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Leguminous service crops in cereal production at high latitudes

Provision of ecosystem services and disservices from a novel intercropping system

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Cover: Late sown service crops (*Vicia villosa* and *Trifolium incarnatum*) in Östergötland 2017 (Exp3)
(photo: E. Lagerquist)

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Leguminous service crops in cereal production at high latitudes

Abstract

Service crops, crops grown to provide services such as soil protection, weed control, or nutrient capture and provision, can reduce the need for mineral fertilisers and intensive weed control. However, high biomass production is necessary in order for these services to be provided. This can be difficult to achieve at high latitudes (*e.g.* Sweden), where growing conditions are poor after main crop harvest, when service crops are usually sown. The aim of this thesis was to increase the understanding of service crop species choice and management on service and disservice delivery. Leguminous service crops were sown into oats in spring/early summer for early establishment of the service crop, and terminated in the following spring in winter wheat. The results showed that species sown at the same time as oats, rather than one month later, and which survived oat harvest and cold temperatures in winter, were best at suppressing weeds and increasing winter wheat yields, although they often also reduced oat yield in the establishment year. Presence of a productive service crop (>0.2 t dry weight (DW) per ha) reduced the occurrence of competitive-stress tolerant weed species, in favour of ruderal species, in the oat row and in combination with oat biomass >8 t DW ha^{-1} the occurrence of competitive perennial species was reduced. Modelling the system using APSIM NG revealed that the service crops mainly provided positive effects on the cropping system, in terms of increased winter wheat yield, increased soil carbon input and reduced losses of nitrogen and water during most of the study period. Drawbacks were reduced oat yield and increased nitrogen losses from winter wheat and in the fallow period after winter wheat. I conclude that the studied intercropping system has potential to provide services and reduce disservices, but that the system needs improvement. These improvements are mainly to ensure even establishment and good termination of the service crops.

Keywords: agroecology; biodiversity; ecological weed management; *Medicago*; modelling; nitrogen; *Trifolium*; water; *Vicia*; yield

Servicegrödor i spannmålsproduktion på nordliga breddgrader

Abstract

Servicegrödor odlas för att bidra med tjänster som att skydda marken mot erosion, bekämpa ogräs och ta upp och leverera näringsämnen, vilket kan minska behovet av mineralgödsel och intensiv ogräsbekämpning. Det krävs dock stor biomassaproduktion för att dessa tjänster ska uppstå. Detta kan vara svårt på nordliga breddgrader (t.ex. Sverige) där tillväxtförhållandena är dåliga efter huvudgrödans skörd, den tid då servicegrödor vanligtvis sås. Syftet med denna doktorsavhandling var att öka förståelsen för hur olika arter av servicegröda och odlingsåtgärder påverkar leverans av tjänster och otjänster. Baljväxter såddes in i havre på våren/försommaren för tidig etablering av servicegrödan, och avdödades påföljande vår i höstvete. Jag fann att arter som såddes samtidigt med havre, jämfört med de som såddes en månad senare, och som överlevde havreskörden och de låga temperaturerna på vintern var bäst på att minska mängden ogräs och öka skörden av höstvete, men de minskade också havreskörden under etableringsåret. Högproduktiva servicegrödor (>0,2 t torrsubstans (TS) per ha) minskade förekomsten av konkurrenskraftiga-stresstoleranta ogräsarter, till förmån för ruderala arter, och i kombination med en biomassa >8 t TS ha^{-1} av havre minskade också förekomsten av konkurrenskraftiga perenna arter. Modellering av systemet med APSIM NG visade att servicegrödorna huvudsakligen gav positiva effekter på odlingssystemet, i form av ökad höstveteskörd, ökad koltillförsel i marken, minskade förluster av kväve och vatten under större delen av studieperioden. Nackdelarna var minskade havreskördar och ökade kväveförluster i höstvete och trädaperioden efter höstvete. Jag drar slutsatsen att det studerade samodlingssystemet har potential att bidra med tjänster och minska otjänster, men att systemet behöver förbättras. Dessa förbättringar är främst att säkerställa jämn etablering och bra avdödning av servicegrödorna.

Nyckelord: agroekologi; biodiversitet; ekologisk ogräsbekämpning; kväve; *Medicago*; modellering; odlingssystem; skörd; *Trifolium*; vatten; *Vicia*

Dedication

To Kjell and Ylwa Sjelin who first introduced me to the idea that agricultural systems should be managed in such a way that it improves the ecosystem. And to all the innovative, visionary farmers I have met on my journey this far, who have inspired me and given me joy!

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text

I Lagerquist E.*, Menegat A., Dahlin A.S., Parsons D., Watson C., Ståhl P., Gunnarsson A., Bergkvist G. (2022). Temporal and Spatial Positioning of Service Crops in Cereals Affects Yield and Weed Control. *Agriculture*, 12(9), 1398. Doi: 10.3390/agriculture12091398

II Lagerquist E.*, Bergkvist G., Menegat A. Management-induced micro-ecosystems in crop fields alter the functional trait composition of arable plant communities. (submitted manuscript)

III Lagerquist E.*, Vogeler I., Kumar U., Bergkvist G., Lana M., Watson C.A., Parsons D. Assessing the effect of intercropped leguminous service crops on main crops and soil processes using APSIM NG. (submitted manuscript)

*Corresponding author.

Paper I is published Open Access.

The contribution of Elsa Lagerquist to the papers included in this thesis was as follows:

I Planned and conducted data collection. Took care of the lab work related to biomass and N15 measurements. Analysed the data. Wrote most of the manuscript with help from co-authors.

II Planned and conducted data collection and lab work. Found and learned the RLQ method. Analysed the data. Wrote most of the manuscript with help from co-authors.

III Planned and conducted data collection and lab work. Calibrated the model, designed and ran the scenarios. Analysed output data. Wrote most of the manuscript with help from co-authors.

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1. Introduction

Cropping system practices and designs are continually being refined to meet the needs of humankind. Increasing productivity has been the highest priority for decades, contributing to the global goal of reducing famine (Byerlee & Fanzo 2019). In many parts of the world this goal has been achieved through improvements in crop varieties and management practices (Alexandratos & Bruinsma 2012). Countries in Europe and North America have experienced great improvements in crop productivity since the mid-20th Century, as indicated by increased annual cereal yields while the annual harvested area has decreased (Giller et al. 2021). These yield improvements can be attributed to plant breeding for high-yielding cultivars (Pronin et al. 2020) and use of large amounts of external resources to provide nutrients and reduce weeds, pests and diseases in cropping systems based on a few high-yielding crops (Schreinemachers & Tipraqsa 2012; Lassaletta et al. 2016; Yuan et al. 2018). However, these intensive, high-yielding systems carry a cost in terms of environmental degradation and contribution to global warming (Foley et al. 2005; Bajželj et al. 2013; Campbell et al. 2017). Agricultural intensification has played a major role in increasing the amount of nitrogen and phosphorus in terrestrial and aquatic ecosystems (Fowler et al. 2013; Muhammed et al. 2018). A considerable proportion of the added nutrients is lost from the system, causing eutrophication (Andersen et al. 2017), contributing to global warming (Hong et al. 2021) and polluting the air and drinking water (Townsend et al. 2003). Today, approximately half of all nitrogen entering crop fields is lost via leaching and gaseous emissions during crop production (Billen et al. 2021). Agriculture contributes 52% of total anthropogenic nitrous oxide (N_2O) emissions worldwide (Tian et al. 2020). Although a large proportion of these anthropogenic N_2O emissions derive from animal production (Bajželj et al. 2013), mainly from ruminant metabolism, annual cropping systems contribute three times more N_2O emissions than perennial systems, and mineral and mixed fertilisers contribute four times more than organic fertilisers (Hergoualc'h et al. 2021). Moreover, agricultural intensification at field and landscape level, with few crops and similar soil and crop management strategies, has greatly reduced the arable flora and fauna. Repeated soil tillage and/or herbicide treatment at similar yearly time points has placed strong selection pressure on weed communities and has shaped them into communities consisting of a few well-adapted and often competitive species (Storkey et al. 2012; Redlich et al. 2018; Sánchez-Bayo & Wyckhuys 2019). This selection pressure is also one of the main drivers of the ongoing loss of several types of organisms in agricultural landscapes (Cowie et al. 2022). Furthermore, the generally intensive use of herbicides in agriculture has resulted in a rapid increase in herbicide resistance in weeds, which will probably make them more difficult to control in the future, at least with existing weed control measures (Peterson et al. 2018). The negative effects of agriculture on the surrounding environment, the loss of efficient pesticides to sustain current management practices and the contribution to climate change mean that the agriculture sector needs to adopt more environment- and climate-friendly practices.

1.1 Ecosystem services

For agriculture to be sustainable, it must meet the needs of both present and future generations (FAO 2023). This means that its environmental impact and resource use must be sustainable, and also that soil physical, chemical and biological functions must be maintained and in many cases improved (Lal 2015). The concept of ecosystem services was established to acknowledge the fundamental value of natural processes for human societies and wellbeing (Daily 1997), and can be applied to guide more sustainable natural resource management (World Resource Institute 2003). Ecosystem services are divided into four groups: provisioning (*e.g.* food, fuel, biomass), regulating (*e.g.* climate regulation, erosion protection, pollination), supporting (*e.g.* soil formation and retention, nutrient and water cycling, provision of habitats) and cultural (*e.g.* spiritual, aesthetic value) (World Resource Institute 2003).

Studies on ecosystem service delivery from agricultural systems (agroecosystems) are rather few compared with corresponding studies on natural systems (Liu et al. 2022). Besides production of food, agroecosystems can supply ecosystem services such as nutrient cycling, carbon sequestration, pollination and biological control (Swinton et al. 2007; Zhang et al. 2007; Bommarco et al. 2013). These services could help reduce the impact of agriculture on the environment and the climate, as well as sustaining agricultural production in the long-term (Bommarco et al. 2013; Lal 2019; Reiss & Drinkwater 2020). Neglecting the importance of supporting and regulating functions in agroecosystems may lead to negative impacts on both the agroecosystem itself and surrounding ecosystems, *e.g.* when lack of vegetation cover results in erosion (Borrelli et al. 2017), lower soil organic matter content results in reduced soil porosity and water-holding capacity (Naveed et al. 2014) or landscape simplification leads to loss of species, reducing the potential for pollination and pest control services (Dainese et al. 2019). Hence, ecosystem services can be beneficial to society at large, to the farmer directly, or to both (Kremen 2020).

Agroecosystems may also cause, or be subjected to, so-called disservices. Ecosystem disservices can be generated in the surrounding environment and act on the agroecosystem, such as pest damage, competition for water or competition for pollination, or can be generated in the agroecosystem and affect the surrounding environment, such as habitat loss, nutrient runoff or leaching, or pesticide poisoning of non-target species (Swinton et al. 2007; Zhang et al. 2007). In highly managed ecosystems, such as agroecosystems, human intrusion and management intensity of the system are key variables for ecosystem processes, and hence have a great influence on both services and disservices from the system (Barot et al. 2017). Some argue that disservices should also include the effect of agricultural management on more distant ecosystems, *e.g.* where external inputs to crop and animal production are manufactured (Barot et al. 2017; Blanco et al. 2019). These disservices include emissions of greenhouse gases during the manufacture of mineral nitrogen (Menegat et al. 2022), pollution of air and water from mining and processing of phosphorus (Reta et al. 2018) and conversion of ecosystems with high natural value into agricultural land for production of livestock feed for the global market (Da Silva et al. 2021). Another complicating factor when addressing ecosystem service and disservices is that the same "provider" can generate both services and disservices, in space and/or time. Examples are insects or animals which may provide services such as pollination or pest and disease control in one crop or at a specific time, while being a crop pest in another crop or at another life stage of either the insect/animal or the crop (Saunders et al. 2016). The term disservices has met with some criticism, especially when applied to natural ecosystems, as disservices are often viewed solely as a consequence of human intrusion or mismanagement of ecosystems (Shapiro & Báldi 2014; Villa et al. 2014). Another criticism is that since the concept of ecosystem services was developed to counterbalance the generally negative view of natural ecosystems as dangerous, highlighting disservices supports this negative perception of nature (Shapiro & Báldi 2014). However, others argue that inclusion of disservices in ecosystem service assessments allows for a more holistic view of ecosystems (Blanco et al. 2019). Moreover, avoiding disservices from a particular ecosystem can sometimes be a stronger incentive than gaining services from that ecosystem (Blanco et al. 2019).

Adapting management practices so that they enhance and make use of ecosystem services in agricultural landscapes is the core of the concepts ecological intensification (Bommarco et al. 2013; Tittonell 2014) and agroecology (Wezel et al. 2020). However, applying an ecosystem service (and disservice) perspective in agriculture is knowledge-intensive and there is still much to learn regarding general processes and how they relate to management, especially at local level (Bommarco et al. 2018).

1.2 Diversifying cropping systems

In-field crop diversification, *i.e.* growing a larger number of species in space or time, has been identified as an important tool to promote ecosystem service provision (Tamburini et al. 2020). Diversification practices, *e.g.* intercropping, crop rotation, service crops and high overall plant diversity at field level (including weeds), have been shown to improve soil fertility, nutrient cycling, water regulation, crop yields, carbon sequestration, pest control and biodiversity (Tamburini et al. 2020). Service crops, which are the research object in this thesis, are crops grown to provide one or several services to the cropping system (Gardarin et al. 2022). The term include *e.g.* cover crops, catch crops and green manures, which generally have their main growth period between two crops. Crop diversification has also been shown to increase the resilience of cropping systems to extreme weather (Smith et al. 2023).

The positive effects of crop diversification are due to the greater diversity of functions these crops provide. Increasing the number of species in a rotation can improve yields by reducing pest pressure (Barzman et al. 2015; Andert et al. 2016) and including deep-rooted crops can improve soil structure (Ball et al. 2005). Growing legumes, especially forage legumes, can increase soil nitrogen content without addition of mineral nitrogen, due to inputs of nitrogen-rich organic material from dinitrogen (N_2) fixing plants, which has a positive effect on the subsequent crop (Jensen et al. 2021). The positive effect of diversified crop rotations is particularly apparent in low-input systems (Smith et al. 2023), where natural processes play a more important role for system functioning (MacLaren et al. 2022). At landscape level, increased crop diversity promotes a higher diversity of flora (Alignier et al. 2020), which in turn can benefit crops through pollination, pest control and potentially less harmful weed communities (Storkey & Neve 2018; Dainese et al. 2019). Diversified crop rotations also increase the variation in management practices, *i.e.* sowing time and weed control, which prevents weed species from adapting to specific management practices (Fried et al. 2012). However, if crop diversification is introduced without increasing crop functional diversity, some of the positive effects of diversification are lost (Smith et al. 2023). Hence, diversification needs to be planned in such a way that essential functions are provided, in the long-term, short-term or both.

