LING ZOU

Crop Rotation as a Tool Towards Sustainable Barley Cropping
CROP ROTATION AS A TOOL TOWARDS SUSTAINABLE BARLEY CROPPING

DOCTORAL THESIS
LING ZOU

ACADEMIC DISSERTATION
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ABSTRACT
Factors limiting barley yield have been reduced since the Green Revolution globally. Nevertheless, higher yields are pursued with the pressure to feed the increasing human population and domestic animals. However, sustainability can be impaired by the cereal monocultures, in particular, cereal-specific pathogen inocula can increase at the cost of diversity of soil microfauna. Use of pesticides has selected for resistance in weeds and pathogens. Use of mineral fertilizers has resulted in waste of resources and pollution. In Finland, about half of the arable land is used for continuous cereal production, and barley (Hordeum vulgare), as an important source for food and feed, is the most cultivated cereal crop. In this dissertation, research focused on evaluating the effects of various rotation crops on weeds, pathogenic fungi, soil fertility and yield of subsequent barley.

The competitive ability of six crops, namely buckwheat (Fagopyrum esculentum), caraway (Carum carvi), faba bean (Vicia faba), hemp (Cannabis sativa), common vetch (Vicia sativa) and white lupin (Lupinus albus) with weeds was evaluated in glasshouse and field experiments. In the glasshouse experiment, couch grass (Elymus repens) grown with buckwheat accumulated the least dry matter. Activated charcoal was used to exclude the effect of root exudates from donor crops. The results indicated that activated charcoal incorporated in the soil deactivated buckwheat root exudates that apparently inhibited the growth of couch grass. In the field experiment, buckwheat’s fast growth and formation of effective leaf area inhibited growth of weeds most effectively among the crops tested.

The effect of turnip rape [Brassica rapa L. ssp. oleifera (DC.) Metzg.] on several soil pathogenic fungi was evaluated in a field trial in two growing seasons involving five different cultivation regimes: mixed culture of barley and turnip rape, turnip rape sown after barley, and turnip rape incorporated as green manure either in autumn or in spring, with continuous barley monoculture as a control. Soil fungal community structure was monitored with capillary-based LH PCR of the ITS region using primers ITS1F/ITS4. The mixed culture and sowing turnip rape after barley did not significantly decrease the relative abundance of Fusarium spp. Incorporation of turnip rape plants into the soil, as a source of organic matter and nutrients, was associated with a low relative abundance of Fusarium spp. Fungal diversity was the lowest and the relative abundance of Fusarium spp. the highest in continuous barley. Higher fertilizer application and organic matter incorporation, leading to high fungal diversity, seemed more important in affecting Fusarium spp. than the allelochemicals of turnip rape, as turnip rape extracts did not detectably inhibit the growth of F. culmorum in an in vitro test. In growth media of different nutrient levels, 10 g soil were suspended in 100 mL sterile water, then 400 μL of the suspended solution was spread on the media of each nutrient level with four replicates. The number of fungal colonies
was counted. The soil *Fusarium* CFU count was higher on nutrient-poor growth medium than on rich medium, supporting the results of field experiments. Results of sequencing indicated that *Penicillium* spp. might also tolerate low nutrient availability.

In two experiments that ran 2010 – 2012 and 2011 – 2013, faba bean, turnip rape and barley were sown as first crops and their residues were tilled into the soil after harvest in blocks in the first year. In the following year, barley, buckwheat, caraway, faba bean, hemp and white lupin were sown in each block and incorporated either at flowering stage (except barley) or after harvest. Barley yield and grain protein concentration were determined. Mineral N concentrations in the plough layer two months after incorporation of crops and before sowing barley in the following spring were determined. In the third year, all the plots were sown with barley. The beneficial effect of faba bean and turnip rape, as first crops, on yields and grain protein concentration of barley was still detectable in the third year. Barley yields after white lupin, faba bean and hemp, as second crops, were higher than in continuous barley. Barley grain protein concentration was increased after faba bean. In contrast, barley yield and grain protein concentration were not improved after buckwheat and caraway. Incorporation of plants at flowering stage posed a risk of increased N leaching, suggesting that incorporation of plants should be delayed or catch crops should be used during winter to reduce N leaching. The response of barley yields to increased mineralized N from green manure or residues after harvest of rotation crops was significant only in the first experiment conducted in 2010 – 2012 suggesting that increased mineral N in the plough layer has minor influence on barley yield.

Thus, rotation with faba bean, white lupin and hemp was beneficial to barley grain yield. In addition, barley grain protein concentration was increased after rotation with faba bean. Weed growth was most strongly suppressed by buckwheat as a complement to current weed management regime, but, rotation with buckwheat and incorporation of its residues did not improve the yield of the successive barley. The effect of glucosinolates of turnip rape on soil-borne pathogens was outweighed by high fungal bio-diversity suggesting that allelochemicals released from plants sown at a realizable density in the field are unlikely to control pathogens. Maintaining high microbial diversity through sufficient nutrient input is crucial to control pathogenic *Fusarium* spp. populations.
**LIST OF ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<td>ANCOVA</td>
<td>Analysis of Covariance</td>
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<tr>
<td>BM</td>
<td>Barley Monoculture</td>
</tr>
<tr>
<td>CA</td>
<td>Correspondence Analysis</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
</tr>
<tr>
<td>DAI</td>
<td>Days After Inoculation</td>
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<td>DAS</td>
<td>Days After Sowing</td>
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<tr>
<td>ETI</td>
<td>Early Turnip rape Incorporation</td>
</tr>
<tr>
<td>FHB</td>
<td><em>Fusarium</em> Head Blight</td>
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<td>GPC</td>
<td>Grain protein concentration</td>
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<td>GR</td>
<td>Growth Rate</td>
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<tr>
<td>GS</td>
<td>Growth Stage</td>
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<td>ITC</td>
<td>Isothiocyanates</td>
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<td>ITS</td>
<td>Internal Transcribed Spacer</td>
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<td>LTI</td>
<td>Late Turnip rape Incorporation</td>
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<tr>
<td>LH-PCR</td>
<td>Length Heterogeneity Polymerase Chain Reaction</td>
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<tr>
<td>LSD</td>
<td>Least Significant Difference</td>
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<tr>
<td>MC</td>
<td>Mixed Culture</td>
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<tr>
<td>NIR</td>
<td>Near Infra-Red</td>
</tr>
<tr>
<td>OTU</td>
<td>Operational Taxonomy Unit</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal Component Analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<tr>
<td>PDP</td>
<td>Potato Dextrose Powder</td>
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<tr>
<td>PGPR</td>
<td>Plant Growth Promoting Rhizobacteria</td>
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<tr>
<td>RGR</td>
<td>Relative Growth Rate</td>
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<tr>
<td>SE</td>
<td>Standard Error</td>
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<td>TAB</td>
<td>Turnip rape After Barley</td>
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</table>
1 INTRODUCTION

1.1 Intensified food production and sustainability of agriculture

Because of the Green Revolution, more people on Earth were relieved from starvation and yield-limiting factors have been gradually reduced (Fig. 1). Particularly in developed countries, effective irrigation systems, use of high-yielding and disease-resistant cultivars, application of mineral fertilizers, pesticides and mechanical farming have increased yields (Gregorich et al., 2001). However, with the increasing human population in the future, the need for more food will require higher yields. From 1961 to 2007 globally, arable land per capita decreased from 0.415 to 0.214 ha, at the same time, cereal (maize, rice and wheat) yields have increased from 1.3 to 3.3 Mg/ha and is projected to be about 4.8 Mg/ha in 2040 (Smith et al., 2010). Therefore, intensifying crop production to meet the demand of humans and animals for food, fodder, fuel and fiber per unit arable land will continue to be the major means because converting natural ecosystems to farm land is not desirable; however, one major concern on intensification is the sustainability (Gregory and Nortcliff, 2013). In agricultural terms, sustainability has been defined as the long-term viability of a particular soil–plant system (or land use), which includes steps to ensure conservation of natural resources, economic viability (including productivity and security) and social acceptability (Gregorich et al., 2001).

