* leaves or mulch with its dry leaves can depress the dry matter production of crops such as *Glycine max* and *Hordeum vulgare* (Bhatt and Todaria, 1990). Aqueous extracts of *Prunus serotina* can reduce the germination, shoot length and root length of *Sinapis alba* (Csiszár, 2009). As the *Prunus* genus was often reported to have allelopathic effects on other plants, BC with a high content of phenolic compounds (Kim et al., 2010), is also likely to have allelopathic potential. In our study, allelopathic effects can be a reasonable explanation of the suppression of seed germination by the BC extracts.

5.2 Drying method

In this study, extracts of OD woodchips always had less suppression on seed germination than FD and UD, which indicated a loss of active compounds during the drying procedure. The properties of the allelochemicals might explain this phenomenon as some allelochemicals are volatile or not heat-stable. The temperatures used in our study for drying the woodchips are very likely to cause losses of these effective constituents.

For example, monoterpenes are considered as the most volatile group of fractions present in wood by the pellet manufacturing industry (Ståhl et al., 2004), and many volatile monoterpenes are also considered as allelochemicals (Vaughn and Spencer, 1993). Such volatile monoterpenes are very likely to be lost during the drying step. Additionally, in a pretest to this study (data not shown), there was an interaction of different extracts on seed germination when petri dishes with different extracts were sealed together in one plastic bag. This also indicated the presence of some volatile allelochemicals in the extracts.

Furthermore, other allelochemicals are heat-labile and might have been inactivated by excessive heating or drying (Macías et al., 2003). A study comparing aqueous extraction procedures of rice (*Oryza sativa* L.) hull at 24 °C and 80 °C found that the extracts under lower extraction temperature had higher allelopathic potential, which might be related to degradation of allelochemicals in the high extraction temperature (Ahn and Chung, 2000). In our study, the low suppression effect in OD105 was probably related to an inactivation of heat-labile allelochemicals, while the low suppression effect in OD25 was possibly related to loss of volatile allelochemicals during the long drying time. OD60 had the strongest suppression effect among the three heat drying treatments, and might be an optimum combination of drying temperature and time.

Although heat drying of woodchips might reduce the allelopathic potential of the extracts, a drying procedure is still necessary for the production of woodchip extracts. After cutting and during the storage of woodchips, the water content can change, and the wood as well as the bioactive compounds can decay over time. At the same time, the water content can strongly influence the decay rate of wood (Brischke and Rapp, 2008). A drying step provided an accurate amount of dry mass for the extraction standardization in this study; in addition it makes the transport and storage of woody material more conducive for a potential use in future.

Freeze drying dries out a frozen product by sublimation, in which the absence of liquid water and the low temperature can stop most of the microbiological reactions (Ratti, 2001). Freeze drying is therefore used more frequently in studies on phytochemicals, and found to be more efficient than other drying methods to maintain bioactive compounds in the dried materials (Bedgood Jr et al., 2005; Lin et al., 2012). Freeze drying was also reported to have better preservation of secondary phenolics in purple willow (*S. purpurea* L.) than other drying methods (Julkunen-Tiitto and Sorsa, 2001). These findings are consistent with our results. In our study, FD extracts had the stronger suppression on seed germination compared UD extracts, indicating more allelochemicals from woodchips were persevered by FD than by OD. Although the cost and energy consumption of freeze drying are often higher than that of air drying (Ratti, 2001), new technologies such as microwave freeze drying and atmospheric freeze drying are developed to save energy and cost (Duan et al., 2016). In conclusion, FD would be the most efficient drying method to maintain the herbicidal activity of aqueous extracts of woodchips but might not be the cheapest.

5.3 Milling and wood to water mixing ratio

At the cell level in plant material, extraction has two main mechanisms: diffusion through cell walls (for relatively small molecules), and rinsing out the cell contents via broken cell walls (Vinatoru, 2001). The extraction of woodchips might combine both processes, as allelochemicals can be either low or high molecular (Brunner et al., 1996; Traversa et al., 2010). The fiber length of wood is normally in the millimeter scale: 0.42 mm for second year *Salix* spp. (Kojima et al., 2009) and 1.03 mm for branches of *P. armeniaca* (Tajik et al., 2015). Milling (4 mm) might be able to break the cell walls in the wood to a certain extent, especially for the BC that have longer fibers. This might explain why the BC extracts became more suppressive for seed germination by milling.

Milling can increase the surface area of wood particles, which comes in contact with water. This might accelerate the dissolution of free allelochemicals from the wood. Beside our study, other authors found that milling increased the phytotoxicity of extracts of material with allelopathic potential (An et al., 1997). However, milling might not always follow “the finer the better” rule for extraction. Beside the energy cost for milling, the fine powder of woodchips can float on the water making mixing difficult. The combination of allelochemicals can have additive, antagonistic or synergistic effects (Einhelling et al., 1982; Yamane et al., 1992; Streibig and Olofsdotter, 2002). As a crude product, the aqueous extracts of woodchips (GW or BC) might contain several effective allelochemicals of which the chemical composition was unclear, but the overall trend was that higher WWRs lead to stronger suppression on seed germination among the tested WWRs (1:10, 1:15 and 1:20).

The suppression effect of BC extracts was already quite strong with the high WWR (1:10), while it was not so strong for the GW extracts with the high WWR (Fig. 3). In the first experiment testing for drying methods, when the WWR was even higher (1:5) though the wood was not milled, the suppression effect of GW extracts was stronger (Fig. 1). Thus, to achieve a strong allelopathic effect of BC extracts, WWR 1:10 can be a suitable choice; while for GW extracts, WWR 1:5 can be more effective.

5.4 Woodchips fraction (bark and core wood)

In a stem, the main role of the xylem is to transport water and mineral nutrients, while in the phloem (the innermost part of the bark) soluble photosynthates are translocated. Inhibitor activity of tree of heaven (*Ailanthus altissima*) on seed germination and seedling growth of garden cress (*Lepidium sativum* L.) was found to be higher in the bark than in the wood, suggesting phloem rather than xylem to be the pathway of allelochemicals (Heisey, 1990). In addition, bark is important for plant defense as phytotoxic secondary metabolites such as phenolic compounds are constitutive or induced (by the exotic wood-boring beetle) in the bark or phloem tissues to resist pests (Eyles et al., 2007). A study on plum trees (*Prunus domestica*) showed that phenolic constituents had higher concentrations in the outer bark, phloem and cambium than in the outer and inner sapwoods and in the heartwood (Hillis and Swain, 1959). Additionally, cutting trees is banned in springtime from the 1st of March in Germany, so the hedgerow pruning time is always before the regrowth of new leaves. Before transporting into the new leaves, allelochemicals are also likely to be distributed in the bark. In our study, the suppressive effect on seed germination was much stronger by the extracts of bark than that of core wood, and the extracts of core wood had very limited effect. This suggested that almost all allelochemicals are distributed in the bark fraction.

5.5 Potential use of woodchip extraction in organic farming

Evidences from the germination test suggested the existence of some bioactive and heat-intolerant organic compounds in the woodchips and especially in the bark, which are very likely to be allelochemicals such as phenolic constituents (Hillis and Swain, 1959). Both the germination suppression in petri dishes and the existence of bioactive compounds are good signs for potential herbicidal uses of woodchips, but still need to be tested on field. Only if its influence on emergence or growth of weeds was confirmed on field, further practical uses of woodchip extracts could be developed (Gruber et al., 2008; Wang et al., 2012; Xu et al., 2018).

Allelopathic activity is often due to the joint action of several allelochemicals rather than a single allelochemical compound (Einhellig, 1995). If suppression effects can also be detected on weeds under field conditions in future studies, both raw woody material and its crude products could be applied on field for herbicidal use, especially in OF. Based on the standardized extraction, a simple liquid product could be developed and used to satisfy practical needs of farmers without purification.

Moreover, simply using woodchips as mulching material is also possible, if the woody species were proved to have strong allelopathic potentials on weeds via standardized extraction and bioassay tests in the lab condition. The application rate of woodchips was always restricted as decomposition of organic materials with high C:N ratios can cause net N immobilization by the decomposers (Manzoni et al., 2008) and may reduce the plant-available N in soil, thus the low thickness of mulch layer could also limit the physical mulching effects (van Donk et al., 2011). In this case, allelopathic effects can be a welcome addition to the physical effects of woodchips mulching.

In all, using woodchips on farms for mulching and weed control, can add additional values to the hedgerows and can also influence farmers’ choices to maintain hedgerows and their biological value on the farm. Furtherly, in future studies, purification and analysis of the functional ingredients can help to find the effective chemical groups and take one step closer to the synthesis of bio-herbicides.

6. Conclusion

As the aqueous extracts of GW and BC woodchips can inhibit or suppress the seed germination of oilseed rape and wheat, these kinds of extracts can be further tested for potential herbicidal use. Freeze drying would be the most efficient drying method, and undried (defrosted) woody material would also be possible to use. Milling and a high wood to water ratio would be favorable for the extraction. In addition, bark wood is a better raw material than core wood for a highly effective extract. To achieve better allelopathic properties of the extracts, the conditions mentioned above should be applied for the extraction.

Although the active constituents in the extracts were not identified or separated, the standardized pre-treatment and extraction method still offers useful information for industrial product development. With this standardized testing approach, woody species showed strong germination suppression effect could be initially used as mulching on the field in OF and add additional values to the hedgerows.

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Physical fractionation of soil organic matter in a long-term trial with annual and perennial energy crops and different tillage intensities

Abstract:

Due to the need to substitute fossil fuels by renewable energies, energy crops become more and more important in German agriculture. So far, little is known about the impact of such cropping systems on soil organic matter dynamics and the distribution of carbon in different density and size fractions of the soil which may be decisive for the overall carbon turnover and the climate impact of such cropping systems. Therefore, the aim of our study was to compare the influence of different energy cropping systems on different physical fractions of soil organic matter. Soil samples were taken from a 12-year long-term trial comparing short rotation coppice (SRC) willow (*Salix schwerinii* E. Wolf × *viminalis* L.) and continuous maize (*Zea mays* L.) mono-cropping. An integrated physical fractionation method was used to separate light fractions (< 1.8 g cm^{-3}) of free- and occluded-particulate organic matter (POM) and heavy fractions (> 1.8 g cm^{-3}) of organo-mineral associations with three size levels (< 2 μm, 2-63 μm, 63-2000 μm) from the soil. After the 12-year cropping period, the soil under SRC willow had higher contents of both free-POM and occluded-POM (0.23% and 0.13%, respectively) than the soil under maize (0.09% and 0.06%, respectively), and also higher contents of sand- and clay-sized organo-mineral associations. The C:N ratios of SOM in the soil under willow were 28, 24, 16, 9 and 13 for free-POM, occluded-POM, sand-, silt- and clay-sized organo-mineral associations, respectively, which were generally higher than that (23, 18, 9, 9 and 11) of the maize soil. Generally, the SRC willow accumulated more OM both in the labile and stable SOM pools than the maize system in the top 20 cm soil.