1.3 Service crops to increase cropping system sustainability

Growing service crops is a potential way to increase cropping system diversity and enhance delivery of ecosystem services (Figure 1). Service crops can enhance in-field nutrient cycling via N_2 fixation (Guiducci et al. 2018), nutrient mining (Wendling et al. 2016; Hallama et al. 2019) and by taking up residual soluble nutrients that are otherwise at risk of being leached (Zhao et al. 2020), or by a combination of all these depending on the species are grown and related management practices. With appropriate management, these nutrients can be available to the subsequent crop, increasing yields and grain nitrogen content (Blackshaw et al. 2010; Bergkvist et al. 2011; Vrignon-Brenas et al. 2016). Growing service crops often leads to increased soil cover, which can increase soil carbon content (Chalise et al. 2018; Blanco-Canqui 2021; Jordon et al. 2022) and microbial biomass and activity

(Schmidt et al. 2019; Garland et al. 2021), and reduce weed pressure through spatial or temporal competition (Vrignon-Brenas et al. 2016; Reiss & Drinkwater 2022). Service crops can also promote growth of mycorrhiza and other beneficial fungi (Hallama et al. 2019; Schmidt et al. 2019), pollinators (Boetzl et al. 2023) and arthropods (Whalen et al. 2022). The main reasons reported by European farmers for growing service crops are improving soil structure, biological soil quality, organic matter content, grain yield and yield quality, and reducing pressure from weeds, pests and diseases (Casagrande et al. 2016; Peltonen-Sainio et al. 2022).

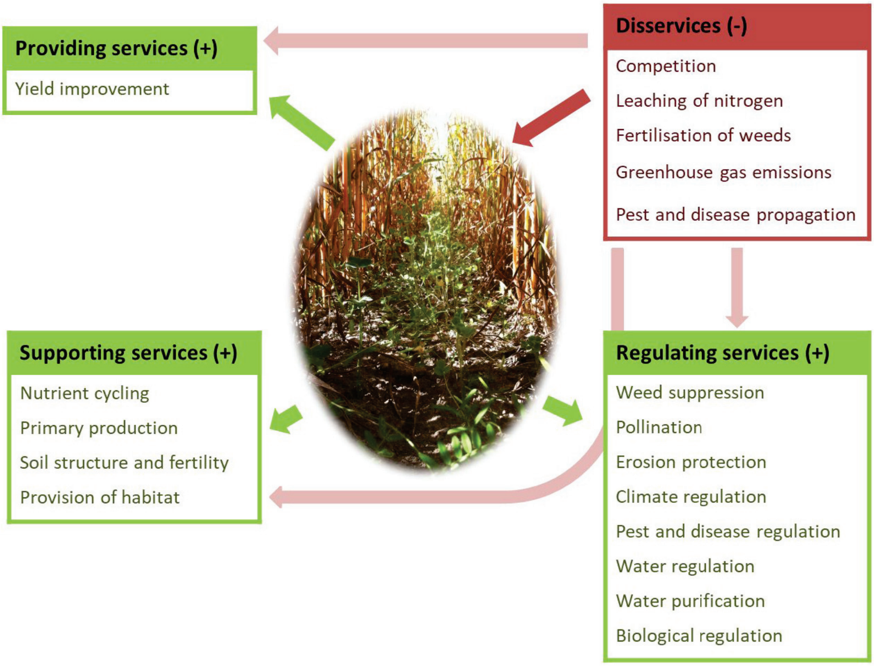


Figure 1. Services (green) and disservices (red) associated with cultivation of leguminous service crops. Source: adapted from World Resource Institute (2003), Zhang *et al.* (2007) and Gardarin *et al.* (2022).

Service crops may also provide disservices. Growing leguminous service crops, either as sole crops or in mixtures where legumes are the dominant species, increases the risk of nitrogen leaching (Vogeler et al. 2019b) and may even promote weed growth (Sjursen et al. 2012). Non-leguminous service crops may be too efficient in taking up and locking in nutrients, so that less nutrients are available to the subsequent crop (Cicek et al. 2015).

Another problem is inefficient termination of the service crop (Casagrande et al. 2016). A common termination method is to use herbicides, which has several limitations, *e.g.* it is prohibited in organic farming and can have a negative effect on non-target organisms (Rose et al. 2016) and increase the risk of weeds developing herbicide resistance (Bourguet et al. 2013). Hence, there are several potential disservices to consider when evaluating the effect of service crops on cropping systems (Figure 1).

1.3.1 Taking local conditions into account – service crops at high latitudes

The ability of service crops to provide services is largely dependent on the amount of biomass produced. Weed suppression, the contribution to soil nitrogen content and yield of the subsequent crop increase with increased service crop biomass (Blackshaw et al. 2010; Reiss & Drinkwater 2022). There is also some evidence that fast growth and high biomass production by service crops is positively correlated with functions relating to nutrient capture, erosion control and soil fertility and structure (Wagg et al. 2021).

In Europe, there is great variation in the extent to which farmers grow service crops. The use of service crops is generally greatest in central western Europe, and lower in southern, northern and eastern Europe (Fendrich et al. 2023). Service crop cultivation in the south is mainly restricted by lack of water, while in the north it is restricted by lack of light and low temperatures during autumn and winter (Peigné et al. 2016; Mills et al. 2020; Garland et al. 2021). In eastern Europe, cold, dry conditions at sowing in autumn and time conflicts with other farm work such as sowing autumn crops are the main barriers to the use of service crops (Mills et al. 2020). A survey on the use of service crops by organic farmers throughout Europe found that the most common practice (54% of respondents) was to grow service crops in autumn (August-September) between two crops (Figure 2a, 2b), while different types of intercropping with service crops over winter were only used by 14-26% of respondents (Peigné et al. 2016). Different methods of intercropping have been developed to extend the growing season of service crops. These include sowing the service crop in conjunction with sowing of a spring-sown main crop (De Notaris et al., 2019) (Figure 2c) or into a standing autumn sown crop (Bergkvist et al. 2011; Amossé et al. 2013; Vrignon-Brenas et al. 2016) (Figure 2d). Another strategy is to sow the service crop at the same time as a winter crop and terminate it in early spring for nutrient release to the main crop (Guiducci et al., 2018) (Figure 2e). Systems with permanent service crops have also been investigated (Bergkvist, 2003; Guiducci et al., 2018) (Figure 2f).

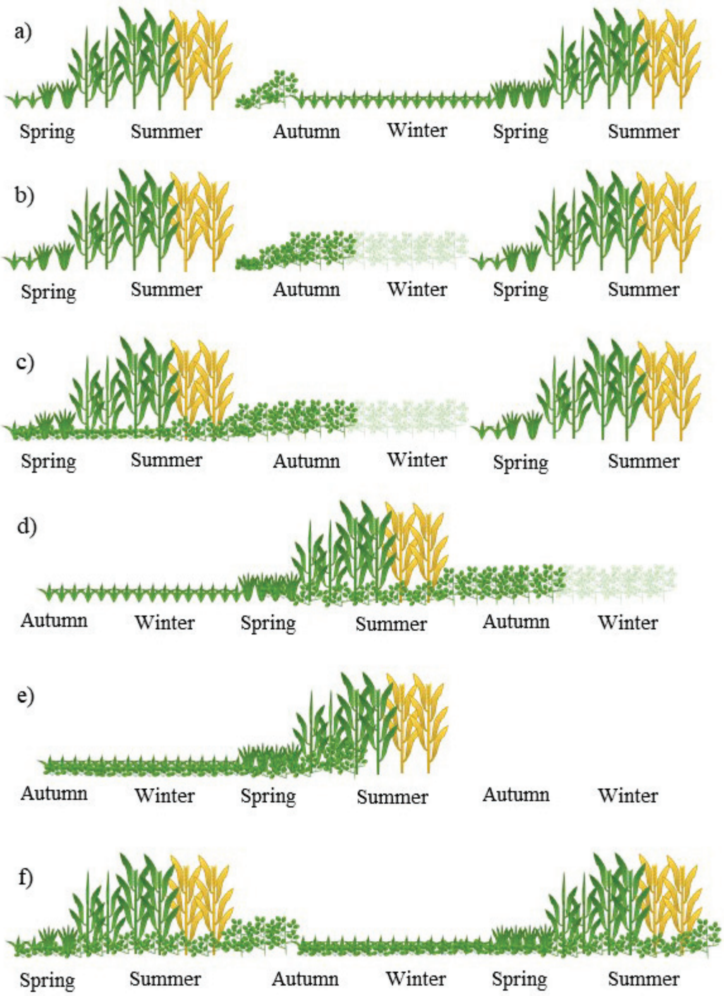


Figure 2. Different methods to include service crops in crop rotations: (a) service crop establishment before sowing of a winter crop, (b) service crop establishment before sowing of a spring crop with service crop termination in late autumn (dark green) or spring (dark + light green), (c) service crop establishment by intercropping in a spring crop, (d) service crop establishment by intercropping in a winter crop in spring, (e) service crop establishment by intercropping in autumn with termination in spring, (f) permanent intercropping with service crops.

The main drawback of intercropping service crops and main crops is the risk of competition reducing yield or grain nitrogen content of the main crop (Carof et al. 2007; Blackshaw et al. 2010; Amossé et al. 2013). In intercropping systems, system performance depends on the degree of cooperation, complementarity, compensation or competition between species (Justes et al. 2021). Cooperation means that the species benefit from each other, such as oilseed rape providing support for peas to climb on (Fletcher et al. 2016). Complementarity occurs when the different species utilise resources in different form, time or space, such as mainly using different forms of nitrogen in cereal-legume intercropping systems (Hu et al. 2016). Compensation occurs when the failure of one species due to environmental conditions is compensated for by growth of another (Creissen et al. 2016), and is mainly favourable when the species provide the same function. Competition occurs when one of the species suppresses the other (Amanullah et al. 2020). Finding species and management practices that provide more cooperation and complementarity and less competition is key to successful intercropping.

1.4 Weeds as service providers

Sown crops are not the only plants that contribute to diversity and service provision from agroecosystems, as indicated in previous sections. Weeds can also supply several ecosystem services, such as maintenance of soil fertility and structure, provision of food resources for pollinators, other insects and birds, and supporting arbuscular mycorrhiza fungi (Storkey 2006; Kubota et al. 2015). There is some evidence that the negative effects of weeds on crop yields are weaker with more diverse weed communities than with less diverse communities (Storkey & Neve 2018; Adeux et al. 2019). However, species traits and weed community trait composition ultimately determine the risk of negative effects of the weed community on the crop (MacLaren et al. 2020). The competitive effect of weed species varies, mainly depending on growth rate and ability to capture resources (DeMalach et al. 2016). Competitive species generally have a negative effect on crop yield, while less competitive species do not notably affect crop yield (Storkey & Cussans 2007). Weeds can also have a positive effect on other organisms, but this effect varies between weed species depending on *e.g.* flowering time and attractiveness as food source (Rollin et al. 2016; Smith et al. 2020a; Morrison et al. 2021).

The ability of weeds to contribute to agroecosystem functioning is a relatively unexplored field of research. Through improved understanding of management approaches that favour a less competitive weed community and of species that provide services to the cropping system, novel systems could be developed, reducing the need for pesticide use and increasing ecosystem services provision from agricultural fields (MacLaren et al. 2020).

1.5 Evaluation of ecosystem service delivery from cropping systems

When developing novel cropping systems, the impact of these cropping systems on multiple ecosystem services must be broadly assessed, to identify both the benefits and the trade-offs that might occur. There is currently no single methodology for assessing ecosystem services from agriculture (Garland et al. 2020; Liu et al. 2022). Instead, a wide range of methods are used, from surveys and expert knowledge to in-field data collection or computer modelling (Liu et al. 2022). Moreover, the types of data collected from in-field studies vary greatly in terms of the categories of ecosystem services assessed and the types of indicators used (functions, pools or properties) (Garland *et al.*, 2020). According to Garland *et al.* (2020), functions (intermediate to fast processes) are the most important variables to assess, while pools and properties should be avoided as they may say more about the state of the site than the function of the cropping system. However, functions can be both costly and time-consuming to assess, as they should preferably be measured over a long period in order to capture daily variations in process rates and inter-annual variations.

Models can be a useful tool for deeper analyses of processes that are difficult to monitor in field studies (Basche et al. 2016; Büchi et al. 2018) and for extending the findings to other environments in terms of management and weather (Ripoche et al. 2011; Chimonyo et al. 2020). Although crop models have some limitations regarding complex systems and processes of ecosystem service delivery (Hernández-Ochoa et al. 2022), assessing a system based on both experiments and models can deepen our understanding of the specific system and help address future challenges to crop production in general (Rötter et al. 2018).

2. Aim and research questions

The aim of my doctorial project was to understand the effects of management and species choice on delivery of multiple services and disservices from a novel intercropping system with cereals and leguminous service crops. The system selected for study was designed for conditions commonly prevailing at high latitudes, with a short period between harvest of one crop and sowing of the next crop in autumn. Leguminous service crops were under-sown in spring oats and left to grow between the rows of the subsequent crop (winter wheat) and killed either by frost or by row-hoeing in early spring. The following research questions (RQ) were addressed in Papers I-III:

- RQ1: How are service crop growth dynamics affected by species choice and sowing time? (Paper I)
- RQ2: Do service crops contribute to nitrogen supply by N_2 fixation? (Paper I)
- RQ3: Are oat yields negatively affected by intercropping with service crops? (Paper I)
- RQ4: Do service crops improve yield (quantity and quality) of the subsequent winter wheat crop? (Paper I)
- RQ5: Does intercropping of service crops in cereals reduce weed biomass compared with sole cropping of cereals? (Paper I)
- RQ6: Does competition from main crop and service crop and within-season soil disturbance alter weed community trait composition towards less competitive weed species? (Paper II)
- RQ7: Can a mechanistic crop model be adapted to simulate the novel cropping system? (Paper III)
- RQ8: Does inclusion of service crops in the crop rotation improve the delivery of multiple services when the system is simulated under different weather conditions? (Paper III)

The starting hypothesis was that a service crop increases service provision, *i.e.* improves winter wheat yield by contributing nitrogen from N_2 fixation, reduces nitrogen losses by staying alive through autumn and suppresses weeds, while causing few disservices such as competing with the crop for light, nutrients and water, increasing nitrogen leaching and promoting weeds. These effects were expected to vary depending on service crop species, sowing time and weather conditions.

Different strategies for service crop establishment aimed at prolonging the growth period of the service crops, and hence biomass production, were compared. The cropping system has been assessed with three methodological approaches and slightly different focus, resulting in three papers (Figure 3). First, the system was evaluated from an agronomic perspective, *i.e.* service crop biomass production and effects on crop yield, soil nitrogen and weed suppression (Paper I). Next, the trait composition of the weed community was assessed, to evaluate whether variations in crop competition and within-season disturbance selected for weed species with different functional traits (Paper II). Finally, the crops and the system were calibrated in the crop model APSIM NG, which was used to assess ecosystem service provision in terms of primary production, carbon input and dynamics of nitrogen and water, using multiple sets of weather data (Paper III).