Figure 1. Yield-limiting factors in crop production. Possible maximum yield can be realized if the yield-limiting factors are excluded (Adapted from Havlin et al., 2005). The relative importance of the yield-limiting factors varies across environments and plant growth stages. In general, the most limiting factor determines yield potential, according to Liebig’s law of the minimum.
Long-term cereal monoculture has reduced fertility of soils and their ability to sustain stable yields (Campbell et al., 1992; Shen et al., 2004). For instance, total soil N in different soils in five regions of Australia decreased, on average, from 280 to 84 kg/ha in sandy soil, from 325 to 203 kg/ha in silty soil and from 640 to 523 kg/ha in clay soil, after 20 to 70 years of continuous cereal production (Dalal and Mayer, 1987). In soils subjected to continuous cereal production from 1880 to 1980 in the UK, organic C concentration decreased from around 50 Mg/ha to 20 Mg/ha. Furthermore, long-term use of pesticides to control pathogens, weeds and insects can induce development of resistance in the target organisms (Gregory and Nortcliff, 2013). Therefore, innovative methods for management of weeds, insects and microbial pathogens are needed to complement the conventional methods and reduce the negative impacts on the sustainability of the ecosystem service (Gregory and Nortcliff, 2013).

1.2 Barley

Barley (Hordeum vulgare L.), one of the most ancient crops, was cultivated 10,000 years ago in the Near East (Smith 1998). Barley is the fourth most-produced cereal crop in the world after maize (Zea mays L.), rice (Oryza sativa L.) and wheat (Triticum aestivum L. emend Thell.) (Table 1). Barley grains contain 50% – 70% starch, 13 – 28% non-starch polysaccharides, 8 – 15% protein, the remaining being minerals, sterols and phytochemicals (MacGregor and Fincher, 1993). The main uses of barley include feed (55 – 60%), malting (30 – 40%), food (2 – 3%) and seed (5%) (Ullrich, 2011).

1.3 Production of barley in Finland

Finland lies between latitudes 60º and 70º N. Most of the cereal production is in the south and west, where the growing season is usually from May to August (Hollins et al., 2004). Barley is the most-produced cereal crop followed by oats, wheat and rye (Table 1) in Finland.

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<tr>
<td>Barley</td>
<td>141.4</td>
<td>132.9</td>
<td>1.8</td>
<td>1.6</td>
</tr>
<tr>
<td>Maize</td>
<td>774.9</td>
<td>872.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oat</td>
<td>24.0</td>
<td>21.1</td>
<td>1.1</td>
<td>1.1</td>
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<tr>
<td>Rice</td>
<td>657.6</td>
<td>719.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rye</td>
<td>15.2</td>
<td>14.6</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Wheat</td>
<td>639.3</td>
<td>670.9</td>
<td>0.8</td>
<td>0.9</td>
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1.4 Factors limiting barley yield in Finland – from perspectives of weeds and pathogens

In Finland, 46% of the arable land is used for continuous production of cereals (Ministry of Agriculture and Forestry of Finland 2010). Because of good infrastructure and economy, fertilizers, machinery and pesticides are generally affordable to farms. Despite the use of pesticides, some pathogen and weed species are not effectively managed and cause yield loss and low-quality products.

Four pathogenic fungi and one weed were selected as targets in this study. All are of worldwide as well as national importance, and the fungi all have wide host ranges.

1.4.1 Diseases caused by the pathogenic fungi

_Fusarium_ spp. can infect a wide range of cereal hosts. _Fusarium_ head blight (FHB) and root rot are common diseases of cereals caused by species of _Fusarium_, particularly _F. graminearum_, _F. culmorum_ and _F. avenaceum_. FHB is the symptom when the spikelets of cereals are infected. Conidia and ascospores are produced and then the disease can be transmitted by wind, also rain splash can transport macroconidia that were too heavy to be carried by wind (Champeil et al., 2004), and conidia and ascospores can be carried by insects (Gordon, 1959). Besides causing FHB, _Fusarium_ spp. can also infect the host at an early growth stage. _F. graminearum_ favors areas with mild winters and warm humid summers. _F. culmorum_ is more widely distributed due to its tolerance to drought and freezing temperature (Champeil et al., 2004).

In a survey on soil-borne pathogenic fungi that cause diseases of cereal stems and roots in Finland, _F. culmorum_, _F. avenaceum_, _Gaeumannomyces graminis_, _Rhizoctonia solani_ and _Septoria nodorum_ were listed as the most common (Mäkelä & Mäki, 1980). Up to 44% of the harvested cereal seeds were infected by _Fusarium_ spp, in 2001 and 2002 (Jestoi et al., 2004). Mycotoxins produced by this genus cause acute poisoning such as nausea, vomiting and stomach pain, and long-term exposure has been associated with weakening of the immune system of humans (Rautala et al., 2008).

*R. solani* is a broad-spectrum pathogen that causes root rot of barley and wheat (Schillinger and Paulitz, 2006), stem canker of potato (_Solanum tuberosum_ L.) and sheath blight of maize (Banville, 1989). The disease is mostly soil- or seed-borne (Tsror, 2010). *R. solani* can survive, grow and remain pathogenic under a wide range of environmental conditions in soils, and pathogenicity of this fungus depends on intrinsic properties of the isolate and the host (Shephard and Wood, 1963).

*B. cinerea* mainly causes diseases on dicotyledonous plants. It produces airborne conidia and ascospores, and forms sclerotia that can survive between growing
seasons on dead plant tissues and soils, and it can remain pathogenic regardless of environmental factors (Williamson et al., 2007). In a field experiment, a 30 cm × 30 cm square containing conidiospores of a benomyl-resistant isolate of B. cinerea was placed on the ground when snap bean (Phaseolus vulgaris L.) initiated blossom, and at harvest, 13% of the pods on the plants that were 4.5 m from the square showed symptoms caused by the isolate (Johnson and Powelson, 1983), demonstrating the rapid spread of the spores. One of the most important diseases it causes in Finland is berry rot of strawberry (Fragaria × ananassa) (Bhaskara et al., 2000). Moreover, B. cinerea causes diseases in vineyards pre- and post-harvest (De Miccolis Angelini et al., 2014), and also causes a pod-rotting disease of oilseed rape (Brassica napus L.) (Williamson et al., 2007).

S. sclerotiorum causes diseases on legumes, sunflower, canola, most vegetables, tobacco, many flowering bedding plants, and stone fruits. Ascospores of this fungus are airborne and can infect flowers and fruits, and soil-borne sclerotia can infect roots and crowns, and sclerotia of this fungus remained infective 5 years after burial in the plough layer (Link and Johnson, 2007). In Finland, it causes yield loss of carrot (Daucus carota L. subsp. sativus Schübl & Martens) (Koponen and Valkonen, 1996).