Keywords: energy crops, willow, maize, soil organic matter, particulate organic matter, carbon, light fraction, heavy fraction, sand, silt, clay

1. Introduction

During the last decades, demand for renewable energy such as bioenergy from energy crops (plants used for energy production) was increasing. In contrast to fossil energy, renewable energy produced by plants can reduce CO_2 concentrations in the atmosphere and thus contribute to slowing down or reduce global warming (Bilgili et al., 2016). In European Union, the biomass needed for energy production is estimated to increase from 0.85×10^8 ton in 2005 to 2.23×10^8 ton of oil equivalents in 2030 (EU-Commission, 2014). There are many promising sources for biomass such as crop residues (Kim and Dale, 2004), perennial grasses (Clifton-Brown et al., 2004; Samson et al., 2005), and woody materials from short rotation coppice on arable land or from forests (Wright, 2006). Besides of the regeneration of bioenergy from energy crops, their carbon neutrality, and even their potential to sequester carbon in soils in the long-term is subject of research (Ma et al., 2000; Lemus and Lal, 2005; Clifton-Brown et al., 2007).

The carbon fractions represented in different soil organic matter (SOM) pools have different mechanism of composition and stabilisation (Lützow et al., 2006). For example, SOM includes plant, animal and microbial residues in different stages of decomposition; and the residence time of plant litter can be as short as 0.1-0.5 years, but the residence time of SOM can vary from 1-2 years (active) to 15-100 years (slow), and it can even remain as long as 500-5000 years (passive; Dungait et al., 2012). Soil organic matter is a changing pool, where the stock is controlled by both, the input of net primary production and by the decomposition. The turnover of SOM can be influenced by vegetation type, soil disturbance, land use change, and some other management practices (Six and Jastrow, 2002). In the case of energy crop production, there are also many management factors which can affect the soil carbon sequestration or release, such as crop species (annual versus perennial), fertilization, soil tillage, and harvest management (Lemus and Lal, 2005; Anderson-Teixeira et al., 2009). So far, various studies describe increased soil organic carbon (SOC) contents under energy crops including both annual and perennial, woody and herbaceous crops (Zan et al., 2001; Tolbert et al., 2002; Mi et al., 2014), but they did not differentiate different fractions of SOC. The distribution of SOM in different soil fractions could determine turnover rates which influence C-storage and C losses (Christensen, 2001).

Among the most frequently used energy crops, there are both herbaceous and woody species, and species with annual or perennial life cycles. Annual energy crops are usually grown on arable land, and in agronomic systems, in which the soil is usually tilled. As tillage results in soil disturbance, it can break up aggregates and expose the organo-mineral surfaces to decomposers (Six et al., 1999). Therefore, much soil carbon would probably be mineralized and oxidized following to tillage operations. Perennial energy crops need primary soil tillage usually only at the time of their establishment on the field (planting), so that soil disturbance is generally low, and SOM (carbon) can be maintained or even accumulated. Additionally, as no mixing of the topsoil takes place in the perennial energy cropping systems that induces mineralization, woody energy crops can have a higher carbon input from leaf-litter and a higher C:N of the leaf-litter compared to herbaceous energy crops and thus a greater SOC accumulation rate in the topsoil layer (Chimento et al., 2016). Besides of leaf-litter and tillage, the rooting system, particularly the amount of fine roots, can also greatly influence the SOC (Chimento and Amaducci, 2015). For annual crops without litter fall the belowground C input is especially important for the SOC stock (Johnson et al., 2006).

Fractionation of SOM was subject in various studies of soils in cropping systems for food production, and for natural ecosystems (Roscoe and Buurman, 2003; Galantini and Rosell, 2006; McLauchlan et al., 2006; Sollins et al., 2006). The fractionation methods used in most studies primarily differentiated in three mechanisms: physical (e.g. density or size), chemical (e.g. solubility, hydrolysability, or resistance to oxidation) or biological (e.g. incubation) (Stevenson, 1965; McLauchlan and Hobbie, 2004; Plante et al., 2011). Among these, the physical fractionations emphasize the important interactions between organic and inorganic soil components, and can separate the free and occluded, but- uncomplexed SOM. These methods can separate the primary and secondary organo-mineral associations which are crucial in the understanding of SOM turnover and storage (Christensen, 2001).

Maize (*Zea mays* L.) is a widely-used energy crop for biogas production (Amon et al., 2007), and was found to be the most high-yielding annual cropping system in a comparison of several annual and perennial energy cropping systems (Xu et al., 2017). Willow (*Salix schwerinii* E. Wolf × *viminalis* L.) short rotation coppice (SRC) is commonly used for combustion to produce energy (Fuentes et al., 2008) and was found to have the highest SOC stock in the 0-90 cm soil profile especially in the topsoil (0-10 cm) in the same experiment (Gauder et al., 2016). As the aboveground biomass yield of the systems is known, the belowground biomass and the organic litter input into soil can be estimated. However, there is a knowledge gap on the relation between the input and the stock of SOM. A study on the different fractions of SOM in these energy cropping systems might help to better understand the SOC sequestration by energy crops and the sustainability of energy cropping systems.

The aim of this study was to compare the influence of two contrasting energy crops on SOM fractions (labile or passive, unprotected or protected by soil minerals). The method for fractionation integrating both, density and particle size fractionations, was established and used on the soil taken from a long-term field trial of annual and perennial energy cropping systems. Soil samples from typical high-yielding annual (maize) and typical high-C-sequestration perennial (willow) energy crops were used in this study.

2. Materials and methods

2.1 Site description and field trial layout

The sampling site is part of a long-term field trial carried out in southwest Germany at the experimental station Ihinger Hof (48.44´N, 8.56´E, 480 m a.s.l.) of the University of Hohenheim. From 2001 to 2013, the field trial with annual and perennial energy crops was run in a split-plot design with cropping system as the main plot factor and the nitrogen fertilization as the subplot factor; for each treatment four replicates were conducted (Xu et al., 2017).

Among all the combinations of cropping systems and nitrogen levels, two cropping systems with a medium nitrogen application (Table 1) were chosen to study the SOC content in different soil fractions: (i) W-SRC: perennial system with willow (*Salix schwerinii* E. Wolf × *viminalis* L.) short rotation coppice (SRC) with three-year cutting intervals; (ii) M-MC: annual system with maize (*Zea mays* L.) in mono-cropping and with reduced tillage.

The climate is characterized as humid temperate. During the experimental period, the mean air temperature was 8.4 °C, and the mean annual precipitation was 713 mm. The soil type is a Haplic Luvisol (Siltic) (IUSS, 2015). The texture of the soil is silty clay covered by loess loam.

Table 1. Energy cropping systems of perennial willow short rotation coppice and annual maize mono-cropping, the tillage management, nitrogen fertilization level, and annual biomass yield in 2001-2013.

Cropping system	Crop	Tillage	N fertilization (kg N ha^{-1} yr^{-1})	Biomass yield (t ha^{-1} yr^{-1})[a]
W-SRC	willow (*Salix schwerinii* E. Wolf × *viminalis* L.)	NT[b]	40	11.3
M-MC	maize (*Zea mays* L.)	RT[c]	120	16.1

[a] From Xu et al., (2017);

[b] NT=no tillage;

[c] RT=reduced tillage to 7-cm soil depth using a rotary harrow.

2.2 Soil sampling, fractionation, and C, N analysis

The soil samples were taken on March 1st 2013. Four cores of soil were taken per plot (4m × 8m) by an auger (2.5 cm Ø) in the middle of the crop rows, in a depth of 0–20 cm. Soil cores were separated into two depths (0–10 and 10–20 cm), and soil from the same depth was mixed as one sample for each plot. Soil samples were sieved to 2 mm and air-dried.

In the laboratory, three sub-samples were taken from the dried and completely mixed soil samples as lab-replicates. A combined procedure of density and particle size fractionation modified from Mueller and Koegel-Knabner (2009) was used. The fractionation was conducted using the following steps (Fig. 1.): An amount of 30.00 g soil was added to 150 mL of sodium polytungstate solution (SPT, 1.8 g cm^{-3}). The soil-SPT suspension was gently stirred and stood overnight to reach full settlement. The free particulate organic matter (f-POM), was extracted by removing the supernatant with a water jet pump. After removing the floating f-POM, this step was repeated once again to remove any leftover f-POM. The f-POM was put on a nylon membrane filter (0.45 μm), rinsed with deionized water and filtered by suction to remove the remaining SPT residues from the f-POM. Washing was stopped when the electric conductivity of the filtrate dropped below 50 μS cm^{-1}. The soil-SPT slurry was again mixed with 150 mL of 1.8 g cm^{-3} SPT, and dispersed using an ultrasonic treatment (60 J mL^{-1}). After the suspension stood overnight, floating occluded POM (o-POM) was obtained as described for f-POM. Washing was done by adding distilled water into the slurry, abundant stirring and centrifuging the mixture in about 250 mL portions for 20 min at 4000 r min^{-1}. The supernatant was removed using a water jet sucking pump. After washing, the remaining soil was wet sieved to > 63 μm to get the sandy fraction. The sandy fraction was again mixed with 150 mL of 1.8 g cm^{-3} SPT to extract more residual o-POM. The remaining part was transferred into a 1 L cylinder with about 1 L distilled water. Silt (sized 2-63 μm) and clay (sized < 2 μm) fractions were separated by sedimentation using the pipette sampling method (Day, 1965).