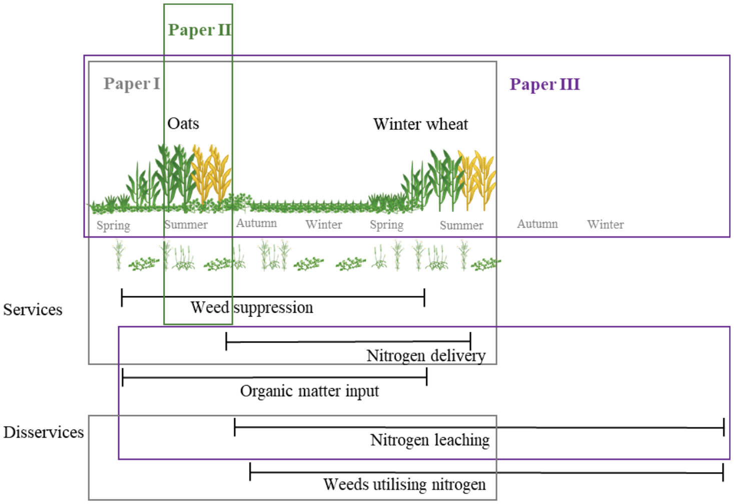


Figure 3. Conceptual cropping system design showing system boundaries (frames) used in Papers I-III. The main services and disservices assessed for service crops are indicated by black lines and their position within frames indicates the main focus areas of each paper.

3. Materials and methods

3.1 Motives of the studied intercropping system

The overall aim of the experimental work was to develop a cropping system that could improve internal recycling of nutrients, carbon sequestration and control weeds, pests and diseases, while still providing the necessary conditions for high main crop yields to be maintained. The system was designed to fit into a cereal-dominated crop rotation, a common cropping system in southern Sweden. Relay intercropping of service crop and cereals was selected to allow the service crop to establish during summer, increasing the likelihood of good soil cover in autumn and winter when winter wheat seedlings would still be too small to protect the soil fully from erosion and weed infestation. Use of a service crop meant that the soil could not be ploughed, which is otherwise common practice between two cereal crops in the study region. Instead, mechanical weeding during the growing season, at sowing of winter wheat and to terminate the service crop in spring of the second year was the only soil tillage operation performed. Wide row spacing and modern row tracing technology made it possible to carry out mechanical weeding in the standing crop and to drill different crops in parallel rows. Oats were sown in 7-8 cm bands rather than in narrow rows, to reduce the impact on yield of wide row spacing and to reduce mechanical damage to the oat plants during row-hoeing. Winter wheat was sown in narrow rows, because winter wheat plants had more time to cover the area between rows than oat plants and because the goosefoot tine used for band sowing does not work as well as a narrow coulter when the soil is moist, which is often the case in autumn in the study region according to local farmers and advisors.

3.2 Overview of experiments

In the experiments my doctoral project is based on, I have been studying a double relay intercropping system, where a leguminous service crop was grown together first with oats (*Avena sativa* L.) and then with winter wheat (*Triticum aestivum* L.) (Figure 3). Legumes were chosen as the service crop as they generally have a better effect on subsequent crops than other common service crop species (Bergkvist et al. 2011). They also compete less with the main crop, as they can fix N_2 from the atmosphere, reducing competition for soil mineral nitrogen (Reiss & Drinkwater 2022). However, competition for light and water probably still occurred, although the service crops ideally replace some weeds that could be highly competitive as well (Westbrook et al. 2022).

Service crops and oats were sown in spring, and winter wheat was sown into the service crop after oat harvest. The service crops were terminated in early spring the second year. However, wet weather conditions at experiment (Exp) 3 in autumn 2017 prevented sowing of winter wheat, so data in winter wheat from this experiment was therefore excluded from the analyses. In-season row-hoeing was carried out in both oats and winter wheat, to control weeds. The soil was not tilled between oats and winter wheat. Exp1 and Exp2 were managed with commercial wide machines and plot size was 8 m x 50 m and 9 m x 50 m, respectively. Exp3-6 were managed with experimental equipment and plot size were therefore smaller, 3.1 m x 36 m. For a more detailed description of crop management, see Paper I (Exp1-4) and Paper III (Exp5-6).

Table 1. Soil properties at the six experimental sites. Textural classes are represented as % of mineral fraction

Experiment	**Clay [%]**	**Silt [%]**	**Sand [%]**	**Organic matter [%]**	**pH H_2O**
1	20	33	47	2	7.1
2	28	42	30	2.7	6.9
3	32	57	11	2.6	6.8
4	41	49	10	3.6	7.1
5	40	46	14	4.3	6.4
6	70	25	5.0	5.4	6.8

3.3 Experimental sites

The experimental sites were located in two regions of southern Sweden characterised by fertile soils and a relatively high proportion of arable land, namely Skåne (Exp1 and Exp2) and Östergötland (Exp3-6) (Figure 4). The soils at the Skåne sites generally had a lower clay and organic matter content than the soils at the Östergötland sites (Table 1). The more southern region of Skåne is slightly warmer (8.8°C as 30-year average) and more humid (712 mm y^{-1} as 30-year average) than Östergötland (7.2°C and 574 mm y^{-1}, respectively).

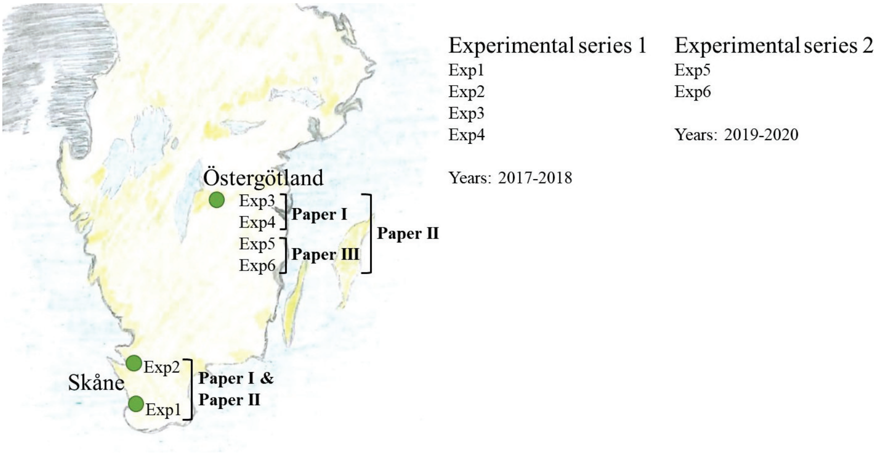


Figure 4. Locations of the six experimental sites, the experimental series they belong to and the papers in which field data were used.

3.4 Experiment design

Data were collected from six field experiments during 2017-2020. The experiments were part of two different projects (referred to as Experimental series 1 and 2), having in common the system strategies explained in section 3.4.1. Experimental series 1 included four of the experiments (Exp1-4), running between 2017 and 2019, and examined the effect of different service crop mixtures and their sowing time. These experiments were managed organically. Experimental series 2 included two of the experiments (Exp5 and Exp6), running between 2019 and 2020, and only used one two-species service crop mixture sown at two different times. These experiments were not managed organically, but no pesticides were used in oats. Data from those experiments were used to assess effects on weed community trait composition in oats (Paper II) and for modelling the system (Paper III), since an extreme drought in 2018 severely affected all plants, and hence cropping outcomes, in Experimental series 1.

3.4.1 Experimental series 1

The experiments in Experimental series 1 were designed in randomised complete blocks with two factors, system strategy and service crop species mixture. In the system strategy factor, three cropping system strategies that differed in the time of sowing and placement of the service crops, as well as in row-hoeing intensity, were compared (Figure 5). In the first system strategy (Early Intra) the service crop and oats were sown simultaneously in the same rows (Figure 5a). In the second strategy (Late Inter), the service crop was sown approximately one month after oats, in the same operation as the first row-hoeing event, in inter-row centres (Figure 5b). In the third strategy (Late Adjacent), the service crop was sown at the same time as in Late Inter, but adjacent to the oat row (Figure 5c). The Early Intra and Late Adjacent strategies allowed for an additional row-hoeing approximately one month after the first, without damaging the service crop. In Late Adjacent, the second row-hoeing was conducted with a slightly narrower hoe than the first, or than in both row-hoeings in Early Intra. With Early Intra and Late Adjacent, winter wheat was sown between the rows of oat stubble, while with Late Inter, winter wheat was sown in the oat stubble. Due to space limitations in the experiments in Skåne (Exp1 and Exp2), the system strategy Late Adjacent was excluded in these experiments.

In the factor service crop species mixture, three mixtures were compared: (i) squarrose clover (*Trifolium squarrosum* L.) and Persian clover (*T. resupinatum*), referred to as 'frost-sensitive annuals'; (ii) red clover (*T. pratense*), white clover (*T. repens*) and the short-lived perennial black medic (*Medicago lupulina* L.), referred to as 'perennials'; and (iii) crimson clover (*T. incarnatum*) and hairy vetch (*Vicia villosa* Roth.), referred to as 'frost-tolerant annuals'. Each system strategy had a control without a service crop. Due to space limitations in the experiments in Skåne, the frost-tolerant annuals mixture was excluded in these experiments.

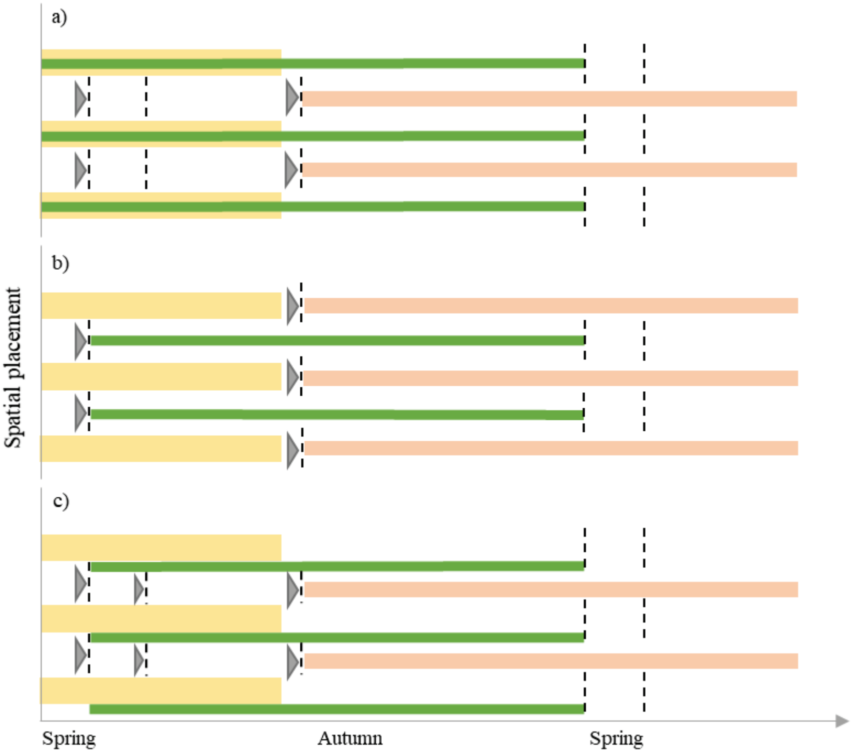


Figure 5. Schematic diagram of the different system strategies: (a) early sowing of service crop (green) in the oat row (yellow) with winter wheat (beige) sown between rows of oat stubble (Early Intra), (b) late sowing of service crop between oat rows with winter wheat sown in oat stubble (Late Inter) and (c) late sowing of service crop adjacent to the oat row with winter wheat sown between rows of oat stubble (Late Adjacent). Dashed lines indicate row-hoeing operations at sowing, for weed control and at termination of service crop.

3.4.2 Experimental series 2

The experiments in Experimental series 2 (Exp5 and Exp6) were designed as randomised complete blocks with two factors, system design and nitrogen dose. The system design included both spatial and temporal arrangement of the crops as in Experimental series 1 (Early Intra and Late Inter strategies; see Figure 5a and 5b) and presence or absence of a service crop. The service crop was always a mixture of *T. squarrosum* and *T. pratense*. The nitrogen (N) dose was either full dose or half dose, where full dose was equivalent to recommended fertiliser doses in the region. Full and half dose was thus 120 and 60 kg N ha^{-1} in oats, and 160 and 80 kg N ha^{-1} in winter wheat. In Paper II, data from both nitrogen doses were included, while in Paper III only data from the full nitrogen dose were included.

3.4.3 Field measurements

Plant data

Numbers and biomass of main crops, service crops and weeds were measured. For the service crops, soil cover was also estimated. For Paper I and Paper II, these measurements were made in four fixed areas in both short ends of the experimental plots, with each area encompassing two crop rows and two inter-row spaces (Figure 6). Within these areas, weeds were counted, biomass samples of all plants were collected before harvest and service crop soil cover was estimated in late autumn. In the oat crop, initial weed numbers were recorded approximately one month after sowing, for use as a covariate in Paper I. For weed counts and weed biomass, each area was further divided into three different subsections: in the crop row (*ir*), close to the crop row (*cr*) and inter-row centre (*ic*) (Figure 6). Biomass samples were cut at ground level, dried at 60 °C for 24 hours and weighed. In Paper I, 10 plants of each service crop species were collected in late autumn for estimation of N_2 fixation using the ^{15}N natural abundance method (Shearer & Kohl 1986). For the model calibration in Paper III, data on 10 randomly selected plants of both main crop and service crop collected from Exp5 and Exp6 at three time points (May, June, July) were used to calculate average plant biomass. Numbers of plants, tillers and heads in the main crop were counted along two 1-m transects covering two crop rows (Figure 6), and used in the analysis in Paper I and to calculate mean crop stand number for the model in Paper III. Yield was determined in a 26-54 m^2 subplot, depending on experiment, and grain quality was estimated for a representative subsample at harvest using the near-infrared transmittance (NIT) method (InfratecTM 1241 Grain Analyzer, Foss, Denmark). Thousand kernel weight was determined for a subsample of harvested grain and then used to calculate number of kernels head^{-1}.