1.4.2 Couch grass
Couch grass (Elymus repens (L.) Gould) is a highly competitive and persistent weed that causes yield losses in temperate regions globally (Werner and Rioux, 1977), and its rhizomes are fast-growing. Raleigh et al. (1962) reported that the diameter of the spread of 14 rhizomes from one parent plant was more than three meters in the soil, which makes couch grass resilient to controlling methods such as tillage and herbicides. Couch grass is the most common weed and accounted for most biomass accumulation in both conventional (163 kg total weed biomass contained 81 kg couch grass in one hectare) and organic farms (678 kg total weed biomass contained 178 kg couch grass in one hectare) in Finland (Salonen et al., 2001).

1.5 Rotation as a tool for management of weeds and pathogens
Rotation with dicotyledonous crops breaks the cycles of the pathogens causing diseases of the main cereal crops. The break crops also provide different rhizodeposits, exudates containing allelochemicals and growth habits, altering the soil environment (Gregory and Nortcliff, 2013).

1.5.1 Role of plant interference in weed management
Plant interference with weed growth can be categorized into two types: the first is physical competition for nutrients, water and light; and the second is allelochemicals released from the plant, most of which are secondary metabolites. Many such secondary metabolites and their modes of action have
been revealed and suggested as a natural alternative for weed management (Macías et al., 2007).

Much research has been conducted in selecting weed-suppressive cultivars of cereal crops. For instance, rice cultivars with allelochemical activity showed significant inhibition of different weeds (Kong et al., 2008). Much research have shown that early crop development, rapid height growth and high specific leaf area were the most effective components for weed suppression (Olesen et al., 2004; Vasilakoglou et al., 2012; Zystro et al., 2012; Dai et al., 2014). Nevertheless the ability of cereals to compete with weeds and high yield were mutually exclusive (Lemerle et al., 2001, Reid et al., 2009). Dicotyledonous crops can be alternative sources of new weed-suppressive allelochemicals. For example, Kumar et al. (2009) found that buckwheat (Fagopyrum esculentum Moench.) residues reduced the emergence of green pigweed (Amaranthus powellii L.). Caraway (Carum carvi L.) allelochemicals (thymol, carvacol, and carvone) inhibited the germination of Alcea pallida Waldst. & Kit., Amaranthus retroflexus L., Centaurea salsotitalis L., Raphanus raphanistrum L., Rumex nepalensis Spreng., Sinapis arvensis L. and Sonchus oleraceus L. (Azirak and Karaman, 2008). Wink (1983) reported that white lupin (Lupinus albus L.) allelochemicals (alkaloids, such as lupanine and sparteine) inhibited the germination of lettuce (Lactuca sativa L.). Brown and Morra (1995) reported that breakdown products of glucosinolates in Brassica sp. inhibited the germination of lettuce (Lactuca sativa L.).

1.5.2 Effects of rotation crops on microbial community

Apart from different field management on rotation crops, the intrinsic properties of rotation crops can influence the structure and composition of soil microbial community that is subjected to long-term monoculture (Gregory and Nortcliff, 2013). Rhizodeposition refers to the release of organic compounds by living plants and residues of plants (Shamoot et al., 1968). The released organic compounds can be mineralized and serve as sources of energy and nutrients for soil microorganisms in soil (Hartmann, et al., 2009). Many rhizosphere activities could be enhanced by sufficient rhizodeposition (Khan et al., 2010), for instance, growth of plant growth-promoting rhizobacteria (PGPR) and other microbes that can improve the rhizospheric environment by inhibiting harmful nematodes and pathogens (Fig. 2).

Also, the impacts of plant exudates on the microbial community of the rhizosphere are substantial, because many studies (Smalla et al., 2001; Kowalchuk et al., 2002; Wardle et al., 2004; Broeckling et al., 2008) have shown that the relationship between plant species and microbial community in the rhizosphere is species-specific. This relationship might be attributed to the varieties in quality and quantity of root exudates of different crops and growth stages of plants (Haichar et al., 2014).
Allelopathy is a biological phenomenon by which an organism produces allelochemicals that affect the growth, survival, and reproduction of other organisms; many allelochemicals are secondary metabolites of plants (Macías et al., 2007). Many identified allelochemicals from some plant species inhibited growth of various pathogenic fungi in vitro, and were used to control some diseases effectively (Inderjit & Mukerji, 2008). Among these allelochemicals, isothiocyanates (ITC), product of hydrolyzed glucosinolates in Brassica sp. (Brown and Morra, 1995, 1997), were widely and deeply investigated. Isothiocyanates are toxic to many nematodes, insects, bacteria and fungi (Borek et al., 1997; Manici et al., 2000; Smolinska et al., 2003; Kirkegaard and Matthiessen, 2004; Matthiessen and Shackleton, 2005). Glucosinolates can be transported through the phloem from leaves to roots (Chen et al. 2001), leading to an impact on microbes in rhizosphere (Bressan et al. 2009).

![Diagram](image.png)

Figure 2. Rhizodeposits, as energy sources, can enhance the activity of different microorganisms. Thus pathogens and nematodes can be inhibited because of competition for nutrients, synthesis of antibiotics and stimulation of host resistance (Adapted from Khan et al., 2010).
1.5.3 Effects of rotation crops on soil fertility

Some plant species can improve soil fertility. For instance, legumes when in effective symbiosis with Rhizobia can add N to the system by biological fixation, and the following crops can benefit from the residual N (Jensen et al., 2004; Rasmussen, et al. 2012). Moreover, some plant species can convert non-available forms of some elements to plant-available forms. Because buckwheat favors nitrate, therefore buckwheat can excrete more protons than other crop species to maintain charge balance (Cakmak and Marschner, 1990). Thus more P can be utilized by buckwheat from phosphate rock (Bekele, et al., 1983). The release of carboxylates from roots of white lupin (Neumann et al., 2000) increased the bioavailability of P (Hinsinger, 2001).

1.6 Effects of green manure on soil nutrient management

Green manure has been mostly used in low-input system, can affect crop and soil nutrient levels (Table 2). For example, maize, with reduced application of mineral fertilizers (100 – 120 kg/ha of N as urea) coupled with incorporation purple vetch (Vicia benghalensis L.) as green manure, produced equivalent yield to a more heavily fertilized crop (220 kg of N as urea) (Kramer, et al., 2002). Apart from the advantages listed in Table 2, green manure of some plant species was used as the source of allelochemicals for management of soil-borne pathogens. Green manure of Brassica has been reported as an effective method to manage soil-borne pathogenic fungi, such as R. solani that causes scab disease of potato (Larkin & Griffin 2007), and species of the genera Cylindrocarpon, Phytophthora, Pythium, and Rhizoctonia that cause apple replant disease (Mazzola & Mullinix 2005).

Table 2. Advantages and disadvantages of using green manure in low-input systems

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increased N supply stability</td>
<td>N release does not coincide with crop demand</td>
</tr>
<tr>
<td>Consistent crop yields</td>
<td>Loss of cash crop as a result of green manuring</td>
</tr>
<tr>
<td>Increased soil organic matter</td>
<td></td>
</tr>
<tr>
<td>Increased soil fertility</td>
<td></td>
</tr>
<tr>
<td>Erosion control</td>
<td></td>
</tr>
<tr>
<td>Improved soil physical conditions</td>
<td></td>
</tr>
<tr>
<td>Improved crop rooting depth</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from Thorup-Kristensen et al. (2003).