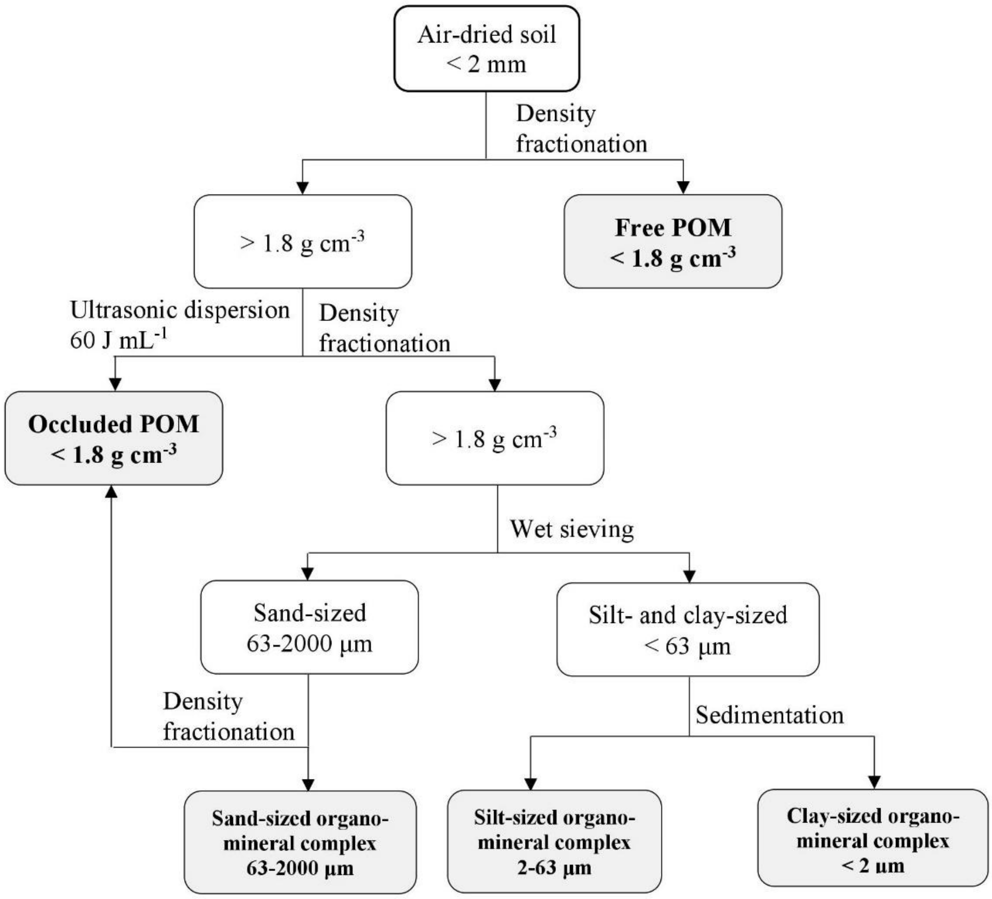

Fig. 1 Flow chart of the procedure of density and size fractionation. POM=particulate organic matter

All the wet fractions derived from the above procedure were frozen (-25°C) and then freeze dried. For each fraction, organic carbon and nitrogen concentrations were measured with a CN analyzer (vario Macro, Elementar Analysesysteme GmbH), and each fraction of one sample was separated into three sub-samples for the measurement.

2.3 Statistical analysis

From the three measurements of organic C and N per fraction the average was calculated for statistical analyses. A two-way ANOVA was used to examine the effects of crop, soil depth and their interaction, on soil fraction weight, organic carbon in soil fractions and C:N ratio of the organic matter in the soil fractions. Multiple comparisons were done based on LSD means only in the case of significant F-tests for the corresponding term. Significances were presented via letter display where means with the same letter are not significantly different from each other. Means and contrasts between the effects were compared at $\alpha < 0.05$. All the tests were performed using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1 Fraction weight

Generally, the recovery rate of the soil sample after fractionation was 97.9% on average. The contents of free-POM (<1%), occluded-POM (<0.5%) and sand (<2%) were much lower than that of silt (about 76%) and clay (about 20%) in the topsoil (0-20 cm). After 12 years of energy crop cultivation, the soil fractions in the topsoil (0-20 cm) were significantly different under maize and willow (Fig. 2). Both the contents of free-POM and the occluded-POM were significantly (107% and 61%, respectively) higher in the willow system than in the maize system; and significantly higher in the top layer (0-10 cm) than in the deeper layer (10-20 cm) of the soil. The sand content was highest in the top 10 cm under willow; the silt content was not significantly different across the crop and depth; and the clay content was significantly higher in the top 10-cm soil of willow than that of maize. In addition, the crop species significantly influence the fractions in the shallow layer (0-10 cm) but not in the deep layer (10-20 cm).

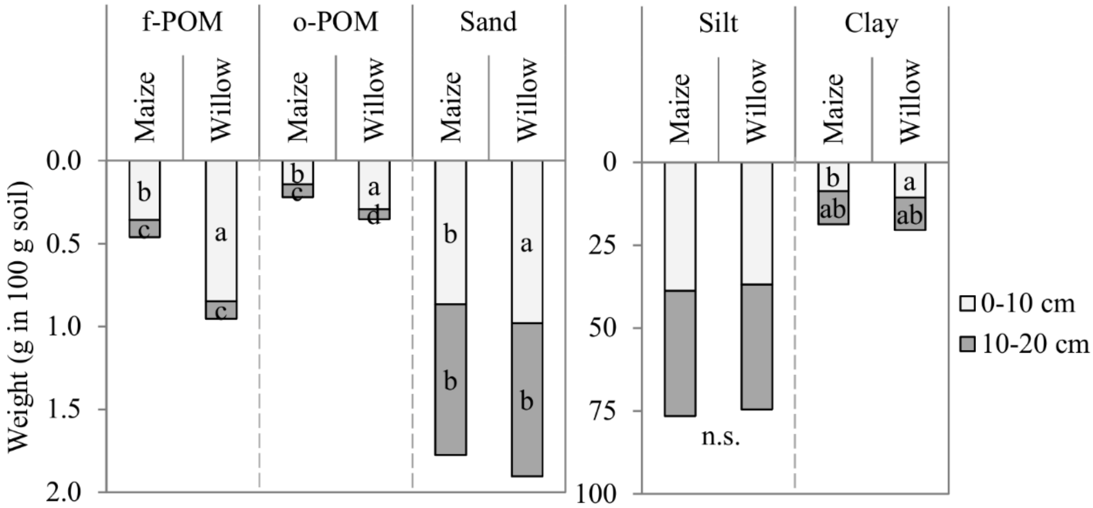

Fig. 2 Weight (g in 100 g soil) of different soil fractions separated by an integrated fractionation method; soils from a 12-year field experiment (mono-cropping of maize and short rotation coppice of willow). For each fraction, significant differences among the crops and soil depths are indicated by letters at $P<0.05$; f-POM=free particulate organic matter; o-POM=occluded particulate organic matter.

3.2 Carbon and nitrogen content

Generally, the mean concentrations of total organic carbon (TOC) were significantly higher in the soil (0-20 cm) under willow (1.39%) than under maize (1.13%), and on average higher in the top layer (0-10 cm, 0.74%) than in the deeper layer (10-20 cm, 0.52%). Among all fractions, the TOC associated with clay and silt accounted for the highest share of the whole SOC; and the TOC proportion of the free-POM and occluded-POM were relatively high compared to their weights (Fig. 3). In the top 10 cm of the soil, the TOC concentrations of both free-POM and occluded-POM were significantly higher in the willow system (36.4% and 19.7%, respectively) than in the maize system (10.7% and 6.8%, respectively) and significantly higher in the top layer (0-10 cm) than in the deeper layer (10-20 cm) of the soil.

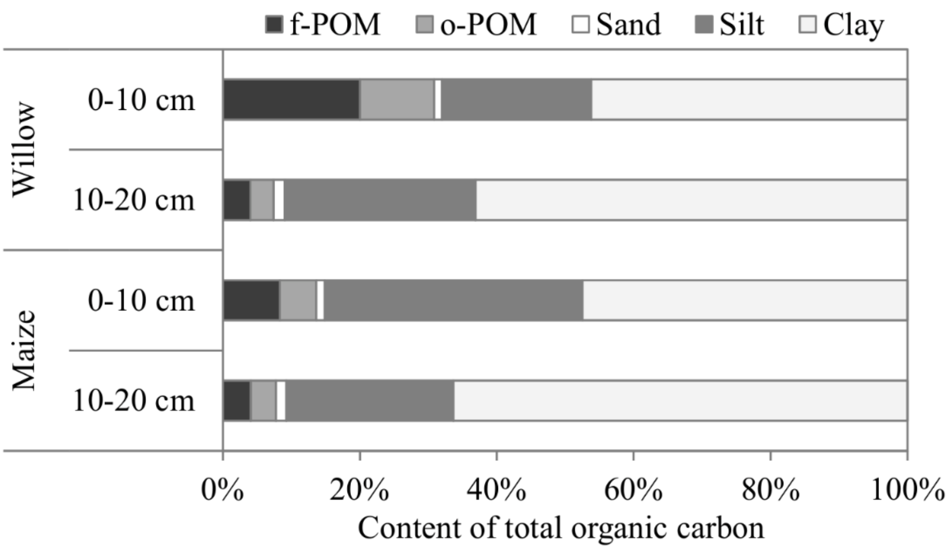

Fig. 3 Content of total organic carbon (%) in different soil fractions accounting for the whole soil; fractionation by an integrated fractionation method; soil samples from 12-year mono-cropping maize and short rotation coppice willow grown for energy biomass production. f-POM=free particulate organic matter; o-POM=occluded particulate organic matter.

After growing the two energy crops for 12 years, the SOC in different soil fractions in the topsoil was also significantly different between maize and willow (Fig. 4). In the top 10 cm of the soil, the content of TOC in all fractions except silt, was significantly higher under willow than under maize (Fig. 4). Particularly the TOC of free-POM under willow was as high as 0.42 g 100 g^{-1} in the top 10 cm of the soil. However, in 10 -20 cm depth, the TOC content in all fractions was not significantly different between willow and maize (Fig. 4).