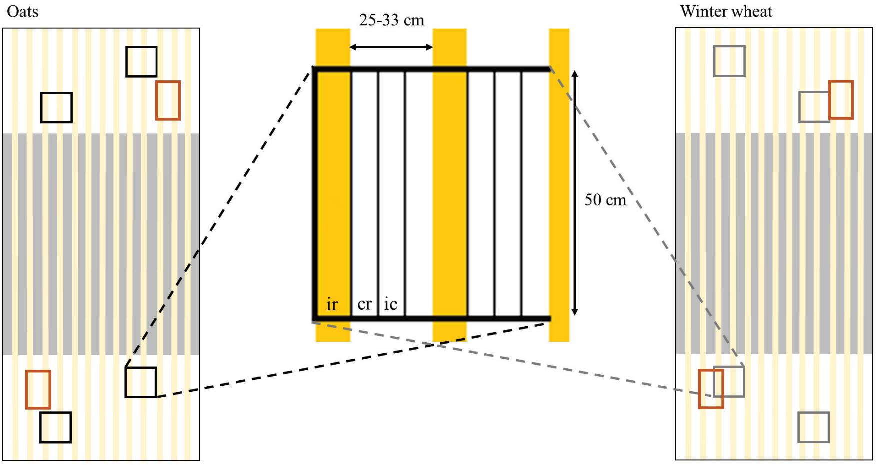


Figure 6. System used for data collection in (left) oats (Paper I and II) and (right) winter wheat (Paper I). Width of sampling area varied depending on the experiment due to different row spacings (25, 32 or 33 cm). Black and grey squares indicate the sampling areas for biomass close to main crop harvest and weed counts, brown rectangles are 1-m transects covering two crop rows for counting plants, tillers and heads of the main crop used in Paper I and III. Sampling of weed biomass and weed counts (centre) included the subsections within the sampling area in which the data were collected: ir = in the crop row, cr = close to the crop row and ic = inter-row centre (middle). Source: modified from Paper I.

Soil data

Initial soil mineral nitrogen and pH were measured in the top 20 cm of the soil at all experiments and used to characterise the sites. Data from Exp5 and Exp6 were also used as input to the model in Paper III. Subsamples of soil were taken over the whole experimental area and pooled to one bulk sample per site. Soil nitrogen was first extracted with 2 M KCl and the solution was analysed for nitrate (NO_3) and ammonium (NH_4) nitrogen using a FOSS TECATOR FIAstar 5000 Analyzer (FOSS GmbH, Hamburg, Germany). Soil pH was measured in aqueous solution.

Soil samples for analysing mineral nitrogen in autumn and spring (Paper I) were collected from the 0-30, 30-60 and 60-90 cm soil layers, except for the autumn sampling at Exp2 which did not include the 60-90 cm layer due to presence of many stones. Subsamples were taken over the whole plot area (10 at Exp1 and Exp2, eight at Exp3 and Exp4) and pooled to one sample per soil layer and plot. Laboratory analyses followed the procedure used in analysis of initial mineral nitrogen.

Samples for analysing soil texture were collected to 80 or 90 cm depth at the different sites. The sieving and sedimentation method (ISO 11277 2020) was used to determine soil texture. The results were used to characterise the sites and, for Exp5 and Exp6, also used as input to the model in Paper III. Using the same samples, initial phosphorus was measured with the ammonium lactate (AL) method (plant-available phosphorus) and by the hydrochloric acid (HCl) method (storage phosphorus) (Egnér et al. 1960). The values obtained were used as input data in the model in Paper III.

3.5 Evaluation of crop performance and service delivery

3.5.1 Service crop performance (RQ1, RQ2, Paper I)

To address research questions RQ1 and RQ2, service crop performance was evaluated based on growth dynamics, *i.e.* summer growth and soil cover in autumn, N_2 fixation and soil mineral nitrogen content in autumn and spring (Paper I). The interacting effects of species mixture, system strategy and experiment (impact of soil and weather) on service crop plant numbers, biomass and soil cover and on soil mineral nitrogen were assessed using linear mixed models. The amount of nitrogen derived from air (%Ndfa) in service crop biomass was also assessed statistically using a linear mixed model, testing the effects of species mixture, experiment and their interaction. Block nested in experiment was used as random factor. Data were tested for normality and homogeneity of variance, and if these criteria were not met, according to plots of residuals versus fitted and normal Q-Q and according to Box-Cox test, the data were transformed with appropriate power transformation.

For the thesis, the growth dynamics of the combination of species mixtures, system strategy and experiment were summarised. The growth dynamics were graded into four levels based on measured biomass and soil cover. Service crop biomass was divided into fast (>0.2 t ha^{-1}) and slow (<0.2 t ha^{-1}) growing, while for soil cover the threshold between large and small was set to 10%.

3.5.2 Effect of service crops on performance of the main crop (RQ3, RQ4, Paper I)

The interacting effect of service crop species mixture, system strategy and experimental site on oat and winter wheat stand development, harvest components and yield variables (RQ3, RQ4) was assessed statistically in Paper I using linear mixed models. Block nested in experiment was used as random factor. Stand development consisted of numbers of plants, tillers and heads, harvest components were heads plant^{-1}, kernels head^{-1} and thousand kernel weight, while yield variables were grain yield, nitrogen yield and nitrogen content in the kernels (the latter two only for winter wheat). All data met the assumptions of normality and homogeneity and were not transformed.

3.5.3 Weed control (RQ5, RQ6, Papers I and II)

Weed control by the service crop was first estimated as the amount of biomass in the different treatments (RQ5) in the experiments in Paper I. Secondly, the type of plant traits selected for by varying degrees of crop competition and disturbance (RQ6) was analysed in Paper II. In Paper II, the term "arable plants" was used instead of weeds, since the latter term has a negative implication of unwanted plants. However, for consistency with the other papers and most literature, the term weeds is used in this thesis.

Weed biomass

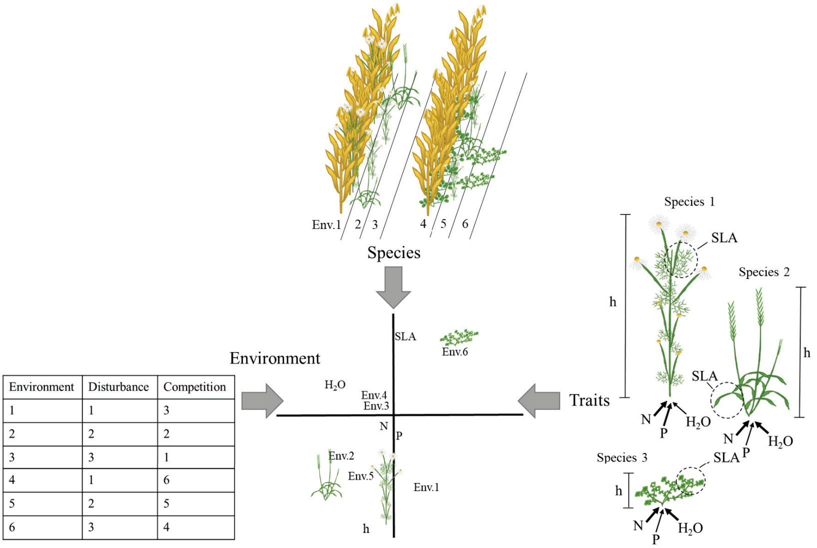
In Paper I, weed biomass at the end of each main crop growing season was analysed both for the full sampling area and for each subsection (Figure 6). The biomass data were analysed statistically with a linear mixed model evaluating the effect of species mixture and system strategy, and their interaction with experimental site, and with block nested in experiment as random factor. As seen for service crop data, the tests for normality and homogeneity indicated that the data required transformation, which was done with appropriate power transformation.

In the thesis, weed biomass collected before oat harvest is shown plotted against service crop biomass collected at the same time, while weed biomass collected before winter wheat harvest is plotted against service crop biomass before oat harvest and service crop soil cover in late autumn. The intention with this was to provide a clearer picture of the direct relationships between service crop and weed biomass.

Weed community trait composition

In Paper II, RLQ analysis was performed to investigate whether differences in management-induced environmental factors affected the weed community in terms of species trait composition (Figure 7). RLQ analysis is a multivariate method that connect environments and species traits through a measure of species occurrence (numbers, biomass or cover) (Dolédec & Chessel, 1994; Dolédec *et al.*, 1996). In the analysis in Paper II, the environmental variables were crop competition (from main crop and service crop) and disturbance intensity (one or two row-hoeing events). Each sampled plot at all sites was assigned a competition level based on the measured biomass of oats (low (<8 t ha^{-1}), intermediate (8-13 t ha^{-1}) or high (>13 t ha^{-1})) and the measured biomass of service crop (low (<0.2 t ha^{-1}) or high (>0.2 t ha^{-1})), as well location in relation to oat rows at which weeds were found (subsection ir, cr or ic) (Figure 6). This resulted in 18 individual competition levels (micro-ecosystems). Each sampled plot was also assigned a disturbance level based on the number of row-hoeing events (one or two) and the subsection, resulting in six individual disturbance levels. The analysis was performed at subsection level, as it was assumed that both competition and disturbance would have different effects in the different subsections, *i.e.* higher crop competition in the crop row (ir) than in inter-row centres (ic) and a greater effect of row-hoeing in the inter-row centre than in the crop row. Data on the species traits were obtained from two databases (Bàrberi et al. 2018; Tyler et al. 2021), and were categorised as related to life strategy, preferred niche/competition and service provision (see Table 2 in Paper II).

Correspondence analysis performed prior to the RLQ analysis revealed that the species composition was site-specific. However, most of the species were found in at least two experiments. To remove site-specific effects, partial RLQ analysis (Wesuls et al. 2012) in which the experimental sites were included as a co-variable was performed. Species that occurred less than three times were excluded from the dataset, since the occurrence of rare species can have a disproportionally large impact that may not make ecological sense (Jongman et al. 1995). As the ordination space created by RLQ analysis has many dimensions, interpreting the results only by the ordination space can be misleading. Therefore, hierarchical cluster analysis was performed to group the species that most commonly occurred together and to investigate whether they shared similar traits.



Environment	Disturbance	Competition
1	1	3
2	2	2
3	3	1
4	1	6
5	2	5
6	3	4

Figure 7. Conceptual diagram of the RLQ analysis. Environmental variables and species traits are linked by species numbers in each environment.

3.5.4 Crop and system modelling (RQ7, RQ8, Paper III)

The Agricultural Productions Systems sIMulator (APSIM) model (Holzworth et al. 2014) was used to simulate the cropping system. APSIM is a dynamic process-based model that simulates the interactions between crop, soil and atmosphere and how these interactions vary over the season (*e.g.* as a function of weather) and with crop development (*e.g.* where processes in the crop change with stage of phenological development). The latest version of APSIM, APSIM Next generation (APSIM NG), was used in all modelling, since it contained a red clover cultivar to use for the service crop. It can also cope better with complex systems (Holzworth et al. 2018), and is taking over from the former version of APSIM (APSIM Classic). APSIM Classic has previously been used to simulate service crops, both as sole crops (Böldt et al. 2021) and intercrops (Bartel et al. 2020).

Three crop modules (oats, winter wheat, red clover) were calibrated to better represent cultivars bred for high latitudes (Sweden) and their growth in the intercropping system. After calibration, the model was used to assess crop productivity and soil processes that are important for ecosystem service and disservice provision from the intercropping system, compared with a control scenario with the oat-winter wheat sequence without a service crop.

Model calibration

Data from the fully fertilised plots (120 kg N ha^{-1} to oats, 160 kg N ha^{-1} to winter wheat) in Exp5 and Exp6 were used in the calibration of oats, winter wheat and red clover. The crops were first calibrated as sole crops and then as intercrops. For red clover, data from other experiments in Sweden and Denmark (Höök 1993; Dhamala et al. 2017, 2018) were used to get an indication of their growth under Northern European conditions, as they were not grown as sole crops in any of my experiments. Several cultivars of winter wheat have already been calibrated for Europe and one of these (cv. Rosario) was used as the basis for calibration of winter wheat. No cultivars of oats and red clover have been calibrated for Europe, and hence default oat and red clover crop parameters were used as the basis for calibration. Calibration was performed in the order phenology, biomass and yield of the two cereals (Seidel et al. 2018), or biomass only of red clover. Total biomass was the main variable considered in the calibration, but the partitioning of the biomass into different plant organs (stem, leaves and head), leaf area index (LAI) and yield were also considered. More details of the calibration can be found in Supplementary Material 2 in Paper III.

Scenario assessment

Two scenarios were compared, one with the intercropped service crop and one without. Outputs related to crop productivity, inputs of organic carbon to the soil and dynamics of nitrogen and water were assessed (Figure 8). The scenarios were simulated with 30 unique combinations of weather-years during the growth periods of the two crops, plus a fallow period ranging from winter wheat harvest until spring when a subsequent spring crop could be sown. The different weather-years were used to generate weather-dependent variation in the productivity of service crop and main crops, as well as their effect on soil processes.

Service crop productivity was evaluated as biomass at oat harvest and in the days before termination in spring. Main crop productivity was evaluated as biomass and grain yield on the day of harvest. Input of organic carbon was evaluated as fresh organic carbon (mainly root biomass) at 0-35 cm soil depth around oat harvest (end of first main crop sequence) and at service crop termination in spring. Nitrogen dynamics were assessed as the sum of nitrogen uptake by all crops, losses of nitrogen via nitrate (NO_3) leaching, gaseous emissions (N_2 and N_2O) at the end of each period, the sum of nitrogen mineralisation in the 0-35 cm layer during different seasons (spring, summer, autumn and winter), and pools of organic and mineral nitrogen in the 0-100 cm layer at the end of each period. Water dynamics were assessed as water uptake by all plants, losses via drainage, evaporation and runoff at the end of each period, and average volumetric soil water content at 0-100 cm in different seasons. The differences between the two scenarios were tested for their significance for all output variables, as was the interaction of scenario and period or season. Linear mixed models were used for the statistical analysis. Most data required power transformation according to the Box-Cox test, and were transformed according to the most suitable power function. These were: main crop biomass, regression between service crop biomass and winter wheat biomass at both sites, regression between service crop biomass and winter wheat yield at Exp6 (Site1 in Paper III), fresh organic carbon, all nitrogen dynamics variables and all water dynamics variables except plant water uptake. Weather-year nested in site was used as random factor in all statistical models.

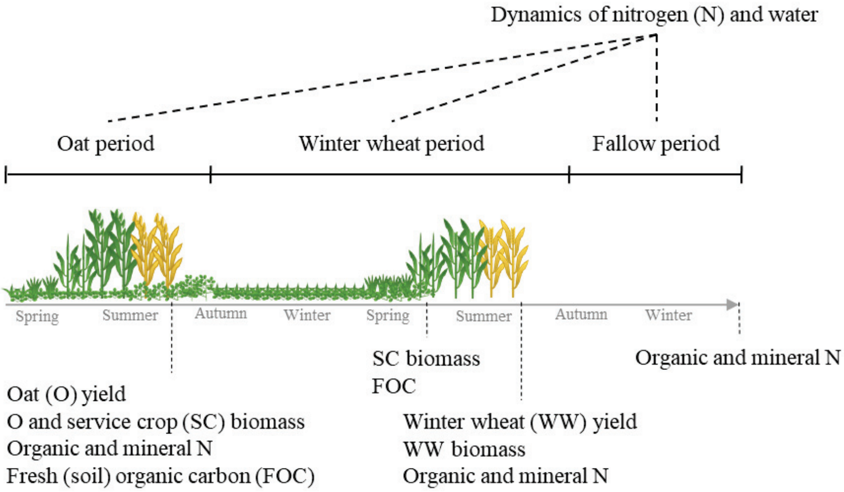


Figure 8. Schematic diagram showing model outputs in Paper III. Dynamics of nitrogen and water were calculated as sums over different periods, while outputs below the timeline were point estimates. Note the different period lengths: oats = 149 days, winter wheat = 365 days, fallow = 216 days. Modified from Figure 1 in Paper III.