Increasing N use efficiency should reduce the costs of farming and pollution. In Finland, Valkama et al., (2013) conducted a meta-analysis including data from 1940–2004 and showed that the current Finnish fertilizer recommendations based on yield expectancy (the higher the expected yield, the higher the N fertilizer recommendation) can be questioned because of low N-use efficiency. Precipitation in winter and early spring can leach mineral N (Jaakkola, 1984). So,
it is important to investigate the timing for incorporation of plant materials to reduce this risk.

2 AIMS OF THE RESEARCH

Introducing new crops in rotation to break continuous cereal cultivation can be beneficial to the successive cereal. Barley was chosen as the successive crop because it is the most-produced cereal in Finland.

The specific research hypotheses were:

Hypothesis 1. Competitive ability of six dicotyledonous crops, namely buckwheat, caraway, faba bean, hemp, common vetch, and white lupin, against weed would vary, and allelochemicals of crops would interfere with growth of weeds additionally (Publication I).

Hypothesis 2. Release of isothiocyanates of turnip rape [Brassica rapa L. ssp. oleifera (DC.) Metzg.] can be realized by cultivation treatments involving turnip rape as rotation or under-sown crop, or as green manure incorporated in soil. We hypothesize that these treatments would affect soil fungal community with focus on pathogenic fungi (F. culmorum, B. cinerea and R. solani). Continuous barley monoculture was used as a control (Publication II).

Hypothesis 3. Rotation crops including faba bean, buckwheat, caraway, hemp and white lupin would improve successive barley yield and grain protein concentration (Publication III).

Hypothesis 4. The timing of incorporation of plant materials into the soil would be a factor influencing soil mineral N availability (Publication III).

3 MATERIALS AND METHODS

The experimental part of this dissertation is described as a general outline. It is presented more thoroughly in the original publications I – III.

3.1. Experimental resources and weather conditions

The glasshouse and field experiments were conducted at different sites (Table 3) in different years on the Viikki Research and Experimental Farm, Viikki, University of Helsinki, Finland. The soil is classified as Vertic Stagnosol (FAO 2014). In Publication I, the top soil was a silty clay loam (silt 30–40%, clay 50–60%, sand 10–20%) with organic matter content of 3.0–5.9% and pH 6.0. In Publications II and III, the top soil is a silty clay loam (31–33% clay, 63% silt and 4–5% sand), pH 6.3. At the first site of field experiment of Publication III, where the experiment was conducted from 2010 – 2012, the organic matter of soil was 3.8%. At the second site, where the experiment was repeated from 2011 – 2013, the organic matter of soil was 7.5%.
Table 3. Aim, Duration and Location of glasshouse and field experiments

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Aim</th>
<th>Duration</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glasshouse (I)</td>
<td>Effect of root exudates of plants on couch grass</td>
<td>2010</td>
<td>60°22' N, 24°02' E</td>
</tr>
<tr>
<td>Field (I)</td>
<td>Weed suppressive ability of plants</td>
<td>2010</td>
<td>60°22' N, 24°02' E</td>
</tr>
<tr>
<td>Field (II)</td>
<td>Turnip rape as a biofumigation tool against pathogenic fungi</td>
<td>2009-2010, repeated in 2010-2011</td>
<td>60°13' N, 25°10' E</td>
</tr>
<tr>
<td>Field (III)</td>
<td>Effect of rotation crops on the yield of the following barley</td>
<td>2010-2012, repeated in 2011-2013</td>
<td>60°22' N, 25°03' E</td>
</tr>
</tbody>
</table>

Roman number refers to publications I, II and III.

The mean air temperatures of growing seasons 2009 – 2013 were, in general, higher than the long-term average (Table 4). Monthly precipitation in the year 2012 was markedly higher than in the other years and the long-term average, whereas precipitation in the year 2011 was similar to the long-term average.

Table 4. Mean air temperature (°C) and monthly precipitation (mm) of growing seasons 2009–2013, together with the long-term (1981–2000) average (FMI 2013, Kaisaniemi) in Helsinki.

<table>
<thead>
<tr>
<th>Month</th>
<th>Mean air temperature (°C)</th>
<th>Monthly precipitation (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May</td>
<td>9.9</td>
<td>11.6</td>
</tr>
<tr>
<td>June</td>
<td>14.8</td>
<td>16.6</td>
</tr>
<tr>
<td>July</td>
<td>17.2</td>
<td>17.2</td>
</tr>
<tr>
<td>August</td>
<td>15.8</td>
<td>16.3</td>
</tr>
<tr>
<td>September</td>
<td>10.9</td>
<td>12.9</td>
</tr>
<tr>
<td>Sum</td>
<td>68.6</td>
<td>74.6</td>
</tr>
</tbody>
</table>

3.2. Plants and fungi used

Plants used for the evaluation of interference with weed and fungal growth were barley, buckwheat, caraway, common vetch, faba bean, turnip rape and white lupin (Publication I). Extracts of plants of turnip rape were tested on reference strains *B. cinerea F. culmorum* and *R. solani* (Publication II). The plant used for the evaluation of the effect of allelochemicals on soil fungal community is turnip rape (cv: Largo) (Publication II). The fungal strains used as references included different species of *Fusarium, Botrytis, Sclerotinia, Rhizoctonia, Penicillium* and *Rhizopus* as detailed in Table A. 1 (Publication II). Plants used for the evaluation of effects on yield and grain protein concentration of the following barley crop were faba bean, turnip rape, barley, buckwheat, caraway, faba bean, hemp and white lupin (Publication III).

3.3 Methods

Methods used in this dissertation were listed in Table 5.
Table 5. Methods that were used in the present study. The Roman numbers refer to the original publications.

<table>
<thead>
<tr>
<th>Aims</th>
<th>Methods</th>
<th>Publications</th>
</tr>
</thead>
<tbody>
<tr>
<td>To deactivate allelochemicals</td>
<td>Activated charcoal</td>
<td>I</td>
</tr>
<tr>
<td>To measure leaf area index</td>
<td>SunScan SS1 ceptometer bar</td>
<td>I</td>
</tr>
<tr>
<td>To measure plant biomass</td>
<td>70°C for 4 days</td>
<td>I</td>
</tr>
<tr>
<td>To evaluate ability of ITS1F/ITS4 to distinguish fungal species</td>
<td>Genbank analysis</td>
<td>II</td>
</tr>
<tr>
<td>To extract DNA from fungi</td>
<td>E.Z.N.A.® Fungal DNA kit</td>
<td>II</td>
</tr>
<tr>
<td>To extract DNA from soil</td>
<td>E.Z.N.A.® Soil DNA kit</td>
<td>II</td>
</tr>
<tr>
<td>To obtain amplicons</td>
<td>LH (Lenth Heterogeneity) PCR</td>
<td>II</td>
</tr>
<tr>
<td>To differentiae amplicons</td>
<td>Capillary eletrophoresis</td>
<td>II</td>
</tr>
<tr>
<td>To quantify soil fungal biomass</td>
<td>Determining Ergosterol by GC/MS</td>
<td>II</td>
</tr>
<tr>
<td>To test turnip rape extracts on fungi</td>
<td>Agar diffusion assay</td>
<td>II</td>
</tr>
<tr>
<td>To identify amplicons of interest</td>
<td>DNA sequencing</td>
<td>II</td>
</tr>
<tr>
<td>To measure soil pH</td>
<td>1:2.5 (v/v) in 0.01 M CaCl₂</td>
<td>II</td>
</tr>
<tr>
<td>To measure soil mineral N</td>
<td>KCl extraction, colorimetry</td>
<td>II, III</td>
</tr>
<tr>
<td>To determine GPC of barley</td>
<td>Near infrared spectrophotometer</td>
<td>III</td>
</tr>
</tbody>
</table>

GPC: Grain protein concentration, GC/MS: Gas-liquid Chromatography/Mass Spectrometry.