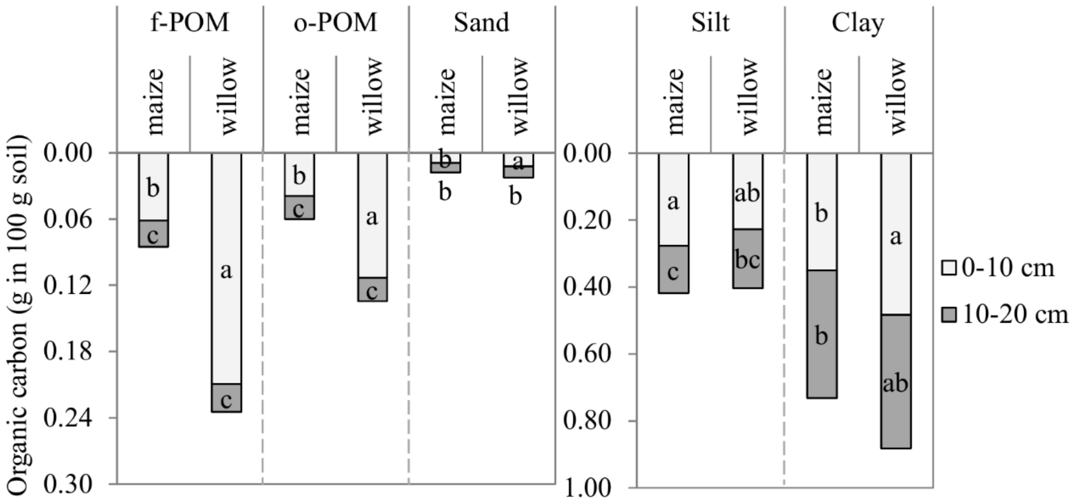

Fig. 4 Concentration of total organic carbon (TOC) in different soil fractions (g in 100 g soil) depending on soil depth; separated by an integrated fractionation method; soils from a 12 year field experiment comparing different energy crops (mono-cropping of maize and short rotation coppice of willow). For each fraction, significant differences among the crops and soil depths are indicated by letters at $P<0.05$; f-POM=free particulate organic matter; o-POM=occluded particulate organic matter.

3.3 C: N ratio of the soil organic matter in different fractions

The C:N ratio of the SOM was significantly higher in the willow system than the maize system (Fig. 5) for the fractions of free-POM, occluded-POM and sand. For both cropping systems, the C:N ratio was always the highest in the free-POM and then the second highest in the occluded-POM. The free-POM of the top 10 cm of the soil under willow was rich in C with a C:N ratio as high as 30.4.

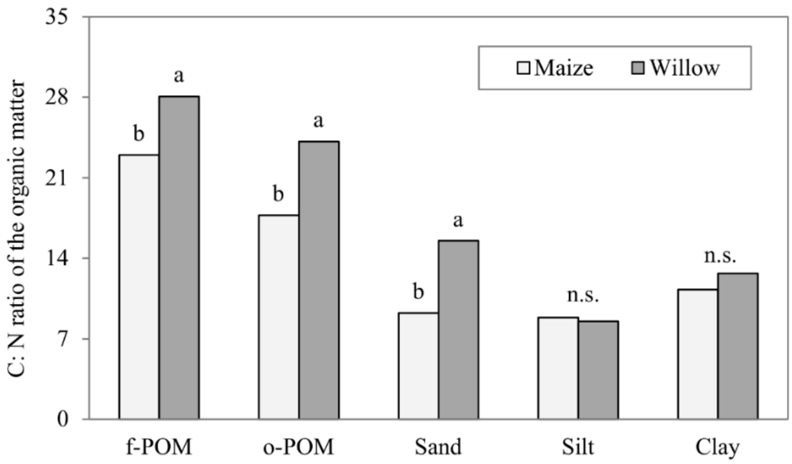

Fig. 5 C:N ratio of soil organic matter in different soil fractions separated by an integrated fractionation method; soils (0-20 cm) from a 12 year field experiment comparing different energy crops (mono-cropping of maize and short rotation coppice of willow). For each fraction, significant differences among the crops are indicated by letters at $P<0.05$; and the two soil depths were merged as the depth did not have significant effect; f-POM=free particulate organic matter; o-POM=occluded particulate organic matter.

4. Discussion

4.1 Fractionation method and recovery rate

Different physical fractionation methods are able to separate uncomplexed OM in soils, primary organo-mineral associations, and secondary organo-mineral associations (aggregates) (Christensen, 2001). In this study, after the integrated fractionation procedure consisting of a density fractionation and particle size fractionation, the uncomplexed OM (free vs. occluded) and the primary organo-mineral associations in different particle size classes (<2 μm, 2-63 μm, and 63-2000 μm) were clearly separated. As a high recovery rate of 97.9% was reached, this fractionation method was efficient. Moreover, C and N contents of the different fractions were clearly distinct, which also hints that the method functioned properly.

4.2 The light fractions: free and occluded particulate organic matter

Separation of POM is usually done via soaking soil in heavy liquids with varying densities (from 1.2 g ml^{-1} to 2.0 g ml^{-1}) (Kölbl and Kögel-Knabner, 2004; Helfrich et al., 2006; Recio-Vazquez et al., 2014; Mani et al., 2017). In this study, a density of 1.8 g ml^{-1} was chosen after adjustments in the pre-tests to separate both f-POM and o-POM. For the o-POM, we assumed that this fraction is not in very close physico-chemical interaction with the mineral phase. For this reason, a destruction of micro-aggregates will release the o-POM and a density separation can be done at the same density of the SPT as used for separating the f-POM. To destroy the micro-aggregates, an ultrasonic treatment on the diluted sample similar to other authors was used after f-POM removal (Kölbl and Kögel-Knabner, 2004). The treatment was done with a low energy input (60 J mL^{-1}) as this considered to be the minimum energy input that avoids any physical damage of organic particles and organo-mineral associations leading to a redistribution of OC in different size classes (Kaiser and Asefaw Berhe, 2014).

As shown in the results, both the f-POM and o-POM contents were significantly higher under willow than under maize and were mostly found in the top 10 cm of the soil. This finding might be explained by the origin and formation of POM: POM (f-POM and o-POM) can be considered as a transitory pool between litter and the mineral-associated OM (Christensen, 2001), so the litter input can greatly influence the POM. Willow is a deciduous perennial plant and can produce large amount of annual litter fall (1-5 t ha^{-1} $year^{-1}$) (Verwijst and Makeschin, 1996). As an energy crop, although maize is more high yielding than willow, the whole aboveground biomass of maize is removed from the field at harvest and little stubble is left on the soil. Additionally, according to the annual biomass yields (Table 1) and shoot: root ratios of willow (1.75, Heller et al., 2003) and maize (6.08, Amos and Walters, 2006), the root biomass of willow (6.5 t ha^{-1}) can be also higher than maize (2.6 t ha^{-1}). As both the potential litter and root biomass return into soil are higher in the willow system, the OM input into the SOM pools can be also higher.

Besides of the input of OM, the decomposition of OM is also important for the SOM stock. Tillage can strongly influence the SOM dynamics by the disruption of the soil structure and releasing physically protected OM, and by changing the soil temperatures and water dynamics as well as the litter incorporation (Balesdent et al., 2000). In this study, for willow, tillage was only done in the year of establishment, while reduced tillage (7-cm rotary harrow) was done every year for maize. It is very likely that less soil disturbance in willow compared to maize favored the POM formation and accumulation in the willow system. In addition, because no tillage was done after the establishment of the woody crop, willow had a relatively dense understory weed vegetation. Although not favorable for biomass production of willow, the weed biomass can also contribute to the SOM stock.

Despite of the similarity of f-POM and o-POM in distribution in depths and the relative pool size in the two cropping systems, the properties of the f-POM and o-POM are different. Free-POM consists of large, undecomposed or partly decomposed root and plant litter (Golchin et al., 1994), it can be either loosely distributed in soil or adhering inter-aggregates (Christensen, 2001). When f-POM is associated with the mineral matrix, only individual primary mineral particles are adsorbed on the POM surface and the association level is low (Golchin et al., 1997). In comparison, o-POM is normally decaying plant debris associated with mineral particles and it is the intermediate between f-POM and high density fractions (Wagai et al., 2009). Commonly, during decomposition of OM, C is oxidized by heterotrophic microbes while N is relatively conserved, as the soil microbial has a much lower C:N ratio (25 in average) than plant biomass (Heal et al., 1997). Thus, the C:N ratio should decrease from f-POM to o-POM because the latter is more degraded. This is consistent with the result of this study that the f-POM generally had higher C:N ratio than the o-POM.

The difference in C:N ratios of the POM between the two cropping systems can also be explained by the litter input of the two systems. The litter of willow is carbon-rich (C:N=47) (Swan and Palmer, 2006) and lignin-rich (20%) (Chauvet, 1987), while the roots of maize are not (C:N=22) (Janssen, 1996). As a result, both the f-POM and o-POM in the soil under willow had higher C:N ratios than that in the maize plots. Furthermore, the C:N ratio is related to the microbial decomposition rate and in other words "the recalcitrance" of OM (Tian et al., 1992). Although the POM is a relative labile SOM pool (Besnard et al., 1996), it still can be inferred that the POM derived from of willow is more resistant to soil microbial decomposition than that of maize.

4.3 The heavy fractions: carbon in sand, silt and clay

The heavy fractions are mineral-dominant, and the OM in these heavy fractions has been intensively transformed by microbial activities and strongly bound to soil minerals resulting in relatively slow turnover rates (Christensen, 1992; Hassink, 1995; Neff et al., 2002). The C:N ratio of the heavy fractions can be smaller than that of the light fractions in the same soil (Sollins et al., 1984), which was also found in this study particularly in the silt- and clay-sized fractions. As the end products of OM decomposition is normally independent of the OM source (e.g. plant species) (Oades et al., 1988; Randall et al., 1995), in our study there were no significant difference of the C:N ratio of the OM in silt and clay-sized fractions between the willow and maize systems.

Clay particles (because of their mineralogy), surface charge characteristics and Fe and Al oxides brought by precipitation, can provide most surface area and high capacity to adsorb OM and so as to stabilize OM (Baldock and Skjemstad, 2000). In our study, the clay-associated OC accounted for the largest proportions of the SOC, indicating that clay might be a more important pool for sequestering OM than silt. There were no significant differences of the silt-sized fraction between cropping systems or between the soil depths, while the clay-sized fraction of willow contained a larger proportion of the whole soil than that of maize, which might be explained by a heterogeneity of the sites.

Tillage can reduce the number and stability of soil aggregates and can cause OM depletion (Six et al., 2000). The maize system had a lower content of the sand-sized fraction and lower C concentrations in the sand-sized fraction in the top 10 cm of the soil which might be explained by the tillage practice. The lower C:N ratio in the sand-sized fraction of maize compared to that of willow might be explained by the difference of OM source.