4. Results and discussion

4.1 Growth dynamics of service crops (RQ1)

Service crop growth dynamics, *i.e.* summer biomass production and soil cover in autumn, were affected by a combination of species mixture, sowing time and year (Figure 9). In general, annuals were more productive than perennials in the first summer, but with delayed sowing time the early growth of annuals and perennials was more similar. The frost-sensitive annual service crops recovered poorly after oat harvest, especially when sown early, resulting in lower or no soil cover in autumn. The perennials generally produced little biomass in summer, except at early sowing in Exp1, but recovered well after harvest and provided some soil cover in autumn. Frost-tolerant annuals were productive during the first summer if sown early and recovered well after oat harvest. From these findings, four growth dynamics categories were identified (Table 2): (i) fast early growth and large soil cover in autumn (fast/large), (ii) fast early growth and small soil cover in autumn (fast/small), (iii) slow early growth and large soil cover in autumn (slow/large) and (iv) slow early growth and small soil cover in autumn (slow/small). The fast/large growth dynamic was mainly found with early-sown frost-tolerant annuals, the fast/small growth dynamic mainly with early-sown frost-sensitive annuals, the slow/large growth dynamic mainly with perennials, at all sowing times, and the slow/small growth dynamic was mainly found in 2018. Hence, growth dynamics varied between years and establishment times, especially for frost-sensitive annuals, which showed fast/small characteristics at Exp1, slow/large characteristics at Exp3 and slow/small characteristics at Exp2 and Exp4 (2018). Hence, the growth dynamics of the service crop species mixtures varied between years, and frost-tolerant annuals and perennials showed greater consistency of performance than frost-sensitive annuals.

Table 2. Growth dynamics categorised based on summer biomass production (fast or slow) and soil cover in autumn (large or small), for data see Figure 9

Growth dynamics	Characteristics	Treatments (Paper I)
Fast/large	Fast growth in summer (>0.2 t ha^{-1}), large soil cover in autumn (>10%)	Perennials sown early in Exp1 Frost-tolerant annuals sown early in Exp3 and Exp4
Fast/small	Fast growth in summer (>0.2 t ha^{-1}), small soil cover in autumn (<10%)	Frost-sensitive annuals sown early in Exp1 and Exp3 Frost-sensitive annuals sown late in Exp1
Slow/large	Slow growth in summer (<0.2 t ha^{-1}), large soil cover in autumn (>10%)	Perennials sown late in Exp1 Perennials sown early in Exp2 Perennials sown either early or late in Exp3 Frost-sensitive and frost-tolerant annuals sown late in Exp3
Slow/small	Slow growth in summer (<0.2 t ha^{-1}), small soil cover in autumn (<10%)	All treatments in Exp2 and Exp4 except early sown perennials (Exp2) and frost-tolerant annuals (Exp4)

In 2017, there was a great difference in productivity between the two regions. At early sowing, the frost-sensitive annuals produced around twice as much biomass in Skåne (Exp1) as in Östergötland (Exp3), while the perennials were 6.5-fold more productive in Skåne (Figure 9). At late sowing, there was a 6.5-fold and 20-fold difference between the two regions for the frost-sensitive annuals and perennials, respectively, with higher biomass production in Skåne than in Östergötland. The difference in soil cover was small except for late-sown frost-sensitive annuals, which had no soil cover in Skåne and almost 40% in Östergötland. The generally higher biomass production in Skåne could be due to more precipitation in summer months in that area, combined with slightly higher temperatures compared with Östergötland (Figure 1 in Paper I), providing more beneficial growth conditions for the service crops. In 2018, the service crop established poorly due to extremely warm and dry weather (Wilcke *et al.*, 2020; Figure 1 in Paper I). Other studies have also found that service crop biomass is drastically reduced when precipitation after sowing is delayed or very low (Hendrickson et al. 2021; Chim et al. 2022). The early-sown frost-tolerant annuals were an exception to the otherwise poor performance of service crops in 2018, as they grew almost equally well in both years. This could be due to their fast establishment, which likely also led to a deeper root system than with other service crops early in the season. The hairy leaves of both species in the mixture, but especially *V. villosa* which constituted the main fraction of the biomass, could also have played a role, by reducing transpiration (Ripley et al. 1999; Wang et al. 2021). The large variation in service crop performance due to annual weather conditions could pose challenges when planning for ecosystem service provision, which is closely related to the amount of biomass produced (Blackshaw et al. 2010; Wagg et al. 2021; Reiss & Drinkwater 2022).

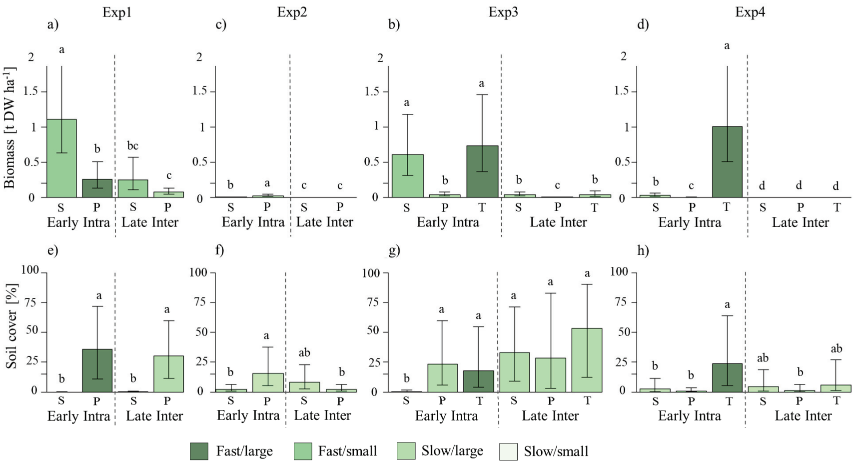


Figure 9. (a-d) Service crop biomass before oat harvest and (e-h) service crop soil cover in late autumn at experimental sites (Exp) 1-4. Colours indicate service crop growth dynamics, for details see Table 2. Data from the Early Intra and Late Inter system strategies, divided within each site with a dashed line. S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. Error bars indicate 95% confidence interval, different letters on bars indicate significant within-site differences ($p<0.05$).

There was a problem with terminating winter-surviving service crops in spring, which led to high competition from the service crops in winter wheat (see section 4.3.2). This was mainly the case for the frost-tolerant annuals and perennials. However, in Exp2, *T. squarrosum*, one of the frost-sensitive annuals, also survived the winter due to mild temperatures in that winter (lowest temperature -7.3°C, temperature >0°C on most days). Hence, on the south east of Skåne and farther south, *T. squarrosum* could be considered winter-surviving.

4.2 Nitrogen contribution by service crops (RQ2)

Most of the service crops studied received the majority (>60%) of their nitrogen from N_2 fixation (Figure 5 in Paper I), as observed previously for these species in other environments (Büchi et al. 2015). The exceptions were the frost-sensitive annuals and *T. pratense* in Exp4 (drought year) and *M. lupulina* in all experiments. *Medicago lupulina* would probably have benefited from seed inoculation, since it is associated with *Ensifer* strains that are often rarer in agricultural soils than the *Rhizobium* strains associated with *Trifolium* species (Roberts et al. 2017). Water availability was likely the main variable affecting N_2 fixation during the experiments, since %Ndfa was relatively high for all species in Exp1 and Exp3 where precipitation was high or normal, while it was lower in the extreme drought year (2018, Exp2 and Exp4). Lack of water has been found by others to reduce N_2 fixation (Peoples et al. 2001; Pandey et al. 2017). The drivers behind this water-induced reduction in N_2 fixation have not been fully identified, but changes in plant gene expression to favour water transport over nutrient reallocation from nodules to other plant parts, seems to be an important driver (Kunert et al. 2016; Sinclair & Nogueira 2018). This leads to accumulation of nitrogenous compounds in the nodules, which inhibits further N_2 fixation (Sinclair & Nogueira 2018). When precipitation finally arrived in late July and early August in 2018, the added nitrogen became available to plants, which could also have reduced N_2 fixation (Guinet et al. 2018; Pampana et al. 2018). In my experiments, *T. pratense* in Exp4 and *M. lupulina* in all experiments all had low biomass and therefore contributed little to competition for or provision of nitrogen. In the drought year, %Ndfa in Exp2 was higher than in Exp4, possibly due to lower fertility in Exp2, *i.e.* low organic matter content and mineral nitrogen, as also observed by Büchi *et al.* (2015) to increase N_2 fixation. Apart from the fertiliser applied before sowing of oats, the potential of the soil to provide nitrogen was lower in Exp2 than in Exp4. Hence, N_2 fixation in the experiments could have been affected by a combination of nitrogen availability and water availability.

A slight increase in soil mineral nitrogen was observed in autumn in treatments with early sown frost-sensitive annuals (Figure 9 in Paper I) indicating an increased risk of nitrogen leaching during the autumn-winter-spring period. In spring, soil mineral nitrogen was higher with early- than late-sown service crops, while perennial service crops reduced mineral nitrogen in the deepest soil layer (60-90 cm) compared with the control.

Higher amounts of soil mineral nitrogen in winter and early spring when frost-sensitive annuals are grown have been seen in other field experiments (Morris et al. 2021; Storr et al. 2021) and could lead to increased nitrogen leaching or promote weed growth, as it seemed to do in Paper I (see section 4.4.1). Hence, having a service crop that recovers after oat harvest is important in this system to prevent nitrogen leaching, as drainage over winter is often high.

4.3 Effects of service crops on main crop performance

4.3.1 Oats (RQ3)

Only the frost-sensitive annuals in Exp3 (fast/small growth dynamics) provided large biomass (0.6 t ha^{-1}) without reducing oat yields compared with the control (Figure 10). In all other cases, service crops with fast biomass production in oats (>0.2 t ha^{-1}, fast/small and fast/large growth dynamics) reduced oat yield. This could be due to wider row spacing in Exp3 (33 cm) compared with Exp1 (25 cm), where oats were more negatively affected by service crop biomass production in summer, and to the perennials and frost-tolerant annuals (especially *V. villosa*) outgrowing oats to a greater extent than the frost-sensitive annuals. The yield components of oats (heads plant^{-1} and kernels head^{-1}) were not affected by intercropping with a service crop. However, thousand kernel weight tended to be lower (p=0.06) when the service crop was sown early compared with late (Table S2.5 in Paper I), indicating that the competition by early-sown service crops mainly occurred during grain filling.

Other studies on intercropping of leguminous service crops and main crops have also observed reductions in main crop yield when the service crop is sown at the same time as the main crop (Guiducci et al. 2018; Taab et al. 2023). However, not all studies have seen a reduction (Ohlander et al. 1996; Blackshaw et al. 2010). With delayed under-sowing of the service crop compared with the main crop, the main crop is generally not significantly affected (Bergkvist et al. 2011; Amossé et al. 2013; De Notaris et al. 2019). In the study by Taab *et al.* (2023), main crop yields were only reduced compared with a weeded control, while compared with an unweeded control, yields were higher when the main crop was intercropped with a forage legume service crop because the service crop replaced the most aggressive weed species. Hence, the relationship between service crop biomass production and main crop performance is not clearly negative, although the risk of competition, both above and below ground, increases with increasing biomass. The competitive impact of the service crop also depends on the overall competitive pressure at the site, *i.e.* weed pressure (see section 4.4.2 and Paper II).

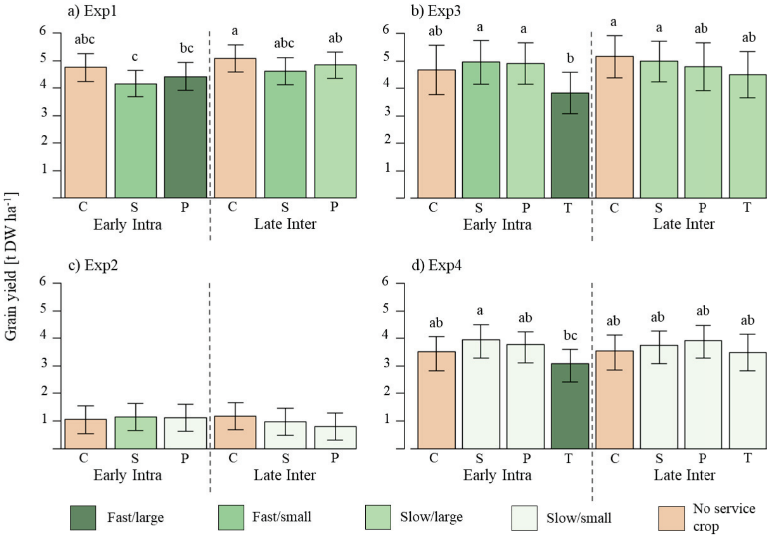


Figure 10. Oat yield at experimental sites (Exp) 1-4. Colours indicate service crop growth dynamics for each treatment, for details see Table 2. Data from the Early Intra and Late Inter system strategies, divided within each site with a dashed line. S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. Error bars indicate the 95% confidence interval, different letters on bars indicate significant within-site differences (p<0.05).