3.4 Design of experiments

3.4.1 Interference potential of crops with weeds (Publication I)

3.4.1.1 Pot experiment

A glasshouse experiment was conducted to investigate whether bio-active compounds in exudates of different plant species can interfere with growth couch grass. Different plant species were used as donors of root exudates. The application of activated charcoal was to exclude the effect of donor crop root exudates. Fourteen couch grass seedlings, used as receivers, (Fig. 3), were transplanted in one row in the middle of each box. Ten cm from each side of the row of couch grass, a row of one of the donor crops was transplanted. In another set of boxes, a trench was dug to the bottom of box on both sides of the couch grass, and filled with mixture of 12.5 g activated charcoal (AC, Sigma-Aldrich, C2889, Sigma-Aldrich Co. LLC., St. Louis, US) and 1000 g soil in a 4 cm wide band, and then donor crops were transplanted in the same way. The experiment was conducted in a randomized complete block design with 4 replicates. Absolute growth rate of crops were regressed against the above-ground biomass of couch grass.
3.4.1.2 Field experiment
In the field experiment, plant samples were collected three times at different growth stages: the first sampling (37 DAS) was at the vegetative stage BBCH (Meier, 2001) growth stage GS35. The second (79 DAS) was at full flowering stage (GS65), and the third (114 DAS) was at the beginning of leaf-drop (GS93). Shoot biomass of weeds and crops (buckwheat, faba bean, hemp and white lupin) were determined from a 0.25 m² quadrat in each plot.

The relative growth rate (RGR) of crops and weeds was calculated based on formula according to Hunt (1982) to minimize the difference resulted from intrinsic properties of different crops. Weed shoot biomass was then regressed step-wise on crop seedling presence, single seed weight, leaf area index and relative growth at different growth stages.

3.4.2 Effects of different cultivation treatments of turnip rape on fungal flora (Publication II)
3.4.2.1 Testing extracts of turnip rape on pathogenic fungi
Each extract was tested on four pathogenic fungi following the procedure of agar diffusion assay (Yu et al., 2002) with four replicates. The four pathogenic fungi were *B. cinerea*, *F. culmorum*, *R. solani* and *S. sclerotiorum* that were isolated in the experimental sites in Viikki. Fungicide (Sportak, Bayer CropScience Pty Ltd, East Hawthorn, Vic., Australia) was diluted 10-fold and added as positive control whereas pure water or hexane was the negative control. The active part of Sportak is rochloraz – imidazole. After inoculation of pathogens and addition of extracts or fungicide or pure water or hexane, petri dishes were sealed with parafilm and incubated at 22°C in alternating periods of 12 h darkness and 12 h
light. After 3 and 9 days, the diameter of the fungal colony in each dish was measured twice and the average was calculated.

3.4.2.2 Field experiment
The experiment, comprised four replicates of five treatments in a randomized complete block design, conducted in 2009 – 2010 and repeated in 2010 – 2011. The treatments were MC (mixed culture) a sown mixture of barley and turnip rape; TAB (turnip rape after barley) consisting of barley sown on the same dates with the MC, followed by turnip rape sown in late July; ETI (early turnip rape incorporation) was the same as MC, but the turnip rape was incorporated into the soil in October; LTI (late turnip rape incorporation), was also same as MC, but the turnip rape was allowed to overwinter and was top-dressed with fertilizer using a precision fertilizer spreader on 4 May in both years, and incorporated at the end of May; and BM (continuous barley monoculture), where barley was sown in the middle of May and harvested in early August, and its stubble was left on the soil. Bulk soil and rhizosphere soil samples were collected periodically (Table 3 in Publication II) and sieved through 2-mm mesh to homogenize the soil and remove plant residues.

3.4.2.3 Soil genomic DNA extraction and capillary-electrophoresis-based LH PCR
Soil genomic DNA and fungal DNA were extracted from 0.8 g of soil and about 0.2 g freeze-dried fungal tissue following the protocol of the E.Z.N.A.® Soil DNA Isolation kit (E.Z.N.A.® Soil DNA kit, Fungal DNA kit, OMEGA bio-tek, Norcross, NC, USA). PCR products amplified by primers ITS1F/ITS4 (ITS1F: 5’-CTTGTCATTAGAGGAAGTAA-3’, ITS4: 5’-TCCTCGCTTATTGATATGC-3’), ITS4 being labeled with FAM, were stored at 4°C overnight before capillary electrophoresis. A mixture of 1 μL of PCR product of each sample, 0.5 of μL 50 bp to 1000 bp size standard labeled with TAMRA (Mapmarker®, MM-1000-TMR, BioVentures, Inc. Murfreesboro, TN, USA) and 10 μL Hi-Di Formamide (Applied Biosystems®, Life Technologies Ltd, Carlsbad, CA, USA) was incubated at 95°C for 3 min, then loaded onto an ABI 3130 (Applied Biosystems®) with the following conditions: injection voltage 1.2 kV, injection time 16 sec, run voltage 15.0 kV, run temperature 60°C, through a 36-cm capillary for 40 minutes.

Length variation of the internal transcribed spacer (ITS) between two subunits of rDNA of different species or even strains enables many finger-printing methods to investigate the microbial community. Capillary-electrophoresis-based length heterogeneity (LH) PCR is used to investigate the dynamics of fungal flora because it is cost-efficient, highly reproducible, technically simple and widely used in microbial community studies (Suzuki et al., 1998; Mills et al., 2003; Connon et al., 2005; Mikkonen et al., 2011). The primer pair ITS1F and ITS4 has been shown to have enhanced fungal specificity and can amplify more species
than other ITS primer pairs (Gardes & Bruns, 1993), and it can differentiate fungi of different species with high reproducibility (Manter & Vivanco, 2007).

3.4.2.4 Measurement of soil properties
On the last sampling date in each experiment, soil samples for the following measurements were taken and stored in -20 °C. The pH was determined by pH meter after suspending 1 part soil in 2.5 parts of 0.01 M CaCl₂ overnight. Fungal biomass in 1 g soil was measured by quantifying ergosterol of fungi (Kontro and Vauramo, 2009). Subsamples of top soil for mineral N concentration analysis were thawed at 4°C overnight before extraction. Soil mineral N was extracted from 30 mL of soil in 100 mL of 2 M KCl (shaken for 1 h) and measured by LACHAT using a Quikchem® 8000 automated analyzer (Hach Company, Loveland, CO, USA) according to Quikchem method 12-107-06-2-A for NH₄⁺ and 12-107-04-1-E for NO₃⁻. Soil water content was determined by the difference between the weight of soil sample and the weight after heating at 105°C overnight. The fungal biomass in soil was corrected to a dry-weight basis. The soil mineral N concentration was determined in mg/L, and then converted to kg/ha on the assumption of 2 million L/ha [100 m * 100 m * 0.2 m (0 – 20 cm, depth of top soil taken)] (Erviö et al., 1990).