4.4 The carbon sequestration potential of the two cropping systems

On arable land, soil carbon stocks were measured to a depth as shallow as 30 cm (Smith et al., 2000), and as deep as 100 cm (Leifeld et al., 2005). The SOC distribution was considered to have an exponential relation with the soil depth (Mishra et al., 2009), and the topsoil could contain most of the SOC in the whole soil profile. Woody energy crops were reported to tend to locate most of the roots in the topsoil layer (0-30 cm): willow had 37% roots in the top 10 cm of soil and 77% in the top 30 cm of soil (Chimento and Amaducci, 2015). Maize root was also reported to be distributed mostly (90%) in the top 30 cm of soil (Dwyer et al., 1996). Together with the litter fall of willow on top of the soil, most of the OM input in the two cropping systems are allocated in the topsoil. Therefore, in our study, only the SOC in the topsoil (0-20 cm) was evaluated and compared between the two contrasting energy crops, because the topsoil interacts with the energy crop production more intensively. The willow system had a higher SOC content in the topsoil compared to the maize system, even when the clay associated SOC was excluded. Thus, there could be a higher potential for the willow system to sequester C in the topsoil.

However, for the deep soil horizons , dissolved OM can be leached from the topsoil through root channels or bioturbation (Rumpel and Kögel-Knabner, 2011). The accumulation of C in the deep soil layer could be also important for the C sequestration (Powlson et al., 2011), as oxygen is limited in the deep soil layer and the mineralization of SOM is reduced (Ekschmitt et al., 2008; Rumpel and Kögel-Knabner, 2011). In future studies, the deeper soil horizons should also be considered to assess the C sequestration potential of energy crop production.

5. Conclusions

The woody energy cropping system (short rotation coppice of willow) seems to provide more labile (light) SOM and stable (heavy) SOM particularly associated with organo-mineral associations in the clay fraction, probably because of the large amount of litter input from willow, the different composition of the biomass and a lower soil disturbance. SOC sequestration induced by certain cropping system was always considered to have reversibility after land use is changed. However, the higher content of heavy SOM in the willow system could last longer after willow was changed to another crop. Therefore, in the view of carbon sequestration in the topsoil, SRC willow can be considered as more efficient than continuous mono-cropping of maize. Besides willow and maize, different other perennial and annual energy crops exist, e.g. switchgrass (*Panicum virgatum*), poplar (*Populus* spp.) or miscanthus (*Miscanthus* × *giganteus).* Currently their cropping is promoted by policy makers to substitute fossil fuels. To gain a better understanding of the overall impact of the use of such crops for energy production, their impact on carbon sequestration is an important factor e.g. when life cycle analyses are done to assess the environmental impact of energy production. So far, little is known about the distribution of SOM in different soil pools in other energy crops and further analyses on the carbon distribution in different soil pools would give a better understanding on the stability of SOM and ultimately on their potential of carbon sequestration which affect the overall climate impact of these systems.

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Yields of Annual and Perennial Energy Crops in a 12-year Field Trial

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ABSTRACT

To find an energy cropping system with low input and high productivity, a 12-yr field trial was conducted in Southwest Germany with perennial crops, and monocropping or rotation of annual crops. The perennials were willow (*Salix schwerinii* E. Wolf × *viminalis* L.) short rotation coppice, miscanthus (*Miscanthus* × *giganteus* Greef et Deu.), and switchgrass (*Panicum virgatum* L.). The monocropped annual was maize (*Zea mays* L.), and the rotation was oilseed rape (*Brassica napus* L. ssp. *oleifera*)–wheat (*Triticum aestivum* L.)–triticale (*Triticale* × *triticosecale* Wittmack). The rotation was split into tillage with moldboard plow or no-till. These systems were implemented with three N fertilization levels. Annual yield trend in years, accumulated yields of biomass, and gross energy were compared across N levels between perennials and annuals, and between tillage managements. Among these cropping systems, maize (18.5 Mg ha^{-1} yr^{-1}) and miscanthus (18.3 Mg ha^{-1} yr^{-1}) were most productive. Without N fertilization, miscanthus was most productive (13.6 Mg ha^{-1} yr^{-1}). In the long run, N fertilization significantly increased the yields of all systems. The long-term yield trends of the perennials were relatively stable, while the annuals without N fertilization showed a prevailing trend of yield decrease. No-till did not significantly lower the yield compared to plowing (8.7 vs. 9.3 Mg ha^{-1} yr^{-1}). Generally, the perennial systems produced higher gross energy yield compared to the annual systems under comparable N fertilizations. Particularly miscanthus gained high yields even with moderate to no N inputs.

Core Ideas

- A long-term field trial of important perennial and annual energy crops was conducted.
- Maize is most high yielding of biomass with high N inputs.
- Miscanthus is most high yielding of biomass without or with moderate N inputs.
- In crop rotations, no-till practice with less input does not reduce the productivity.
- Nitrogen input is more important in annuals than perennials to maintain the yield level.

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Renewable energy (RE) sources have become more important in recent years, since it is assumed that their development can slow down climate change and reduce human dependence on fossil fuels (Cornelissen et al., 2012). The utilization of biomass as an energy source is a promising option for achieving this goal. Biomass has attractive advantages compared to other RE sources, such as multiple approaches of energy conversion as well as being the only source among RE which can easily be converted to transport fuels (Edenhofer et al., 2013). The newly set out vision for European Union climate and energy policy until 2030 was also predicted to support the biomass-based RE production (Allen et al., 2014). The biomass demand for energy purpose can be increased to 166 to 223 Tg of oil equivalent in 2030 according to the impact assessment of the European Commission (EU-Commission, 2014).

The biomass for RE can originate from various sources, and the energy crops are predicted to have the greatest contribution in the future (Deng et al., 2012). Nowadays, species and cultivars are selected and further developed to adapt to specific conditions and for specific purposes in energy production. Diversified bioenergy cropping systems are being used worldwide.

However, it is still not easy to predict the possible contribution of biomass toward the future global energy supply since one of the most crucial parameters, the yield level, remains partially uncertain on a regional scale (Berndes et al., 2003). The existing studies about energy crop yield mostly compare different genotypes of one species (Piscioneri et al., 2000; Clifton-Brown and Lewandowski, 2002), or groups of species with similar physiological or growing patterns (C_4 grasses, short rotation coppice [SRC], forest, grain crops). Direct comparisons of cropping systems such as annuals vs. perennials are often not analyzed beyond a 3-yr time span (Propheter et al., 2010; Zeri et al., 2013; Wilson et al., 2014). Though many energy crops have been studied in separate approaches, there is a lack of long-term experience with simultaneous evaluation of different energy crops and cropping systems at the same location.

Due to the different growth patterns of annual and perennial species, plant development, productivity, the use of resources, and the energy stored in the biomass can vary considerably. Perennial plants need higher labor and capital inputs during the initial growth stage (Powlson et al., 2005); willow, for instance, is established by planting twigs and miscanthus by

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Abbreviations: CR, crop rotation; DMY, dry matter yield; HHV, higher heating values; HV, heating value; RE, renewable energy; SOC, soil organic carbon; SRC, short rotation coppice.

7. General discussion

In the previous chapters (I-IV), several aspects of plant-soil interactions were evaluated, in the case where woody and herbaceous plants or plant materials are used on arable land. Results of the individual studies were presented and discussed specifically for each study. In the following chapter, the results from chapter I-IV are taken up again, for a general discussion with an extended perspective and a linkage between the chapters, and answers are given to the initially raised objectives of the overall study.

7.1 Effects of residues from woody plants on farmland

7.1.1 Effects on crop yield

Several potential effects of residues of woody plants can be considered to affect crop yield, and a selection of them is discussed in this chapter here, namely allelopathic effects, nitrogen availability, soil temperature and soil water.

Allelopathic effects can improve the growing conditions of a crop so as to increase the yield, by natural control of weeds (Cheema and Khaliq, 2000) or pests (Farooq et al., 2011), by a reduction of plant parasites in soil (D'Addabbo et al., 2011), or by a suppression of plant pathogens and diseases (Liu et al., 2009). On the contrary, allelopathic effects can also be harmful to the crop itself and thus decreasing the crop yield, by direct impediment of crop establishment or inhibiting growth, e.g. reducing germination, reducing root elongation and decreasing leaf area expansion (Oyun, 2006; Siddiqui et al., 2009).

In the long-term study on wood chip mulching (chapter I), the straw and biomass yields were significantly lower in the plots with wood chip mulching (WCM), and the relative yield in the mulched plots compared to the unmulched ones had a decreasing trend with the year in general. This unfavorable effect on vegetative growth of the crops can be ascribed to allelopathic effects of the woody material, and, moreover, probably also to an accumulation of allelochemicals over time in the soil. The indication that there actually exist effects of wood chips ingredients on plants is demonstrated in chapter II. Seed germination was inhibited by aqueous extracts from wood chips; therefore, substances which interfere with plant growth can be assumed to be leached from wood chips also in the field, and thus assumed to affect crop yield, even though there was no clear evidence of accumulation of allelochemicals in soil in former studies. However, some allelochemicals, such as phenolic compounds which are common components in woody tissues (Loomis and Battaile, 1966), were reported to inhibit decomposer activity (e.g. soil fauna and fungi) from decomposing the plant litter in soil (Chomel et al., 2014). A slowed down decomposition of plant residues with allelopathic effects may lead to their accumulation, and together with newly input residues they may increase the allelochemical concentration in soil resulting in a self-reinforcing process.

Besides, when woody plants such as hedgerows, or short rotation coppice, are cultivated on a farm, allelopathic interactions with adjacent crop fields can develop (Kidanu et al., 2005). Allelochemicals can be dissolved in rain water and move with flows in the soil or with the mycorrhizal networks (Kobayashi, 2004; Achatz et al., 2014). As the study showed that not all crops responded to wood chips application in terms of yields, growing crops tolerant to allelopathic effects or using woody species with low or without allelopathic effects on farmland could help avoiding any unfavorable effects. That would allow to benefit from ecological effects of woody plants on farmland (e.g. hedgerows, or other agroforestry systems), and prevent crop loss because of interactions between crops and woody plants.

Woody residues could also influence the crop yield by reducing the plant available N in soil. Compared to herbaceous plants, woody plants have residues with both high C and lignin contents (McKendry, 2002). In chapter III, SOM derived from willow (C:N ratio of leaf and root litter can be as high as 60; Jug et al., 1999) accumulated in soil. It can lead to reduced availability of nitrogen when woody residues are decomposed to form soil microbial biomass with a much lower C:N (Heal et al., 1997; Cleveland and Liptzin, 2007). Some common constituents in woody tissues, such as terpenoids, was reported to decrease nitrification potential and aggravate the N unavailability for crops (Adamczyk et al., 2013).