4.3.2 Winter wheat (RQ4)

The most positive effects on winter wheat grain yield were seen for the most productive service crop mixtures, *i.e.* all service crops tested at Exp1 and early sown frost-tolerant annuals at Exp4 (Figure 11). The growth dynamics of the service crop were less important. All growth dynamics except slow/small, with very little biomass production over the whole growth period, showed potential for yield improvement. Early sown frost-tolerant annuals at Exp4 (fast/large) had the greatest effect on nitrogen concentration in harvested wheat grain and nitrogen yields. Improved yield of the subsequent crop is an important benefit of service crops, and many studies report improved grain yield and/or nitrogen yield following a service crop (Bergkvist et al. 2011; Amossé et al. 2014; De Notaris et al. 2019; Peterson et al. 2021). However, service crops do not always increase yield of the subsequent crop, possibly due to too high fertilisation rates of that crop (Vogeler et al. 2019b), nitrogen leaching or gaseous emissions before the subsequent crop has started to take up nitrogen (Thorup-Kristensen 1994; Basche et al. 2014) or delayed decomposition of service crop residues (Thorup-Kristensen & Dresbøll 2010; Chim et al. 2022). Chim *et al.* (2022) found no effect on yield of the main crop grown directly after the service crop, but saw an effect for the second subsequent crop. Hence, the positive effect of the service crop on the subsequent crop largely depends on when the nutrients (mainly nitrogen) become available, which in turn depends on both the chemical composition of the service crop (Wivstad 1999; Ghimire et al. 2017) and weather conditions (Thorup-Kristensen & Dresbøll 2010), affecting decomposition before and during the time in which the crop will take up nutrients. Soil structure and texture also affect decomposition and the associated risk of losses through leaching or denitrification. Soils with large pore space promote decomposition compared with soils with small pore space (Negassa et al. 2015), and pH determines microbial community composition (Wang et al. 2019), plant nutrient availability (Hartemink & Barrow 2023) and nutrient uptake (Barrow & Hartemink 2023). The risk of nutrient leaching is highest on soils with low water-holding capacity, typically sandy soils (Askegaard et al. 2011; Vogeler et al. 2019b), while the risk of denitrification and gaseous nitrogen losses is highest on soils that are compacted, have a high clay content and are rich in organic matter (Skiba & Ball 2002; Rochette et al. 2008, 2018). Hence, the effect of service crops on subsequent main crops is a complex interaction between service crop biomass, biomass quality, weather and soil properties.

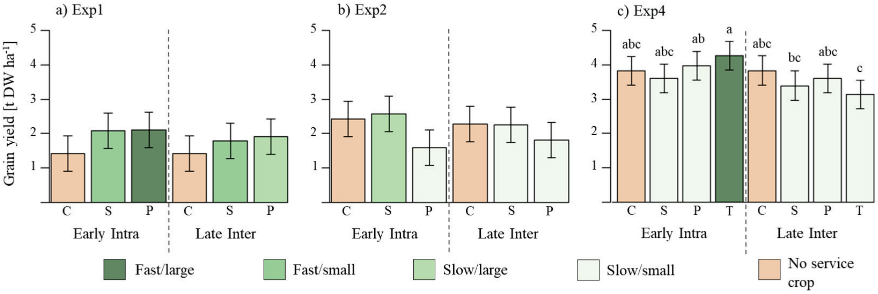


Figure 11. Winter wheat yields at experimental sites (Exp) 1, 2 and 4 (it was not possible to sow winter wheat at site 3, due to wet soil conditions in autumn). Colours indicate service crop growth dynamics for each treatment; for details see Table 2. Data from the Early Intra and Late Inter system strategies, divided within each site with a dashed line. S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. Error bars indicate the 95% confidence interval, different letters on bars indicate significant within-site differences (*p*<0.05).

4.4 Effect of service crops on weeds

4.4.1 Weed biomass (RQ5)

In oats, weed biomass was significantly reduced (*p*=0.03) with frost-tolerant annual service crops (fast-growing) compared with perennial service crops (slow-growing) (Figure 8 in Paper I). The regression line between service crop biomass and weed biomass indicated only a slight negative relationship (Figure 12a, 12d). However, the highest weed biomass was found in plots with no service crop (grey symbols in Figure 12) or a slow-growing service crop (left side of dashed line in Figure 12), while plots with a fast-growing service crop never had the highest amounts of weeds. Hence, the fast-growing service crops seemed to be able to prevent the most vigorous growth of weeds, although some of the differences likely were due to natural variation in weed abundance within the field. In winter wheat, weed biomass was reduced in treatments with perennial service crops in Exp1 (*p*=0.005 and *p*=0.04 compared with frost-sensitive annuals and no service crop, respectively) and frost-tolerant annual service crops in Exp4 (*p*=0.03 compared with frost-sensitive annuals) (Figure 8 in Paper I), with all having large soil cover in autumn. There was a neutral to slightly negative correlation between service crop biomass at oat harvest and weed biomass in winter wheat (Figure 12b, 12e). The relationship between weed biomass in winter wheat and service crop soil cover in autumn was negative at Exp2 and Exp4 (establishment year 2018; Figure 12c) and slightly negative at Exp5 and Exp6 (establishment year 2019, not included in Paper I; Figure 12f). In winter wheat the generally low weed biomass in plots where service crops were established in 2017 (circles in Figure 12) was due to the drought.

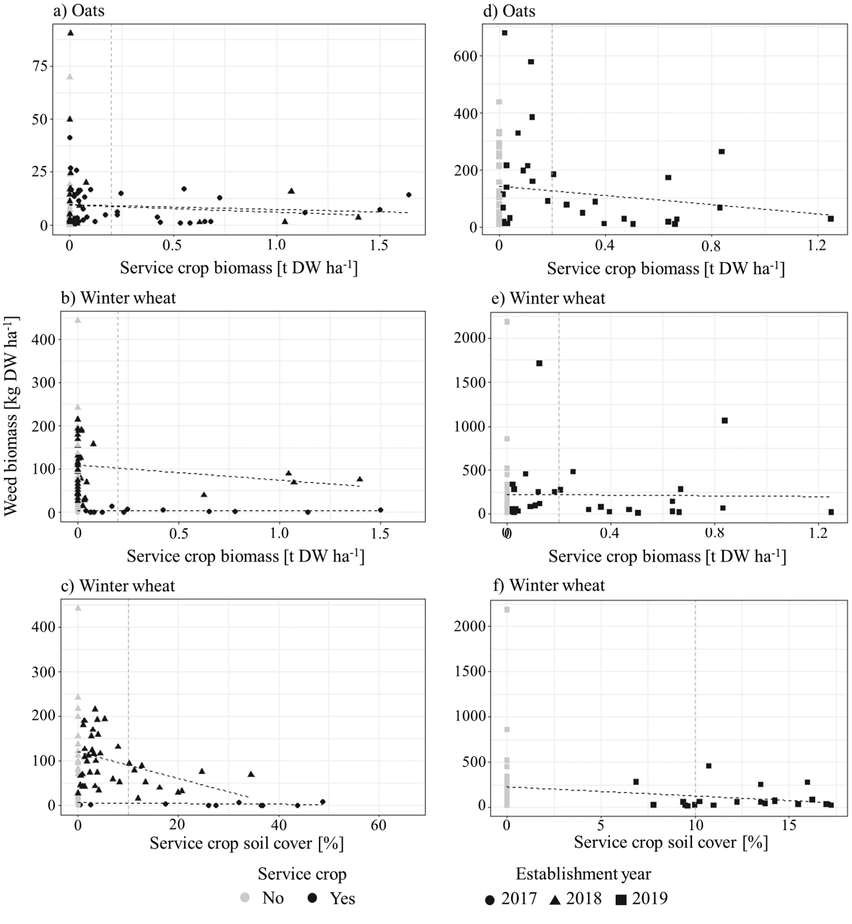


Figure 12. Weed biomass dry weight (DW) in (a, d) oats and (b, c, e, f) winter wheat at different levels of (a, b, d, e) service crop biomass at oat harvest and (c, f) service crop soil cover in autumn in (a-c) Experimental series 1 (Exp1-4), and (d-f) Experimental series 2 (Exp5 and Exp6, not included in Paper I). Vertical dashed lines indicate the breakpoint between fast and slow growth in the first summer (a, b, d, e), or large and small soil cover in autumn (c, f). Symbol colours indicate whether the treatment included a service crop or not, and shape and regression lines indicate service crop establishment year.

The lack of a clear effect of service crops on weed suppression in observations from my experiments, compared to what have been reported in previous studies (*e.g.* Reiss & Drinkwater 2022; Taab et al. 2023), could be due to that service crop biomass at all sites was generally low and that the service crops only covered part of the soil surface.

Differences in weed biomass within subsections (in crop row, close to crop row and inter-row centre) due to competition were seen for both oats and winter wheat. In oats, there was a tendency for less weed biomass in inter-row centres with the Early Intra system strategy than with the Late Inter strategy over all experiments (p=0.06). This was expected, since the Early Intra system was hoed twice and the Late Inter system only once. Row-hoeing intensity close to the crop row did not have a significant impact. In winter wheat in Exp1, weed biomass production in winter wheat rows was lower in the Early Intra system compared with Late Inter (p=0.014). This could either be a legacy effect of higher competition in the previous year or because the area of the crop row had been hoed more frequently in oats, since management was the same in all treatments in winter wheat. Moreover, this difference was larger with perennial service crops than with no service crop or with frost-sensitive annuals (p=0.001). The perennial service crops covered the soil relatively well, and survived winter (data not shown).

4.4.2 Weed community trait composition (RQ6)

The influence of a fast-growing service crop (>0.2 t dry weight (DW) per ha at harvest of oats) was most pronounced in the crop row (position ir), where it reduced the occurrence of tall-growing species with high specific leaf area (SLA), low light requirements, relatively high moisture requirements and mainly a Grime's competitive-stress tolerant (CS) life strategy (Figure 2 in Paper II, Figure 13). Instead, the weed community was shifted towards a more numerous group of low-growing species favoured by frequent soil disturbance and with Grime's ruderal (R) life strategy. Another driver of weed community trait composition was competition from oats, with intermediate and high oat biomass reducing the occurrence of perennial species with Grime's competitive (C) life strategy compared with when oat biomass was low. Instead, the weed species community was generally associated with species with a ruderal life strategy. Hence, with increasing competition, from both oats and service crop, the weed species community shifted from being dominated by relatively highly competitive species (C and

CS) to including less competitive species, as well as a larger number of species. Other studies have found that crop competition, whether from a cereal (Gaba et al. 2018) or an intercropped leguminous service crop (Taab et al. 2023), mainly suppresses the dominant weed species and leaves the more rare species less affected. Intercropping with service crops (legumes and/or grasses) also effectively suppresses the same perennial weeds when found in my experiments (Brandsæter et al. 2012; Ringselle et al. 2017). Among the competitive and competitive-stress tolerant weed species some agronomic highly problematic species belonged, such as *Circium arvense*, *Elymus repens* and *Galium aparine*. However, some ruderal species could also be considered highly problematic from an agronomic perspective, *e.g.* *Chenopodium album*, *Fallopia convolvulus* and *Tripleurospermum inodorum*. These plants grow tall and/or wide if they have sufficient resources and produce many seeds. Hence, it seems that inclusion of a service crop and a competitive main crop eliminates the preferred niche of some problematic weeds, but not all. Other measures might be needed to manage the fast-growing ruderals, *e.g.* repeated row-hoeing, which reduced ruderals in inter-row centres in Paper II (see Figure 2b, 2d in Paper II).

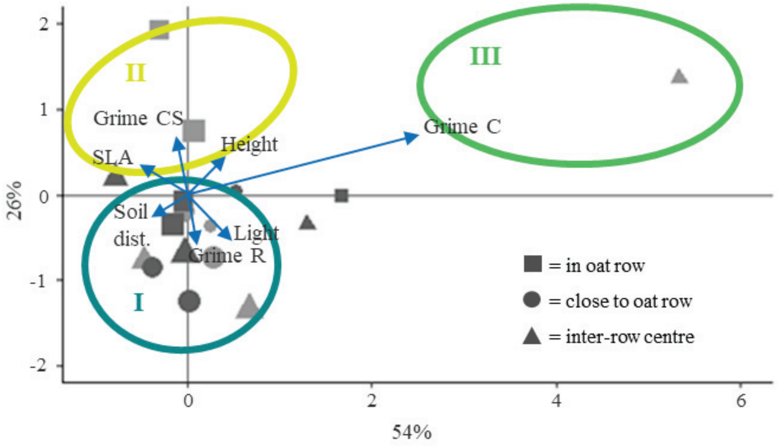


Figure 13. Ordination plot of the environmental variable competition (symbols), trait variables (arrows and labels) and species clusters (coloured ellipses). For the environmental variables, symbol shape indicates plot subsection sampled (see legend), size indicates oat biomass (low <8 t ha^{-1}, intermediate 8-13 t ha^{-1}, high >13 t ha^{-1}) and colour indicates service crop biomass (light grey = low <0.2 t ha^{-1}, dark grey = high >0.2 t ha^{-1}). Cluster I contain 15 species, cluster II four species and cluster III three species. Modified from Figure 2 in Paper II.

The species found in the experiments are all common in agricultural fields in northern Europe (Salonen et al. 2001; Goerke et al. 2008), which could explain the quite similar scores for most species traits (Figure 2d-2f in Paper II). However, there were certain similarities between species often found in the same micro-ecosystem and hence ending up in the same clusters in the hierarchical cluster analysis. Species in cluster II, which were mainly associated with the area in the crop row, were more shade-tolerant than species in the other clusters (Figures 2 and 3 in Paper II). On the other hand, species associated with intermediate to high competition (cluster I) tolerated drier conditions than those found where competition was lower (Figure S1 in Paper II). These species also had a higher requirement for light, which could be why they were mainly found between crop rows. The specific leaf area of plants could also be related to the need for tolerating competition, as species with the highest SLA were mainly found in the crop row (cluster II), followed by the micro-ecosystems with intermediate to high competition (cluster I), while the species with the lowest SLA were found where oat competition was low (cluster III). Hence, the type of species found in the different micro-ecosystems appeared to be adapted to at least some of the environmental constraints expected in these micro-ecosystems.

All species found are adapted to modern high-intensity systems and generally have a high requirement for disturbance and nutrients. No species showed solely stress-tolerant Grime’s life strategy or stress tolerant-ruderal life strategy, which have been suggested to be the most desirable plants in a weed community (MacLaren et al. 2020). One of the databases from which data on species traits were obtained in this thesis (that by Bàrberi *et al.* (2018)) contains very few species showing these life strategies. Of 240 species in the database, two have a stress-tolerant strategy and 12 a stress tolerant-ruderal life strategy. Over the years, the weed species community in arable fields has changed towards species that are adapted to the management regime of the field (Fried et al. 2012; Storkey et al. 2012; Trichard et al. 2013), while less adapted species have been lost (Fried et al. 2009). To allow rarer weed species to establish viable populations, long-term changes in crop management are needed (Rotchés-Ribalta et al. 2015; Albrecht et al. 2016). The surrounding landscape also has a great impact on weed species diversity, with higher diversity in the crop mosaic increasing weed diversity in the field (Alignier et al. 2017). Hence, landscape components also need to be managed to promote diversity in agricultural fields.

4.5 Simulation of the crops and the system (RQ7)

Three crops were calibrated for use in simulations of the study system with APSIM NG in Paper III, the two main crops (oats and winter wheat) and a one-species service crop modelled using a module for red clover. The crops were first calibrated as sole crops, after which some of the best parameter combinations were tested in intercropping (Supplementary Material 2 in Paper III). The parameter combinations that worked well in both sole cropping and intercropping were used in the scenario assessment. In Paper III, Site1 correspond to Exp6 and Site2 to Exp5.