3.4.2.5 Nutrient gradient experiment
To test how nutrient concentration can affect soil-borne fungi, four growth media were prepared (Table 6), and 50-mL aliquots were poured into 15-cm (diameter) Petri dishes. A 10-g soil sample from a turnip rape plot was suspended in 100 mL sterile water and further diluted ten-fold with sterile water, then 400 μL of the suspension was spread on each of four replicate dishes per nutrient level. After the dishes were incubated for 4 d at room temperature (approx. 22°C), the number of colony forming units (CFUs) with pigment (from light pink to dark purple) typical of *Fusarium* spp. was counted. Two pigmented colonies were selected from each dish, spread on a microscope slide, and examined using a 40× objective lens of a Leica Laborlux S microscope (Leica Microsystems GmbH, Wetzlar, Germany).

<table>
<thead>
<tr>
<th>PDP (g)</th>
<th>Streptomycin Sulfate (g)</th>
<th>Agar (g)</th>
<th>Sterile water (L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0.3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>0.3</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>0.3</td>
<td>16</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 6. Growth media containing different PDP for nutrient gradient experiment

PDP: Potato Dextrose Powder
3.4.3 Effect of rotation crops on successive barley (Publication III)

3.4.3.1 Field experiment

In two three-year rotation experiments (Fig. 4), barley, faba bean and turnip rape, as first crops, were grown in one block (40 m × 25 m) in the first year. In the second year, second crops were sown in randomized complete block designs with four replicates in each block of first crops. At flowering stage, the whole plots (6 m × 1.25 m) of caraway and white lupin were incorporated into uppermost 10–15 cm soil with a rotary power harrow (Fig. 5). Half plots (3 m × 1.25 m) of buckwheat, faba bean and hemp were incorporated into the soil at flowering stage and the residues of the other half were incorporated after harvest. In the third year, all the plots were sown with barley.

Throughout the experiment, the synthetic fertilizer, banded to the seed-bed at the time of sowing, was N-P-K 23-3-5 (Cemagro Oy, Lohja, Finland) for the non-legume crops and N-P-K 16-7-13 for the legumes (Table 1 in publication III). Both faba bean and white lupin were inoculated with appropriate commercial strains of rhizobium (Elomestari Oy, Kukkola, Finland) before sowing.

Figure 4. Field experimental setup (Publication III).
3.4.3.2 Barley yield and grain protein content

Barley was harvested and its yield was determined (kg/ha) from the plots at maturity. The grain yield was dried, sorted, weighed, and 13% moisture content was converted to weight and reduced from the total weight. The protein concentration of the grain was determined with a near infra-red analyser (DA 7200, Perten Instruments AB, Segeltorp, Sweden).

3.4.3.3 Soil mineral N measurement

Soil samples (0–30 cm) were taken two months after the crop residues were tilled and again before sowing barley in the next growing season for mineral N (NO$_3^-$ and NH$_4^+$) measurement. Samples were stored at -20 °C until they were analysed at the Finnish Environmental Services (Suomen Ympäristöpalvelu Oy, Oulu, Finland). The difference in soil mineral N concentration between sampling times was recorded as N loss (leaching, immobilization, gaseous losses). Results were converted from mg/mL to kg/ha using the same method in 3.3.2.4, with the depth of top soil changed to 0.3 m.

Weather data were collected including accumulated rainfall, temperature, number of days with freezing temperature (\( \leq 0^\circ\text{C} \)) and accumulated snow depth (FMI 2013).

3.5. Statistics

3.5.1 Analyses by Bionumerics

The range of PCR products was limited between 150 bp and 1000 bp length standards by GeneScan version 3.7 (Applied Biosystems®). The area of each peak representing an OTU was calculated using BioNumerics 5.0 (Applied Maths NV, Sint-Martens-Latem, Belgium) to investigate the change of OTUs under different cultivation treatments. The Shannon diversity index (SDI) of each analyzed sample was calculated as: \( H' = -\sum p_i \ln p_i \), where \( p_i \) is the relative area of the \( i \)th OTU peak.

Cluster analysis of the normalized fingerprints of all samples of different dates was performed in BioNumerics to visualise clustering of fungal communities. Pairwise similarities for the electrophoretograms were calculated with Pearson
correlation and a dendrogram was drawn with Ward's method taking the presence, absence, and relative abundance of peaks into account.

3.5.2 Univariate and multivariate analyses

The statistical analyses were conducted using R (R Core Team, 2013). The effects of treatments (Publications I–III) were determined by ANOVA, and when \( P < 0.05 \), the effect of treatment was considered as statistically significant. Means of treatments were compared using the LSD test (\( P = 0.05 \), Publications I–III). Principal component analysis (PCA) and Correspondence analysis (CA) were conducted based on correlation matrix to reveal the relationship among variables and how observations change with variables (Publication II). Step-wise simplification of models was conducted to exclude factors that did not significantly (\( P < 0.05 \)) affect barley yield, mineral N two months after incorporation of plant materials, mineral N before sowing barley, and mineral N loss (Publication III).

4 RESULTS AND DISCUSSION

4.1 Evaluation of ability of different crops to interfere with growth of weeds

The results of the present research showed that growth rate is an important factor determining the weed-suppressive ability of crops. Due to its slow growth, caraway did not seem to be promising rotation crops to supplement weed management (Fig. 6). In contrast, buckwheat was the fastest-growing crop which was related to the lowest biomass of couch grass growing in the same box. Also, leaf area was important factor determining the weed-suppressive ability because in hemp plots, the male plants senesced after flowering, reducing overall leaf area and allowing weeds to grow. Monoecious hemp cultivars may provide more effective weed suppression. Couch grass accumulated much less dry mass when grown with faba bean than with the other crops (Table 1 in Publication I), even though, there was no other evidence in the present study showed any bio-active compounds in faba bean could inhibit weed growth. In field experiments, several studies concluded that early vigor and fast growth of crops are the most important factor determining weed-suppressive ability (Reid et al., 2009; Zystro et al., 2012; Dai et al., 2014).

In addition to the physical competition that buckwheat provided, the results of the glasshouse experiment showed that buckwheat root exudates could inhibit couch grass (Fig. 1 in Publication I). Kalinova et al (2007) showed that 4-hydroxyacetophenone and vanillic and gallic acids extracted from buckwheat roots could inhibit the growth of *Amaranthus retroflexus* L., *Achillea millefolium* L. and *Sinapis alba* L. in agar. In contrast, weed-suppressive ability of some identified allelochemicals in some crops was not detectable in this research. For instance, thymol, carvacol, and carvone in caraway inhibited the germination of *Amaranthus retroflexus* L., *Centaurea solstitialis* L., *Raphanus raphanistrum* L., *Rumex nepalensis* Spreng., *Sinapis arvensis* L. and *Sonchus oleraceus* L. Azirik...
and Karaman, 2008). Alkaloids of white lupin such as lupanine and sparteine inhibited germination of lettuce (*Lactuca sativa* L.) (Wink, 1983). This might be attributable to concentration of allelochemicals in different cultivars and parts of plant vary or deactivation of allelochemicals by soils (Poulsen et al., 2008). Buckwheat appears to be the ideal candidate for weed management, because in the field experiment, it rapidly developed large leaf area and effectively shaded weeds and its weed-suppressive root exudates.