When woody residues are used as mulching on the soil, they can influence the soil microclimate in several ways. Organic mulching can lower the soil temperature by reflection of sunlight; decrease or increase the soil temperature by reducing heat-exchange with warmer or cooler surface air; increase the soil temperature by accumulating humus in the topsoil and deepening the soil color in the long run (Bussière and Cellier, 1994). On one side, as found in the field study of chapter I and in other studies (Gruda, 2008; van Donk et al., 2011), the wood chip mulch wood retain more water in the soil and this would also benefit crop yield, particularly in dry years. On the other side, increased soil water content might also result in lower soil temperatures and higher denitrification which might affect N mineralization, crop germination and development in spring.

7.1.2 Effects on weeds

Generally, the effects of woody plants and their residues on crops (chapter 7.1.1) are supposed also to apply for weeds. All the crop yield promoting effects (e.g. water retention, increased soil temperatures) could also increase weeds, and the yield reduction effects (e.g. allelopathic and N-immobilization effects) could also decrease the number of weeds. While these effects that disturb the crop growth or reduce crop yields should be avoided in crop production, the same effects are wanted for weed control. In conventional farms, there are various synthetic herbicides available for an effective weed control. In organic farms, where chemical synthetic herbicides are not permitted the potential effects of woody residues on weeds could be considered for the integration in the overall weed management of a farm (Wang et al., 2012).

In the current studies the hedgerow wood chips were shown to have suppressing effects on seed germination (chapter II) and on weed numbers in the field (chapter I). The weeds suppression is likely to be related to three impacts including physical impediment, allopathic effects and N deficiency. In the experimental design of the underlying study (chapter I) physical impediment is expected to have a stronger effect on weeds than on crops, because the wood chips mulch has been applied several weeks after crop emergence by hand between the rows. When used as mulch, woody residues (e.g. bark) can reduce weeds emergence by covering the soil with obstructing particles and by reducing the exposure of the soil surface to light which triggers weed germination (Teasdale and Mohler, 2000). Besides of the direct physical effects on weeds and the indirect physical effects on the soil surface microclimate, allelopathic effects of woody material or woody residues have been studied and described. Extracts and leachates of woody materials were found to have allelopathic effects on weeds in laboratory, pot and field studies (Heisey, 1990; Khanh et al., 2005; Rathinasabapathi et al., 2005). There are various typical allelochemicals, e.g. juglone from black walnut (*Juglans nigra* L.), phenolic compounds and terpenoids from many tree species (Kuiters and Denneman, 1987; Jose and Gillespie, 1998; Heisey, 1999). Additionally, soil mineral nitrogen availability for weeds can be also decrease because of the high C:N ratio of the woody residues used on soil.

7.1.3 Effects on soil properties

Woody residues applied on soil can increase the soil organic matter (SOM), which has been demonstrated in many studies (Salomon, 1953; Yao et al., 2005; Scharenbroch, 2009). An elevated level of SOM can contribute to carbon sequestration in the soil and mitigation of climate change. In chapter III, the soil under SRC willow had higher SOM and SOC content in the upper soil layer (0-20 cm) than the soil under mono-cropping of maize. The SOM associated with light fractions (free- and occluded- particulate organic matter) represent a labile pool of SOM, and were always higher in the soil under willow than that under maize (chapter III). This can be related to the large amount of annual leaf litter shed on soil and to the large root system of willow. Soil disturbance such as tillage can break the organo-mineral associations and aggregates in soil, so that OM associated with or protected by minerals can be released and exposed to decomposers. Willow as a perennial crop is usually not tilled after plant establishment, so soil disturbance is reduced, and accumulation of SOM and SOC takes place. Additionally, the SOM in the light fractions is less decomposed, the high C:N ratio of the willow compared to maize residues also lead to relatively high C:N ratio of the OM in light fractions. High C:N of the SOM can cause slower decomposition rates of the OM (Nicolardot et al., 2001) and thus also to SOM accumulation.

In chapter I, if amounts of 80 and 160 m^3 ha^{-1}, respectively, of wood chips from hedgerow (C:N=47; Gruber et al., 2008) were annually spread onto the soil for more than 16 years until today; an accumulation of SOM and SOC, similar to SRC willow can be assumed. The main differences between SRC willow and WCM are soil tillage and crop species: in the study of WCM, soil was usually tilled by a moldboard plough, while in SRC willow no tillage was applied; the rooting depth are deep and litterfall are thick with perennial willow, while annual crops have shallow roots and less litterfall. Although in the study of WCM, SOM and SOC were not yet measured, the issue of carbon sequestration can be part for future studies and may add extra ecological values to wood chips used for mulching.

7.2 Effects of short rotation coppice willow on the following crops and weeds

Willow (*Salix schwerinii* E. Wolf × *viminalis* L.) grown in short rotation coppice (SRC) turned out to be promising in terms of long-term productivity, even with low levels of nitrogen fertilization (chapter IV), and in terms of relatively high rates of carbon sequestration in the soil (chapter III). Willow, on the other hand, was also found to have an allelopathic potential (chapter II). Although the aboveground biomass in a willow SRC system is mainly removed from the field for energy production, there are still large amounts of roots (Heller et al., 2003) and leaf litter (Verwijst and Makeschin, 1996) left in/on soil. As they may also have allelopathic effect, all these residues should not be ignored for the following crop, even after clearing the land after several years of willow SRC. Either for adjacent crop fields, or for any land use change to crop production after the cultivation of willow stands, yield depression may happen due to the allelopathic effects of the residues. Thus, integration of SRC energy trees with allelopathic potentials (e.g. willow and eucalyptus) on arable land should be taken with caution (Kidanu et al., 2005). Field clearing should be thoroughly conducted, or fallow time should be extended after planting SRC willow. As discussed for the crop production, the weeds could also be reduced because of the residual effect from the former SRC willow cropping system. In a best case, allelochemical-tolerant crops are grown on the field, and the associating weed population will be relatively small as they were suppressed by the residual effect from willow root and litter residues.

7.3 Options for woody energy plants on arable land in comparison to arable cropping systems

Woody energy plants including willow, poplar and eucalyptus trees are widely planted all over the world (FAO, 2003). Taking willow as an example, although it is not the most high-yielding energy crop in this study (chapter III) on a high nitrogen fertilization-level, its biomass yield can compete with all other energy crops except miscanthus without additional fertilization. On comparatively poor and marginal arable land energy trees can be an alternative to annual crops (Niu and Duiker, 2006); fertile soils, however, will probably not be chosen for growing woody plants. Besides of the growing of trees species for energy use, woody material such as stems above a certain diameter as by-product of trees or hedgerows on farmland can also be used for example for combustion.

7.4 Outlook

- For the study on **wood chip mulching on crop fields**, WCM seems to have weed suppressing effects and might be used on-farm for weed control, particular in organic farming. But from a practical perspective, technical solutions must be found to apply wood chips using machinery and without damaging the crops. The particle size of wood chips might influence the release of allelochemicals and should be standardized to an optimal size. Studies on the soil properties under WCM would be valuable for the understanding of long-term effect of woody residues applied to the soil in a cropping system based on annual crops. For this purpose, the density fractionation method can be modified and applied on soils under WCM. The effect of WCM on other soil properties such as pH, nutrient contents (e.g. P, Ca, and Mg), and cation exchange capacity are also important to assess as these properties can influence crop production, too. In future studies, it will also be interesting to do large scale estimation of the woody resources from local farms, from forests and from industrial and communal waste (bioenergy production, timber industry, or gardening). If woody residues are not considered as waste but as a valuable material, the preservation and restoration of farm trees and structural elements like hedgerows is done and their ecological benefits is supported.

- For the study of **watery extracts from wood chips**, if in future studies the "active ingredients" of woody residues would be identified, bio-herbicides could be produced from them, if large scale elution methods are developed commercially. It might be an option to use such products on organic farms, particularly for high-value food crops.
- For the study of **soil organic matter under different energy cropping systems**, because of carbon sequestration, if more information about its benefits to the soil would be available, farmers would probably grow woody energy trees more frequently. If the C-sequestration potential in such cropping systems could be confirmed by other studies, a reassessment of SRC could be considered by policymakers.
- For the study of **annual and perennial energy cropping systems**, perennial energy plants, particularly energy trees, would be suitable in low-input systems and on marginal land. Up to now there is not much information available on what is happening with subsequent crops after removing SRC from fields. Therefore, it should be tested whether there is any potential residual effects of the SRC willow on the following crops. Possible interactions (e.g. allelopathic effects and resource competition) of woody plant and adjacent crops should also be considered and cropping systems then should be modified accordingly.

8. Summary

Woody plants on arable land can be grown for their products (e.g. trees for energy biomass) or simply as field borders (e.g. hedgerows). Establishing woody plants on arable land can increase biodiversity, reduce soil erosion, diminish nitrate leaching and improve drinking water quality. Woody plants have the potential to increase soil organic matter and sequestrate carbon in soils and biomass, which is important for the mitigation of climate change. However, the residues of woody plants can also have unfavorable influences (e.g. allelopathic effects, or competition for nitrogen) on crop production. In the past, woody plants have often been removed from arable land because of intensification and mechanization of agriculture; while nowadays, they are restored on arable land to achieve various ecological benefits, particularly in developed countries. The aim of the current study was to investigate selected aspects of plant and soil responses and their interactions with woody plants and their residues on arable land. Four publications describe and discuss the results of laboratory and field experiments with woody residues from hedgerow pruning (wood chips) and the effect of short rotation coppice willow in comparison to other energy crops, and their effects on yields, weeds, and selected soil characteristics.

The first publication (accepted by Agronomy Journal) describes a study about long-term effects of wood chips application from hedgerows (mainly *Acer pseudoplatanus* L., *Prunus avium* L., *Prunus padus* L., *Salix caprea* L., *Ligustrum vulgare* L., and *Fraxinus excelsior* L.) on arable land, with a focus on weed infestation and yield. Data were collected from a 16-year field trial at the organic research station Kleinhohenheim, in Southwest Germany, with wood chips mulching (WCM) on a typical crop rotation (cereal-based, grain legume and fodder included). The wood chips were derived from in-situ hedgerow prunings and annually applied in three rates (0, 80 and 160 m^3 ha^{-1}). Wood chip mulching reduced the weed density in spring significantly by 9%, and the high mulching rate resulted generally in lower weed numbers than the low mulching rate, while WCM caused no significant grain yield loss of cereals and grain legumes. However, the relative crop yield of plots with WCM compared to the control showed a decreasing trend over time, which might be related to unfavorable effects of WCM on the vegetative growth of crops. The weed suppression by WCM is presumably a result of several impacts such as a physical barrier, changes in soil temperature, lower nitrogen availability and allelopathic effects. Hence, woody residues can be used for weed control in arable crops but care should be taken concerning their potentially unfavorable effects on crops.