The calibration resulted in parameter combinations that gave satisfactory biomass at main crop harvest for all crops (Figure 2, Table 2 in Paper III, Figure 14). However, to obtain similar simulated biomass levels at main crop harvest as observed in the field, early growth had to be overestimated because the model could not capture rapid growth early in the season. For oats, base phyllocron, how fast new leaves emerge, and minimum leaf number were reduced in the model to hasten phenological development (Table SM2.1 in Paper III). The latter also had a positive effect on LAI, which was overestimated (Table SM2.4 in Paper III). In addition, the grain filling period was prolonged in the model, to better match the actual time to maturity and grain yield. For winter wheat, simulations of early development were better than for oats prior to calibration, as winter wheat cultivars have already been calibrated for European conditions. However, a small reduction in maximum leaf number improved the simulation of early development, leaf biomass and LAI (Figure SM2.1, Table SM2.2, Figure SM2.4 and Table SM2.5 in Paper III). For red clover, the temperature response of photosynthesis and nitrogen re-translocation were changed so that these processes would be faster at lower temperatures, while radiation use efficiency (RUE) was increased to further accelerate early biomass production (Table SM2.7, Figure SM2.7, and Figure SM2.8 in Paper III). The increase in RUE was motivated by findings in several previous studies in similar environments showing higher RUE than the default value (Jannink et al. 1996; Singer et al. 2007; Torssell et al. 2007; Kiniry & Evers 2008; Riesinger et al. 2009; Harbo et al. 2022). Radiation use efficiency of all crops considered can vary greatly both within seasons and between years (Jannink et al. 1996; Kiniry & Evers 2008; Riesinger et al. 2009). However, in those studies RUE was rarely lower than 1.5 (APSIM red clover default value) and often approached 2, so an increase was considered realistic. Moreover, RUE has been shown to increase with increasing distance from the equator (Rodriguez & Sadras 2007), supporting the use of a higher value at high latitudes. Modifying RUE could potentially also have improved the simulation of early development of oats and a slight increase, from 1.6 to 1.7, was tested, but gave minor effects. Larger changes were not tested, as RUE was not a parameter changed for other cultivars. Moreover, the slow growth and lack of response to increased RUE could be because the model does not simulate diffuse light, which is positively correlated with RUE (Rodriguez & Sadras 2007). Similarly to RUE, diffuse light increases with increasing distance from the equator (Rodriguez & Sadras 2007).

In a study simulating maize development and growth at similar latitudes, Morel *et al.* (2020) found that maize biomass allocation could not be adequately simulated, although phenological development was well simulated. Simulated maize growth was similar to that observed in field in the early part of the season, but was greatly underestimated in the latter part, as was also observed during the calibration of the crops for Paper III. By increasing RUE and the coefficient of extinction, Morel *et al.* (2020) increased both early growth and improved the estimation of late-season biomass (Morel et al. 2020).

In the calibration in Paper III, the grain filling period of oats and winter wheat was also prolonged to meet the time for harvest and improve the simulation of grain yield. Similarly, Knörzer *et al.* (2011) found it necessary to prolong the life span of wheat simulated under German climate conditions by modifying thermal time response and time to maturity. They also found that increasing leaf senescence rate improved simulations of grain yield. This was however not done in the calibrations in Paper III, since data on leaf senescence were not available.

Vernalisation sensitivity and photoperiod sensitivity have been modified in some APSIM oat cultivars, and in the APSIM wheat cultivars adapted to Europe. However, changing vernalisation sensitivity and photoperiod sensitivity in the default oat cultivar or further modifying them in winter wheat did not lead to any clear improvements in the simulated biomass, and thus the default values were used.

The simulated red clover derived 82.5% and 84.3% of its nitrogen (cumulative over the whole life of the crop) from nitrogen fixation, at the two sites considered in Paper III. Those values are well in line with the nitrogen fixation estimated in Paper I (Figure 5 in Paper I), and with those observed by Büchi *et al.* (2015). The site with the higher value had a less fertile soil (Exp5, called Site2 in Paper III), which also agrees with field observations (Büchi et al. 2015).

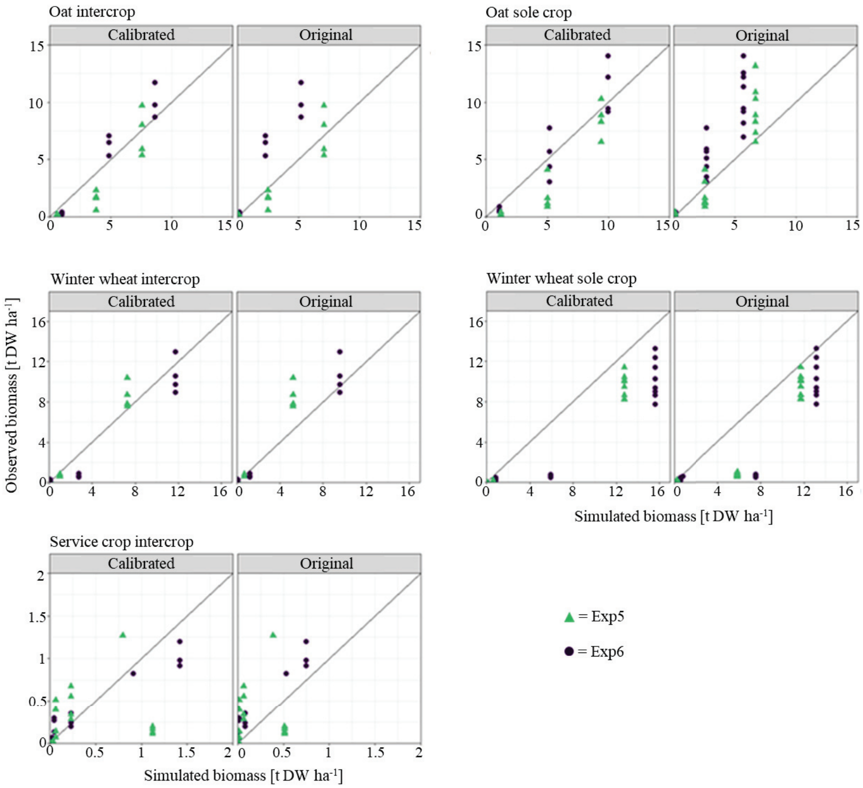


Figure 14. Simulated versus observed biomass in dry weight (DW) at experimental sites (Exp) 5 and 6 of the three crops (oats winter wheat, red clover service crop) assessed as sole crops and intercrops in Paper III. Diagrams show the difference between simulations with original parameters and parameters used in the calibrated versions. The different values for observed biomass are derived from the different plots data was collected from.

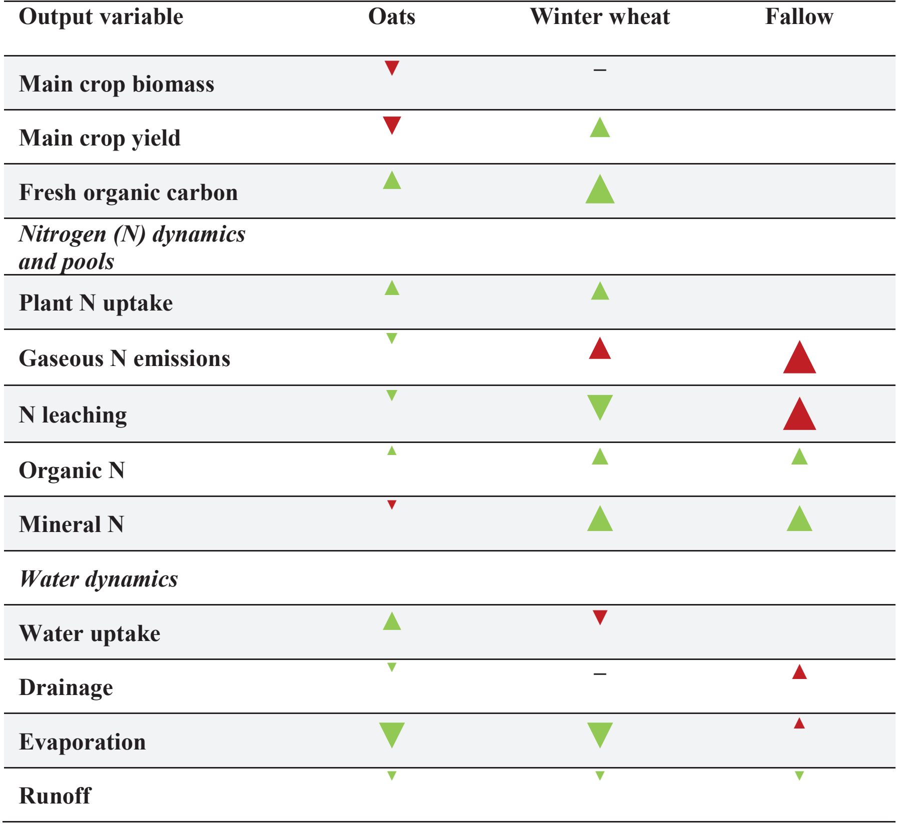
The identified overestimation of oat biomass early in the season is likely to have biased the calibration of the service crop in the intercrop to some extent, as the simulated resource use by oats is also higher due to this overestimation. When using the calibrated crops in the scenario assessment this was considered to be of minor importance, as the biomass in late summer and autumn is more important for service provision than biomass during early establishment. The simulated biomass of winter wheat was also more affected by intercropping than observed in field experiments. This could be partly because of differences in crop arrangement, since in the field experiments the winter wheat and service crop were grown in separate rows, with approximately 25 cm distance between winter wheat row centres (Figure 6), while in the model the plants were simulated using the same resources, and hence growing at the same spot. In the early stage of winter wheat growth, the plant canopies and root systems would not have been in contact, since there was some distance between the two crops. However, the reduction in winter wheat biomass in the intercropping system made the simulated early biomass much more similar to observed biomass and improved model fit (Figure 2 and Table 2 in Paper III). The calibration of the service crop resulted in good estimation of biomass in mid-August and late October, but not earlier in the season (Figure 3 and Table 2 in Paper III). As the biomass at the end of the service crop period was most important for the scenario assessment, the calibrated crop module was considered to be satisfactory.

4.6 Assessing multiple services from intercropping with service crops using APSIM (RQ8)

The simulated service crop was a fast/large type according to the classification in section 3.5.1, with mean biomass production until oat harvest of 445±215 and 410±164 kg ha^{-1} at Exp5 (Site2 in Paper III) and Exp6 (Site1 in Paper III), respectively, and survival during winter. Similarly to observation in Paper I, oat yields were significantly (p<0.001) reduced by the service crop when it produced this amount of biomass (Figure 3b in Paper III, Table 3), while winter wheat yields were generally increased (p=0.01) in the service crop scenario compared with the control (Figure 3b in Paper III, Table 3). However, there was only a slight negative correlation between service crop biomass and oat biomass (Figure 3c in Paper III), and the relationship between service crop biomass and oat yield was negative at Exp6 and positive at Exp5 (Figure 3c in Paper III). This reflects the varying effect of a productive service crop on oat performance observed in Paper I (see Figure 10). Since the main variables that differed between the repeated model simulations were the weather variables temperature, rainfall and radiation, it is likely that a favourable year (high precipitation and warm temperatures) supports growth of both crops. No crop data were available for the other weather-years used in simulations, so it was not possible to draw conclusions regarding the accuracy of the model in each individual year. However, the general pattern seems realistic in relation to that observed previously regarding the relations between the service crop and the first and second main crop (De Notaris et al. 2019; Shackelford et al. 2019; Taab et al. 2023).

Intercropping with a service crop increased soil fresh organic carbon, mainly root residues, compared with the control scenario, especially in spring when the service crop had been growing for a longer time (Figure 4 in Paper III, Table 3). Long-term effects on carbon stocks were not assessed in Paper III because the decomposition rate of organic matter was underestimated (section 4.2.2 and Supplementary Material 1 in Paper III), as also observed by Vogeler et al. (2019a) under Danish climate conditions. However, the contribution of the service crop, with approximately 30-70% more fresh organic carbon in the soil than in the control at service crop termination, indicates good potential of the service crop to increase soil carbon stocks. The average increase in soil fresh organic carbon was approximately 250 kg ha^{-1}, similar to the mean annual carbon sequestration of 320±8 kg ha^{-1} from using service crops reported in a recent review (Poeplau & Don 2015). Although the service crops in that review were not grown as intercrops in widely spaced rows, allowing for higher plant density and potentially higher biomass production than in the system under study in my thesis, the simulated fresh organic carbon input seems to be realistic. Not all of the fresh organic carbon input will contribute to long-term carbon stocks, but most of the modelled soil fresh organic carbon was derived from roots, which have been shown to contribute more to soil carbon stocks than aboveground biomass (Kätterer et al. 2011). Determining the long-term effects of growing service crops as sole crops and less frequently, since at high latitudes establishing a service crop after harvest of a main crop would only be possible before a spring-sown crop, compared to more frequent intercropping of the service crop as in Paper III, would provide a better understanding of the relative potential of these two types of service crops when integrated in cropping systems. Modelling is a tool with great potential in such studies, due to its ability to compare many different scenarios over a long period. However, for accurate results, better simulation of organic matter decomposition in cold temperatures than the present module in APSIM NG is required.

Table 3. Change in output variable values in the service crop scenario compared with the control scenario in Paper III. ▲ = increase and ▼ = decrease, size indicates approximate magnitude of change, colour indicates positive (green) or negative (red) change from an ecosystem service perspective



Output variable	**Oats**	**Winter wheat**	**Fallow**
Main crop biomass	🔻	–	
Main crop yield	🔻	▲	
Fresh organic carbon	▲	▲	
Nitrogen (N) dynamics and pools			
Plant N uptake	▲	▲	
Gaseous N emissions	▼	🔺	🔺
N leaching	▼	▼	🔺
Organic N	▲	▲	▲
Mineral N	🔻	▲	▲
Water dynamics			
Water uptake	▲	🔻	
Drainage	▼	–	🔺
Evaporation	▼	▼	🔺
Runoff	▼	▼	▼

Overall, uptake of soil mineral nitrogen by plants was higher in the service crop scenario than in the control scenario, especially in winter wheat (Figure 5 in Paper III, Table 3). However, the contribution of the service crops to uptake of soil nitrogen was minor, as they obtained around 75% of their nitrogen from N_2 fixation. This supports the suggestion that addition of a service crop improves crop growth and yield by adding easily available nitrogen to the system. In oats, simulated nitrogen losses were similar between the two scenarios, while in winter wheat gaseous emissions were slightly higher in the service crop scenario compared with the control scenario, while leaching was slightly lower (Figure 5 in Paper III, Table 3).