4.2 Capillary-electrophoresis-based LH-PCR using ITS1F/ITS4 as a method in investigating fungal flora

The primers ITS1F/ITS4 used in the present study could differentiate amplicons from different reference strains (Table A.1 in Publication II), and size-calling based on capillary electrophoresis produced consistent results for all the amplicons of the species, with standard deviations (SD) of less than 1 bp. However, when a difference of less than 2 bp between fungi could not be resolved, indicating the risk of underestimating fungal diversity. The results of Genbank analysis (Table 1 in Publication II) also indicated that there is a risk of underestimating fungal diversity, since the CIs between some species of *Fusarium* overlapped. Therefore, the number of species of *Fusarium* can be underestimated. Anderson et al. (2003) reported that species numbers were less underestimated by ITS1F/ITS4 than by other ITS primers. However, to identify an unknown amplicon, a reference strain is helpful, and to confirm it, sequencing is necessary. For systematic investigation of microbial flora, high-throughput sequencing will be needed to reveal the constitution and phylogeny of OTUs.
4.3 Role of turnip rape on fungal pathogen management

4.3.1 Effect of extracts of turnip rape on pathogenic fungi

Many of the turnip rape extracts inhibited the growth of fungi 3 days after inoculation (DAI) in comparison with water and hexane controls (Table A. 3 in Publication II). Water extract from the shoot inhibited the growth of *F. culmorum* and *R. solani* more than that of other species at 3 DAI, and still mildly inhibited the growth of *F. culmorum* and *R. solani* 9 DAI. Most extracts had no detectable effect on the growth of fungi at 9 DAI. Thus, the effect of turnip rape extracts was transient. The volatile nature of isothiocyanates (ITC) (Buttery et al., 1976) may explain this transient inhibition of turnip rape extracts. ITC is toxic to a wide range of different fungi (Kirkegaard & Matthiessen, 2004), which would have inhibited growth of fungi after turnip rape was incorporated in the soil in the present study. However, in the treatments ETI (Early turnip rape incorporation) and LTI (Late turnip rape incorporation), fungal biomass was higher than in other treatments (Table 4 in Publication II). Poulsen et al. (2008) showed that large amounts of glucosinolate and isothiocyanates were incorporated in organic fractions in both sandy and clayey soils with low availability to microorganisms. Matthiessen and Shackleton (2005) suggested that properties of soil such as pH, calcium level and soil texture could affect the efficacy of using isothiocyanates derived from *Brassica* crops in soil-borne pest management. Therefore, the role of isothiocyanates in soil-borne pathogen management is limited because of its volatile nature and susceptibility to deactivation in soils.

4.3.2 Low nutrient availability resulted in low fungal diversity

Soil samples of treatment BM (barley monoculture) were low in soluble N and Shannon diversity index in bi-plots (Fig. 4 in Publication II) of both experiments. The change of different OTUs representing different fungi was more drastic in BM than in other treatments (Fig. A. 1 in Publication II). This indicated that low nutrient availability created a competitive environment for fungi. The plant, as a competitor for nutrients with microbes, can reduce the population of fungi, and N can be sequestered for much longer by plants than microbes, so plants generally win the competition (Hodge et al., 2000). The presence of barley roots depressed microbial growth in an N-poor soil layer enriched with barley stubble but not in an N-sufficient layer enriched with clover (Wang & Bakken, 1997). Mycorrhizal fungi, as symbionts of plants, compete with other soil microbes for carbon and N sources (Chalot & Brun, 1998). Therefore low nutrient availability can create a more competitive environment for plants and microbes in the soil and hence may result in elimination of some fungal species. Sufficient nutrient input is important in maintaining soil microbial diversity. As Turner et al. (2013) indicated, the higher soil N nutrient status in pea plots resulted in much higher diversity of eukaryotes than in wheat plots.
4.3.3 *Fusarium* spp. thrives in low nutrient availability

In the field experiment, operational taxonomic units (OTUs) indicative of *B. cinerea*, *R. solani* and *S. sclerotiorum* did not respond to different treatments unlike those for *Fusarium*. The lower relative abundance of *Fusarium* spp. was associated with treatments ETI (early turnip rape incorporation) and LTI (Late turnip rape incorporation), whereas higher relative abundance of *Fusarium* spp. was associated with treatment BM (continuous barley monoculture) (Fig. 3 in Publication II). Moreover, in soil samples of the last sampling date of both experiments under BM, SDI, fungal biomass and soil mineral N were negatively related to the relative abundance of *Fusarium* spp. The results of sequencing confirmed the presence of *Fusarium* spp. (Table A. 2 in Publication II). This indicated that *Fusarium* spp. thrived in the competition for nutrients. The higher soil *Fusarium* CFU count on nutrient-poor growth medium than on rich medium supported the results of the field experiments (Fig. 7). The number of pigmented colonies, which was markedly higher on the nutrient-poor growth medium than on the nutrient-rich one (Fig. 8), were identified as *Fusarium* spp. by microscope. The pigment of the *Fusarium* colonies may have been bikaverin, production of which is usually induced in N-limited or acidic conditions (Limón et al., 2010). Its biological function remains unknown. *Fusarium* remains viable for a long time. For example, *F. culmorum* has been isolated from air-dried inoculated soil after 13 months (Vakalounakis & Chalkias, 2004) and some species of *Fusarium* have been recovered after 10 years storage in anhydrous silica gel or sterilized soil (Windels, 1993). The mechanisms of the survival and competitiveness of *Fusarium* in adverse environments will be an interesting topic to investigate in the future. The incorporation of turnip rape at the flowering stage did not inhibit the growth of fungi. On the contrary, it enhanced their growth, resulting in higher fungal diversity and biomass (Table 4 in Publication II).

OTU 613 increased in relative abundance in treatment BM in both experiments. The length of this OTU is only 1 bp less than that of the reference *Penicillium* strain, and the sequence of OTU 600 of treatment BM in the second experiment was most similar to that of *P. ochrochloron*, showing that species of this genus might also be able to tolerate an environment with low nutrient availability. Dong et al. (2006) found that *Penicillium* sp. could effectively reduce the incidence of cotton wilt disease caused by *F. culmorum*. Morales et al. (2007) observed that *Penicillium* sp. was able to solubilize phosphates. Therefore, the genus may have further potential as both a biofertilizer and a bio-control agent for diseases caused by *Fusarium* spp.
Figure 7. Effect of the growth media with 2 g PDP (Potato dextrose powder) (the lower 4 replicates) in comparison with that with 8 g PDP (the upper 4 replicates) on the number of pigmented colonies in petri dishes.

Figure 8. Effect of different PDP concentrations on the relative abundance of *Fusarium* spp. in two experiments. Bars show means with LSD, n = 4. LSD was calculated based on P < 0.05, D.F. = 10.
4.4 Effect of rotation crops on yield of successive barley

The effects of faba bean and turnip rape as first crops on increasing barley yield and grain protein concentration (GPC) were both still detectable after two years (Table 3 in Publication III). Kraljević et al. (2007) found that rapeseed enhanced the growth and grain yield of subsequent wheat. Faba bean increased soil mineral N and enhanced N uptake by the subsequent wheat in Germany (Kaul, 2004).

Faba bean, hemp and white lupin as second crops were all associated with higher third-year barley yields (Table 3 in Publication III). Moreover, rotation with faba bean was associated with higher grain protein concentration. In contrast, buckwheat and caraway did not improve barley yields. This indicated that the beneficial effects of rotation crops differed. Buckwheat can inhibit weed growth because of its large leaf area and root exudates containing allelochemicals (Results of Publication I), but it is not evident if the residual effect of these exudates or other secondary chemicals caused the low barley yield in this study.