The second publication (submitted to Seed Science Research) is directly related to the WCM in publication I. The study aimed at gaining a first insight in potential allelopathic effects of

the wood chips used in experiment I and their influence on seed germination under laboratory conditions. Watery extracts of wood chips from goat willow (*Salix caprea* L.) and black cherry (*Prunus padus* L.) were tested on seed germination of oilseed rape (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). The extraction procedure was standardized for varying conditions including drying methods (freeze drying at -50 °C, oven drying at 25, 60 or 105 °C), milling, wood to water mixing ratio (WWR=1:10, 1:15 or 1:20) and fraction of the material used (bark or core wood), in order to produce extracts with a high capacity to suppress germination. Extracts of freeze dried and undried (defrosted) wood chips resulted in the lowest germination rate (<6%) of both crops after two weeks, compared to the relatively higher germination rates (12%–53%) in extracts of oven dried wood chips. In a high WWR ratio (1:10) with black cherry, the germination rate of oilseed rape was significantly lower in the extracts of milled wood chips (26%) compared to un-milled ones (49%), and similarly for wheat seeds (milled 1% germination rate, un-milled 19% germination rate). The suppression effect increased with increasing concentration of the extract: germination rates were 86% (WWR=1:20), 71% (WWR=1:15) and 35% (WWR=1:10) with milled wood chips. Extracts from the bark resulted in significantly lower germination rates (<4%) than that from core wood (>88%). The combination of freeze drying, with milled wood chips, high WWR and wood bark turned out to be the most suitable method to inhibit seed germination and thus to retain effective allelopathic compounds in the extract. The effects of germination inhibition was influenced by the interaction of tree species, species used in the germination trials and extraction method. The findings from this study can be applied for a systematic testing of different woody species, particularly for choosing woody residues with allelopathic potential to suppress weeds.

The third publication (in preparation) is about soil organic matter (SOM) responses to long-term cultivation of a woody perennial energy crop versus an herbaceous annual energy crop on arable land. Soil samples (0-10, 10-20 cm) were taken after 12 years of continuous cropping of short rotation coppice (SRC) willow (*Salix schwerinii* E. Wolf × *viminalis* L.) and mono-cropping of silage maize (*Zea mays* L.) at the research station Ihinger Hof, Southwest Germany. Soil fractions with different physical properties (density and weight) were separated in the laboratory and the total organic carbon was determined in these fractions. The integrated fractionation method separated light fractions (<1.8 g cm^{-3}) including free- and occluded-particulate organic matter (f-POM and o-POM), and heavy fractions with three particle-size classes (63-2000 μm for sand, 2-63 μm for silt, and <2 μm for clay). Generally, SOC contents were significantly higher in the upper soil under willow (1.39%) than under maize (1.13%). The soil (0-20 cm) under willow accumulated 154% more labile SOM (f-POM and o-POM) than the soil under maize did. The results can be explained by the continuous input of leaf litter and root turnover under the willows, and by the fact that the willows were not tilled except in the year of plant establishment. The C:N ratios of the SOM in POM and in the sand-sized fractions were also significantly higher under willow (28, 24, and 16) than under maize (23, 18, and 9). The findings indicate a slower turnover of SOM and a higher potential of carbon sequestration in soil under SRC willow, as an example for a woody energy crop, compared to silage maize, a typical annual energy crop grown with soil tillage.

The fourth publication (Published in Agronomy Journal) is based on the same 12-year field experiment as publication III, and it evaluates the biomass and gross energy yield of six annual and perennial energy cropping systems with different nitrogen fertilization levels, including SRC willow and silage maize (selected soil properties of both presented in publication III). The annual systems included mono-cropping maize with reduced tillage, a crop rotation of oilseed rape (*Brassica napus* L. ssp. *oleifera*)–wheat (*Triticum aestivum* L.)–triticale (*Triticale* ×*triticosecale* Wittmack) with moldboard plough or with no-tillage. The perennial systems included SRC willow, miscanthus (*Miscanthus* × *giganteus* Greef et Deu.) and switchgrass (*Panicum virgatum* L.). For each cropping system, three levels of nitrogen fertilization (0, 50% and 100% of crop-specific best management practice) were applied. The mean annual biomass yield was found to be highest in maize (18.5 Mg ha^{-1}) and miscanthus (18.3 Mg ha^{-1}) with 100% nitrogen, and without nitrogen highest in miscanthus (13.6 Mg ha^{-1}). However, only higher nitrogen levels could maintain the high yield potential of these two crops. Without or with reduced nitrogen fertilization, annual yield of maize was decreasing continuously; without nitrogen fertilization, the annual yield of miscanthus was also decreasing after eight years. Willow had a mean annual yield of 11.0 Mg ha^{-1} in average for three nitrogen levels, and its biomass yield was not significantly reduced when the nitrogen fertilization was reduced from 100% to 50%, suggesting it is less dependent on nitrogen fertilization compared to other energy crops (except for miscanthus) in this study. A yield trend of willow was only visible within the three-year coppice rotation but so far not with plantation age, except the establishment period. Overall, both growing of woody plants and applying woody residues on arable land can influence the plant growth (crops and weeds), soil properties and their interactions in many ways. Favorable influences include (i) reducing weed density by WCM, (ii) achieving promising biomass yield by growing SRC tree with low soil nitrogen, and (iii) increasing soil organic matter (carbon sequestration) by growing SRC willow; unfavorable influence includes reducing biomass production of crops by WCM. According to the findings with wood chips extracts, it is very likely that the main weed-suppressing effect is due to allelopathic effects.

Short rotation coppice willow seems to be a favorable low-input system with a medium but constant yield. Residues of SRC willow from clearing the land after several years of growing might have similar effects on crops and weeds in the following years, as the wood chips from hedgerows showed. It also can be assumed that the carbon sequestration which was found under SRC willow also takes place in the hedgerows. All favorable influences on crop production might motivate farmers to keep or restore woody plants on farm, and enhance ecosystem services within the farming system; while the unfavorable influences should be avoided if woody plants are to be used on arable land.

Future research objectives can be related to (i) technological improvement of the wood chips application on fields for weed control; (ii) determination and isolation of allelopathic ingredients from woody materials, and using them for a new generation of natural herbicides; (iii) study on potential long-term residue effects and interference of SRC woody plants on following crop growth; and (iv) studies on carbon sequestration in the soil under hedgerows.

9. Zusammenfassung

Gehölze können auf Ackerflächen zu Produktionszwecken angebaut werden (z.B. Bäume zur Biomasseproduktion) oder dienen als Feldgrenzen (z.B. Hecken). Gehölzpflanzen auf Ackerflächen wirken sich dabei positiv auf die Biodiversität aus, verringern die Bodenerosion sowie die Nitratauswaschung und haben einen positiven Einfluss auf die Trinkwasserqualität. Des Weiteren tragen sie zu einer Zunahme der organischen Bodensubstanz und zur Kohlenstoffsequestrierung im Boden bei und leisten damit einen Beitrag zum Klimaschutz. Die Gehölzpflanzen selber und auch deren Rückstände wie z.B. Häckselgut von Hecken können aber auch ungewünschte Auswirkungen auf die Kulturpflanzen nach sich ziehen, die beispielsweise durch allelopathische Effekte oder durch die Konkurrenz um Ressourcen (z.B. Licht) hervorgerufen werden. In der Vergangenheit fielen Gehölzpflanzen auf Ackerflächen vermehrt der Intensivierung und Mechanisierung in der Landwirtschaft zum Opfer, während heutzutage Bestrebungen bestehen, deren Zahl zu erhalten, um Ökosystemleistungen zu sichern. Das Ziel dieser wissenschaftlichen Arbeit war, Wechselwirkungen zwischen Pflanze und Boden bei ausgewählten Gehölzen sowie deren Ernterückständen auf Ackerflächen zu untersuchen.

Die vorgelegte Arbeit besteht aus vier Publikationen und umfasst Labor- und Feldexperimente, die sich zum einen mit den Effekten von Hackschnitzeln aus Heckenrückschnitt auf die landwirtschaftliche Produktion und zum anderen mit dem Vergleich einer Kurzumtriebsplantage mit anderen „Energiepflanzen“ in unterschiedlichen Anbausystemen beschäftigen. In den Untersuchungen werden relevante Aspekte zu Erträgen der Kulturpflanzen, Unkräutern und ausgewählten Bodenparametern herausgegriffen.

Die erste Publikation (veröffentlicht im Agronomy Journal) beschreibt Langzeiteffekte der Ausbringung von Hackschnitzeln von Hecken (hauptsächlich *Acer pseudoplatanus* L., *Prunus avium* L., *Prunus padus* L., *Salix caprea* L., *Ligustrum vulgare* L., und *Fraxinus excelsior* L.) auf den Ertrag und den Unkrautbesatz auf einer ökologisch bewirtschafteten Ackerfläche. Hierfür wurden Daten eines 16-jährigen Versuchs auf der ökologisch bewirtschafteten Versuchsstation Kleinhohenheim in Südwestdeutschland gesammelt. Untersucht wurde der Effekt von Hackschnitzelmulch (HSM) auf eine typische Fruchtfolge (Getreide, Leguminosen und Ackerfutter). Die Hackschnitzel stammten vom Rückschnitt der Hecken des Betriebs und wurden jährlich in drei verschiedenen Mengen ausgebracht (0, 80 und 160 m^3 ha^{-1}). HSM führte zu einer Reduktion des Unkrautbesatzes um 9 % im Frühjahr, wobei höhere Ausbringungsmengen im Vergleich zu niedrigeren generell in geringerem Unkrautbesatz resultierten. Der Einfluss auf den Ertrag war statistisch nicht signifikant, jedoch wurden über die Versuchszeit tendenziell sinkende Erträge auf mit HSM behandelten Parzellen gegenüber der Kontrolle beobachtet. Die unkrautunterdrückende Wirkung des HSM könnte auf verschiedenen Effekten beruhen, nämlich der mechanischen Behinderung des Auflaufens von Unkräutern, einer geänderten Bodentemperatur, einer reduzierten Stickstoffverfügbarkeit durch die Gabe von Material mit vergleichsweise weitem C:N-Verhältnis sowie allelopathischen Effekten. Hackschnitzel können daher zwar zur Unkrautkontrolle auf Ackerflächen verwendet werden, es müssen jedoch potentiell ungewünschte Effekte auf die Kulturpflanzen berücksichtigt werden.

Die zweite Publikation (eingereicht bei Seed Science Research) basiert direkt auf der ersten und beschäftigt sich mit möglichen allelopathischen Effekten von HSM und deren Einfluss auf die Samenkeimung unter Laborbedingungen. Getestet wurden die Auswirkungen wässriger Extrakte von Hackschnitzeln der Salweide (*Salix caprea* L.) und der Gewöhnlichen Traubenkirsche (*Prunus padus* L.) auf die Keimung von Raps (*Brassica napus* L.) und Weizen (*Triticum aestivum* L.). Ziel dieser Arbeit war die Entwicklung einer standardisierten Extraktionsmethode, wobei die Trocknung (Gefriertrocknung, Ofentrocknung mit 25, 60 oder 105 °C), das Mahlverfahren, das Holz-Wasser-Verhältnis bei der Extraktion (HWV; 1:10, 1:15 oder 1:20) und das Ausgangsmaterial (Rinde oder Kernholz) variiert wurden. Die Extrakte aus der Gefriertrocknung und die des ungetrockneten Holzes führten nach zwei Wochen zu der geringsten Keimrate (<6 %) bei beiden Kulturarten. Die ofengetrockneten Varianten besaßen eine höhere Keimrate von 12 bis 53 %. Die Keimrate von Raps lag bei einer hohen HWV (1:10) mit Extrakten aus gemahlenen Hackschnitzeln der Gewöhnlichen Traubenkirsche bei 26 % und damit signifikant niedriger als mit Extrakten aus ungemahlenem Material (49 % Keimung). Weizenkörner keimten unter diesen Bedingungen in geringerer Anzahl als Raps, aber die Keimung war mit Extrakten aus gemahlenem Material (1%) auch geringer als mit Extrakten aus ungemahlenem Material (19 %). Der Effekt der Keimungsunterdrückung stieg mit erhöhtem HWV bzw. höherer Konzentration der Extrakte. Die Keimraten betrugen durchschnittlich für HWV 1:20 86 %, für HWV 1:15 71 % und für HWV 1:10 35 % mit gemahlenen Hackschnitzeln. Aus der Rinde gewonnene Extrakte führten zu einer signifikant geringeren Keimrate (<4 %) als die des Kernholzes (<88 %). Die effektivste Methode zur Erhaltung offensichtlich allelopathisch wirksamer Verbindungen war die Kombination aus gemahlenen Hackschnitzeln aus Rindenholz, Gefriertrocknung (-50 °C) und einem hohen HWV. Diese hatte den größten Effekt auf die Unterdrückung der Keimung. Die Ergebnisse aus dieser Publikation können zur Untersuchung weiterer Gehölzarten angewandt werden und bieten eine Grundlage für die Auswahl geeigneter Substrate mit einem möglichst hohen allelopathischen Potential zur Unterdrückung von Unkraut.

Die dritte Publikation (in Vorbereitung) beschäftigt sich mit der organischen Substanz (OS) beim Anbau mit Gehölzen zur energetischen Nutzung im Vergleich zum Anbau annueller Energiepflanzen auf Ackerland. Untersucht wurde ein 12-jähriger Dauerversuch auf der Versuchsstation Ihinger Hof in Südwestdeutschland mit einer Weiden-Kurzumtriebsplantage (*Salix schwerinii* E. Wolf x *viminalis* L.) und einer 12-jährigen Maismonokultur (*Zea mays* L.). In diesem Versuch wurden Bodenproben im Bereich 0 –10 cm und 10 – 20 cm gezogen. An jeder Probe wurden im Labor eine Dichtetrennung sowie eine Fraktionierung nach Korngröße durchgeführt, und der Kohlenstoffgehalt jeder Fraktion bestimmt. Die Dichtefraktionierung resultierte in einer leichten Fraktion (<1,8 g cm^{-3}), die sich aus freier partikulärer und in Bodenaggregaten eingeschlossener OS „occluded- particulate organic matter" (f-POM und o-POM) zusammensetzte sowie der schweren Fraktion, bestehend aus drei Klassen verschiedener Partikelgrößen: Sand (63-2000 µm), Lehm (2-63 µm) und Ton (<2 µm). Generell fanden sich höhere Gehalte an OS in der oberen Bodenschicht unter Weiden (1,39 %) als im Maisanbau (1,13 %). Im Boden unter Weiden war die leichte Fraktion (f-POM und o-POM) um 154 % höher als beim Maisanbau. Grund dafür war der kontinuierliche Zufluss von Streu und von Wurzelresten sowie die fehlende Bodenbearbeitung. Ebenso war das C:N Verhältnis der OS in den Sandfraktionen unter Weide (28, 24 und 16) höher als unter Mais (23, 18 und 9). Die Ergebnisse deuten auf einen langsamen Umsatz von OS und damit auf ein höheres Kohlenstoffsequestrierungspotential unter Weiden in Kurzumtriebsplantage als beim Maisanbau hin.

Die vierte Publikation (veröffentlicht im Agronomy Journal) nutzt denselben 12-jährigen Feldversuch wie die dritten Publikation. Es erfolgte eine Bewertung des Biomasse- und des Bruttoenergieertrags von sechs annuellen und perennierenden Energiefruchtfolgen mit verschiedenen Stickstoffdüngungsstufen. Die annuellen Systeme bestanden aus Mais in Monokultur mit reduzierter Bodenbearbeitung; einer Fruchtfolge mit Raps (*B. napus* L. ssp. *oleifera*) – Weizen (*Triticum aestivum* L.) – Triticale (*Triticale x triticosecale* Wittmack) mit wendender bzw. keiner Bodenbearbeitung. Die perennierenden Systeme umfassten eine Kurzumtriebsplantage mit Weiden (*S. schwerinii* E. Wolf x *viminalis* L.), Miscanthus (*Miscanthus x giganteus* Greef et Deu.) und Ruthenhirse (*Panicum virgatum* L.). Für jedes Anbausystem wurden drei Stickstoffdüngungsstufen (0, 50 und 100 % der praxisüblichen Düngemenge) etabliert. In Mais wurde im Mittel der höchste jährliche Biomasseertrag festgestellt (18,5 Mg ha^{-1}), gefolgt von Miscanthus (18,3 Mg ha^{-1}) jeweils bei einem N-

Düngeniveau von 100 %. Ohne Stickstoffdüngung lag der jährliche Biomasseertrag bei Miscanthus mit 13,6 Mg ha^{-1} am höchsten. Das hohe Ertragsniveau konnte bei beiden Kulturen über die 12-jährige Versuchslaufzeit nur mit der höchsten N-Düngerstufe gehalten werden. In den Fruchtfolgen und bei Rutenhirse sanken die Erträge über die Jahre auch mit hoher Stickstoffgabe. Je geringer die Stickstoffdüngung ausfiel, desto stärker war der Ertragsrückgang. Die Weiden in Kurzumtriebsplantage zeigten unabhängig von der Stickstoffdüngung und der Versuchslaufzeit im Mittel gleichbleibende Erträge von 11 Mg ha^{-1}. Offenbar ist die Stickstoffdüngung für Weiden in Kurzumtriebsplantagen im Vergleich zu den anderen untersuchten Kulturen und Anbausystemen ein weniger wichtiger Produktionsfaktor.

Das Ausbringen von Hackschnitzel von Hecken auf Ackerflächen und der Anbau von Gehölzpflanzen (Weide in Kurzumtriebsplantage) zeigten Effekte im oberirdischen Pflanzenaufwuchs und hatten Auswirkungen auf die Bodeneigenschaften. Gewünschte Auswirkungen der Managementmaßnahmen waren (i) die Verringerung des Unkrautbesatzes, (ii) der geringe Stickstoffinput für eine zufriedenstellende Produktivität von Weiden in Kurzumtriebsplantage, und (iii) und die Erhöhung der OS (Kohlenstoffsequestrierung). Unerwünschte Effekte äußerten sich in der tendenziellen Reduktion der Biomasseproduktion der Kulturpflanzen

Wie die Studie zu Extrakten aus den Hackschnitzeln zeigt, scheinen tatsächlich allelopathische Effekte eine mögliche Ursache für die Unkrautunterdrückung bei der Hackschnitzelapplikation zu sein. Diese oder ähnliche Effekte könnten auch nach der Rodung von Kurzumtriebsplantagen auf die Nachfrüchte auftreten, z.B. aus Rückständen von Wurzeln und Stamm. Weiterhin könnte beim Erhalt von Heckenbiotopen auch mit einer Kohlenstoffsequenzierung gerechnet werden, ähnlich wie es bei den Weiden in Kurzumtriebsplantage gezeigt wurde. Die günstigen Effekte des Anbaus von Gehölzen könnten Landwirte motivieren, Gehölzpflanzen auf ihren Ackerflächen zu belassen bzw. zu etablieren und die Ökosystemleistungen auf dem Betrieb zu erhöhen.

Weiterführende Forschung könnte darauf abzielen (i) technische Lösungen für eine praktikable Hackschnitzelausbringung zur Unkrautbekämpfung zu finden, (ii) die allelopathisch wirksamen Substanzen von Gehölzen zu identifizieren und zu isolieren und so gegebenenfalls Grundlage für eine neue Generation von Herbiziden zu schaffen, (iii) Langzeitfolgen von Ernterückständen nach dem Anbau von Kurzumtriebsplantagen auf die nachfolgenden Kulturen zu untersuchen, und (iv) Studien zur C-Sequestrierung unter naturnahen Hecken vorzunehmen.

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Publications

Xu, J., Gauder, M., Zikeli, S., Möhring, J., Gruber, S., & Claupein, W. (2018). Effects of 16-year Woodchip Mulching on Weeds and Yield in Organic Farming. *Agronomy Journal*, *110*(1), 359-368.

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Xu, J., M. Gauder, S. Zikeli, J. Moehring, S. Gruber, and W. Claupein. Fractionation of soil organic matter in a long-term trial with annual and perennial energy crops. (In preparation)

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