4.5 Incorporation of green manure did not result in more N.BS and barley yields

Plants incorporated at the flowering stage contained more nutrients than those incorporated after harvest, but third-year barley yield did not show differences due to incorporation stage. Nevertheless, GPC of third-year barley was generally higher after plants incorporated at the flowering stage than when their post-residues were incorporated (Table 3 in Publication III). Despite the increase of GPC, incorporation at flowering posed great potential for N loss (N.D) (Table 3 in Publication III), with the mean value of N.D being positive when plants were incorporated at flowering, negative when plants were incorporated after harvest. Thus, incorporation of plant residues after harvest resulted in less mineral N loss. Likewise, it was found that delaying the incorporation of ryegrass as catch crop in Sweden and Denmark effectively reduced N leaching (Stenberg et al., 1999; Thorup-Kristensen & Dresbøll, 2010).

Temperatures from May to October in 2010–2012 were broadly similar, except that temperatures in late October 2010 and 2012 dropped below 0°C (Fig. 6 A–C). Thus the climate is warm enough for immobilization, denitrification and mineralization by microbial activity (Pietikainen et al., 2005). This can explain why delayed incorporation reduced mineral N losses. The temperature dropped sharply after October. The average temperature of top soil (at 20 cm depth) between harvesting in autumn and sowing of the following spring is about 0.5°C (Heikinheimo and Fougstedt, 1992), which limits volatilization, and microbial activity that can lead to immobilization.

Furthermore, the large amount of precipitation from November to the following May in Finland could cause more N loss due to absence of plant cover and
release of N caused by freezing and thawing. A two-fold increase of N mineralization was observed in arable soils 20 days after thawing, attributed to the lysis of cells of plant materials and microorganisms (Joseph & Henry, 2008). The number of freeze-and-thaw cycles (Fig. 10 D-F) in winter-spring of 2011 – 2013 was 12, which was more than the other two winter-springs and related to the lowest N.BS. It had been known that more number of freeze-and-thaw cycles can cause more N leaching which may in turn affects N availability to plants (DeLuca et al., 1992, Joseph & Henry, 2008). Furthermore, other nutrients than N can also be affected by freeze-and-thaw cycles (Sjursen et al., 2005; Liu et al., 2010; Wang et al., 2014). It would be interesting to investigate effects of freeze-and-thaw cycles on soil nutrient level and its contribution to crop yields in Finland for longer time and in multiple locations.

4.6 The effect of mineral N in plough layer on barley yield was minor
Mineral N concentrations before sowing barley (N.BS) (Fig. 9) were lower in 2012 than in 2013. However, the third-year-barley yield in 2012 was, in general, higher than that in 2013. Furthermore, coefficients of regression models using N.BS to predict barley yield were significant in site I (2010 – 2012), but not in site II (2011 – 2013). However, the $R^2$ value of the significant model was low, and adding N.BS as covariate in the simplified model did not cause any increase in the proportion of variation explained. Furthermore, in site II, organic matter was more than in site I, which may allow more N to mineralize. Nevertheless, yield in site II was remarkably lower than in site I (Fig. 9). Mineral N in the soil was 23 kg/ha at site I and 29 kg/ha at site II (Table 3 in Publication III), which can be classified as medium in terms of the amount of top-soil mineral N compared with results of soil mineral N (0 – 30 cm) in some research conducted in Finland, where top-soil mineral N averaged 38 kg/ha in western Finland (Paasonen-Kivekäs & Yli-Halla, 2005), and 25.4 kg/ha in southern Finland (Tuulos et al., 2014). A meta-analysis of Finnish data from 1940–2004 (Valkama et al., 2013) showed that barley yield response to N fertilization and N derived from soil organic matter varied between years, and that the variation in yield was largely due to unidentified factors.
Figure 9. Response of barley yields to mineral N in the plough layer (N.BS) before sowing third-year barley. The correlation was significant at site I (***: $P < 0.001$), with the S.E. of the slope being 0.008 and that of the intercept 0.19.

4.7 What is the most-weighted tool in sustainable barley-cropping?

Buckwheat showed superior weed-suppressive ability among the crops tested (publication I). Crop rotation in agro-ecosystem can change weed flora in comparison to long-term monocultures (Andersson and Milberg, 1998). However, barley yield and grain protein concentration was not improved after buckwheat (publication III). Fungal diversity remained higher and *Fusarium* populations lower in treatments involved turnip rape and incorporation of its green manure than barley monoculture (publication II), but the yields of the following barley after these treatments were not significantly different from barley monoculture (data not published because only one-year data in 2010 is available). This is not surprising because it has been proved that increased *Fusarium* pathogen populations do not necessarily lead to more disease incidence (Edwards et al., 2001; Steinkellner and Langer, 2005). Nevertheless, the effects of turnip rape, as the first crop, improved barley yields at both experimental sites in 2012 and 2013 (publication III). Therefore, contributions of rotation crops to successive barley can be influenced by climates. Finding out the most yield-limiting factor seems to be an important step towards barley sustainable cropping.

It would be interesting to investigate other effects of rotation crops in further research. For example, legume crops can enhance the growth of beneficial soil microbes (Peoples et al., 2009) and mobilize P by root exudates in soils, thus enhancing uptake of P by subsequent non-legume crops (Hinsinger 2001; Li et
al., 2009). Many rhizosphere activities can be enhanced by sufficient rhizodeposition for instance, growth of PGPR (plant growth-promoting rhizobacteria) that can improve plant nutrition (Hartmann et al., 2009; Richardson et al., 2009).
Figure 10. Rainfall (RF) of each month and sum of temperatures (ST.1) from May to October, and temperatures sum (ST.2), the number of days with temperature below 0 °C (FD), and the number of freeze-thaw cycles (CYC) from November to May of in 2011, 2012, 2013. One freeze-thaw cycle (Figure 6D) included the time when the temperature started to increase from temperature below 0 °C, to temperature above 0 °C and decrease to or below 0 °C.
5 CONCLUSIONS
The results of this dissertation demonstrate that buckwheat is an ideal candidate for weed management, because of its fast growth to form effective higher LAI that could shade weeds and root exudates containing allelochemicals that could inhibit weed growth additionally. Purifying the allelochemicals from buckwheat root exudates and investigating the mode of actions is interesting for further research.

Higher fungal diversity, maintained through sufficient nutrient and organic matter input, was more closely related to lower *Fusarium* population than the presence of bio-active compounds from turnip rape, because of the transient effect of these compounds on growth of fungi. Higher fungal diversity can be associated with presence of more microbes that are antagonistic to pathogens. Thus, concluding allelochemicals exist in natural forms from donor plants to control pathogens can be confounded, and using bio-fertilizers including inocula of antagonistic fungi can help to control *Fusarium* populations.

Rotation with faba bean, white lupin and hemp increased successive barley grain yield. In addition, rotation with faba bean and turnip rape increased barley grain protein concentration. In contrast, rotation with buckwheat and caraway did not affect the yield and grain protein concentration of the following barley crop. Increased mineralized N from green manure or residues of rotation crops significantly affected barley yields in only one case of different experiments. Mineral N supply before sowing barley is highly dependent on weather conditions in Finland. Cold winter weather with more days with freezing temperature resulted in more N in soil. It would be interesting to investigate changes of rhizodeposits in other forms related to rotations with faba bean, hemp and white lupin. Early incorporation of green manures of crops cannot be recommended because more mineral N derived from crop residue can be subjected to leaching in long winter-spring period compared with delayed incorporation after harvest.
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