In the fallow period, nitrogen losses through both leaching and gaseous emissions were greatly increased in the service crop scenario compared with the control. However, the effect of service crops on nitrogen mineralisation mainly emerged in summer in winter wheat (Figure 6 in Paper III). These simulation results support findings in field experiments that the service crop reduces both gaseous emissions and leaching while it is living (De Notaris et al. 2018; Vogeler et al. 2019b), but when it dies and residues decompose, both sources of nitrogen losses increase (Storr et al. 2021; Olofsson & Ernfors 2022). The relatively large nitrogen losses, combined with the increased pools of both organic and mineral nitrogen in the fallow period (Table 4 in Paper III, Table 3), suggest that this period requires more attention in research regarding service crops for improved cropping system sustainability and a perspective on nitrogen management that covers a long period, both in policy and practice. The poor simulation of decomposition also affected nitrogen dynamics and potentially simulated nitrogen losses. With a slightly faster decomposition rate, nitrogen mineralisation and related losses would probably have been slightly higher in the service crop scenario, due to the higher organic matter content. However, this should mainly occur in winter wheat and the fallow period, which were already identified as risk periods.

Water losses, through evaporation, drainage and runoff, were lower in the service crop scenario during the two crop periods, but slightly higher in the fallow period (Figure 7 in Paper III, Table 3). The greatest difference was seen for evaporation, where introduction of a service crop seemed to have prevented some losses from the soil directly to the atmosphere. The higher water uptake by oats and service crop in the first summer led to slightly lower soil water content in the service crop scenario during autumn, but soil water was recharged during winter. In winter wheat, soil water content was lowest in the non-service crop scenario, mainly due to higher evaporation losses and greater water uptake. In the fallow period, water losses were elevated due to higher soil water content at the end of the winter wheat period (Figure 7 and Figure 8 in Paper III, Table 3). The lower soil water content in the non-service crop scenario than in the service crop scenario was due to a combination of greater losses, especially from evaporation, and slightly higher plant uptake. Similarly, a modelling study by Yang *et al.* (2020) found reductions in drainage (11-21%) in the service crop period and in evaporation in the subsequent crop (32% and 24% for maize and soybean, respectively) when a wheat service crop was grown from autumn to spring. Other studies have shown that APSIM is able to simulate fluctuations in soil water (Smith et al. 2020b; Ma et al. 2022) and evaporation (Vogeler et al. 2020; Guo et al. 2021). The results in Paper III indicate that under relatively humid conditions similar to those in southern Sweden, intercropping with a service crop will not cause major issues with water stress. In fact, the service crop will contribute to active use of soil water for biomass production, thus reducing direct water losses from the soil.

Although some important negative effects of including a service crop in the oat-winter wheat rotation were observed, such as reduced oat yield and increased nitrogen losses in the fallow period, most of the services studied were positively affected by the service crop (Table 3).

Using mechanistic models can help reveal many crop-soil interactions and provide insights into how different types of management affect productivity, the surrounding environment and the climate. A drawback of modelling with regard to assessing ecosystem service provision from cropping systems is the lack of dynamic response of soil physical and biological conditions to crop management practices. For example, APSIM does not take into account changes in bulk density or the microbial community (Maharjan et al. 2018; Peng et al. 2022), which can occur (often intentionally) with changes in crop management (Chalise et al. 2018; Martínez-García et al. 2018; Schmidt et al. 2018; Qi et al. 2022). Moreover, crop models can only be really reliable when they are properly calibrated and validated for specific environmental situations (Gaydon et al. 2017; Hao et al. 2021; Baum et al. 2023). Since existing crop models have mainly been developed using data from conventional cropping systems, they might be poorer at simulating systems reliant on organic fertilisers and non-use of pesticides. For example, weeds, pests and diseases are not simulated in APSIM by default, which could lead to overestimation of crop performance (Hochman et al. 2014; Snow et al. 2014). For weeds, an additional crop module representing one weed species or a simple weed community can be added (Grenz et al. 2006; Chikowo et al. 2008; Zeleke 2017). External population dynamics models of pests have been connected to APSIM in some studies, to simulate the effect of pest outbreaks on the crop (Brown et al. 2011; Whish et al. 2015). APSIM simulations have also been used to feed population dynamics models (Barton et al. 2021). In future work, models simulating the occurrence of weed species and their functional traits

(Colbach et al. 2021) or weed population dynamics models (Daouti et al. 2022) could likely be linked to APSIM for better assessments of weed competition in the cropping system. However, adding more complexity to the model should be balanced against greater difficulty in generalisability.

4.7 Limitations of the work

The studies reported in Papers I-III were designed to answer applied research questions and focused on comparing different alternatives of the proposed cropping system, mainly with regard to sowing time and species of service crops. Hence, unweeded controls and treatments with service crops grown as sole crops were not included, since these were not regarded as realistic in practical management. The unweeded control would also not have been accepted by the farmers who hosted the field experiments. Unweeded controls could have helped reveal the effect of competition on weeds in Papers I and II, especially whether the late-established service crop could compete with the weeds between crop rows. However, it was not possible to have a completely unweeded treatment for the late-sown service crops, since the establishment method involved one row-hoeing event at service crop sowing. Data on sole-crop service crops would have been useful in calibration of the service crop in Paper III, and would also have provided a better understanding of how competition with the main crop and weeds at the two sowing times affected service crop growth in Paper I.

In model calibration, all data were used for calibration and no separate validation step was included. Comparing simulations with calibrated parameters to observations from a separate dataset is common practice to ensure that the calibrated variables work well in other environments. Since data were only available for two experiments, model calibration and validation was not done separately but in parallel in Paper III, where it is referred to simply as calibration. Using all available data in the calibration step can improve calibration strength and ensure that the model is applicable in a broad set of environments (Raymundo et al. 2017; Brown et al. 2018).

4.8 Cropping system improvements

It was clear from the findings in Paper I that the novel cropping system needed further development to become more robust and to optimise service provision and reduce the risk of disservices. To some extent, this development requires operational skills, improved establishment and knowing when to terminate the service crop with regard to its biomass and weather conditions. Thus, future research on improving service crop management would benefit from being carried out in close collaboration with farmers, as local conditions and operational skills play a major role in optimal management of service crops.

4.8.1 Improved evenness and area covered by service crops

One reason for the varying effect of the service crops in Paper I was likely the varying success of establishment of those service crops (Table S3.1 in Paper I, Figure 9), affecting both weed suppression and delivery of nitrogen from service crops to winter wheat. The confidence interval of service crop plant density was 5-287 plants m^{-2}, indicating great variation within and between plots. The establishment method was intended to provide fairly distinct service crop rows (Figure 15a), so that it would be possible to terminate one crop at a time. However, due to issues with clogging, the drill sometimes spread the seeds unevenly, with gaps and larger patches as a result (Figure 15b). Widespread seeding, combined with increased seed rate, would likely improve weed suppression, as it would increase soil cover and total biomass production. However, a more widespread seed distribution might cause problems with service crop termination (see section 4.8.3).

Experiments in which the effects of seed rate and/or width of seed distribution on service crop biomass production, termination success, weed suppression and effect on the main crops can be better assessed are needed to provide better guidance to farmers on how to sow intercropped service crops. These studies should also assess the economic impact of service crop cultivation, as service crop seeds are costly, which is one reason why farmers are hesitant about their use (Casagrande et al. 2016; Shah et al. 2022). Crop models could be useful in reducing the experimental costs of identifying appropriate seed rates, as has been done to identify best sowing and termination time for service crops in sole crop stands to reduce nitrogen leaching (Constantin et al. 2015). Before APSIM is used in such studies, it must be improved in terms of simulating early growth of all crops, compared with the status of the model in Paper III.

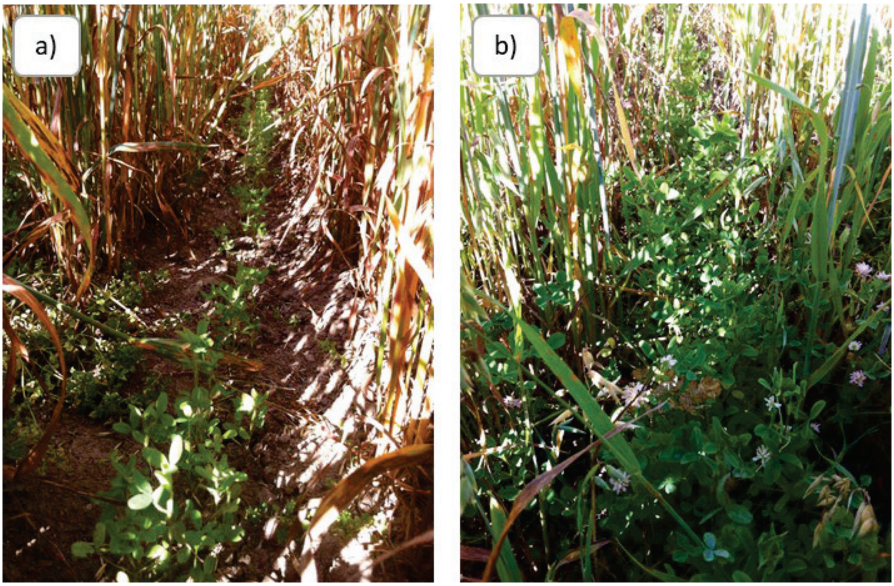


Figure 15. Differences in establishment within the same plot in Exp3. (a) Service crop sown in a narrow row as planned and (b) service crop seeds spread out beyond the row due to unequal distribution by the drill. Species mixture: *Trifolium resupinatum* and *T. squarrosum*. Photos taken in mid-July 2017.

4.8.2 Modified sowing time and plant arrangement

In Paper I, two sowing times were compared: sowing the service crop at the same time as oats and sowing it approximately one month later, at the first row-hoeing event. The second sowing time was chosen so that the oat crop would be large enough to avoid damage from soil pushed into the crop row by the hoe, which is part of the weed control mechanism in row-hoeing (Lötjönen & Mikkola 2000). Delayed sowing into a main crop drastically reduces service crop biomass, as competition with legumes, mainly for light and water, increases during the vegetative period of the main crop (Ohlander et al. 1996). In the intercropping system studied in this thesis, a shorter delay in sowing the service crop (one or two weeks after sowing of oats) would have resulted in somewhat intermediate biomass production to that observed in the experiments. This would have reduced the competition with the main crop compared with early sowing, but increased the competition with weeds (De Notaris et al. 2019). However, this might require a different sowing method to avoid damage to the main crop, since it would be smaller and possibly less resistant to row-hoeing.

Reducing the distance between crop rows or crop and service crop rows is another management strategy which could increase the competitive advantage over weeds. In sole crop cereals, smaller row distance (stepwise reduced from 50 cm to 12 cm) and increased seed rate (normal compared with 150% of normal) of winter wheat and oats have been shown to reduce weed biomass (Boström et al. 2012). More detailed studies on combinations of optimal distances between main crop and service crop and service crop sowing time would help reveal the true potential of service crop competition to reduce weeds, especially the most damaging weed species.

4.8.3 Improved termination of service crops

In the novel system studied in this thesis, service crops were both sown and terminated using a row-hoeing tool, as a non-chemical service crop management technique. However, there were some issues with termination of the service crop. The hoe was not as efficient in terminating large service crop plants if they were not growing in the inter-row centre (as defined in Figure 6). Plants growing toward the edge were often pushed farther into the crop row, where they survived and grew tall during spring and flowered in early summer (Figure 16). Using a wider hoe when sowing winter wheat, to remove service crop plants growing close to the crop row or at least push them more to the middle, could potentially ease later termination of the service crop. Large weeds were also sometimes pushed to the side by the hoe instead of being uprooted. Poor termination of service crops is one of the main problems farmers report regarding growing service crops (Casagrande et al. 2016) and is hence an important factor in system improvement to increase the use of service crops among farmers.

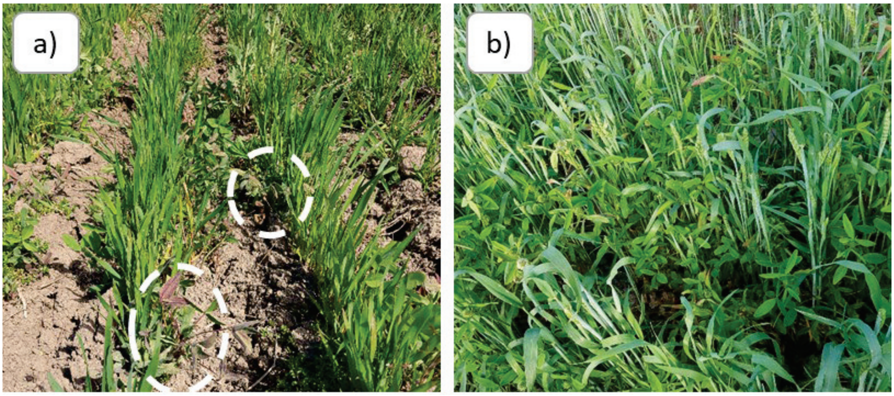


Figure 16. Unsuccessful termination of winter-surviving *Trifolium squarrosum.* (a) Plants pushed to the side by row-hoeing in mid-May in year two and (b) plants about to flower in mid-June in year two. Photos taken in Exp2 in 2019.

4.8.4 Other species and species combinations

In Paper I, three mixtures with relatively similar species were used, to enable identification of benefits and disadvantages of mixtures with different expected growth dynamics. Good establishment and ability to continue to grow after harvest of the main crop and in the following winter were identified as growth dynamics giving the best outcome with regard to weed control, risk of nitrogen leaching and improved winter wheat yield. Service crop mixtures showing such growth dynamics were mainly early sown frost-tolerant annuals in Östergötland and perennials in Skåne. Frost-sensitive annuals also showed potential for nitrogen delivery, but their poor recovery after oat harvest and the fact that recovered plants were later killed by frost pose a great risk of nitrogen leaching and promotion of weeds (Thorup-Kristensen 1994; Storr et al. 2021). However, winters in southern Sweden can also be too mild to kill *T. squarrosum* and this will probably occur more often with future temperature rises (IPCC 2021). Other species or species mixtures showing similar growth dynamic as the frost-tolerant annuals and the perennials would probably also be suitable for the system, with candidate species varying depending on location. Combining frost-sensitive annuals with a slower frost-tolerant species could be a way to ensure both early biomass production and regrowth. Another approach could be to include a non-legume that is better at taking up the released nitrogen in autumn. Diversifying service crop mixtures has been proposed as a way to increase total biomass production, resource utilisation and many of the above-mentioned ecosystem services. There is some evidence supporting this suggestion (Wendling et al. 2017; McKenzie-Gopsill et al. 2022; Rouge et al. 2022). The positive effect of diversification has been shown to be especially strong in situations with low availability of nitrogen and when no single species dominates the mixture, due to higher complementarity in resource use (Wendling et al. 2017). However, species that easily become dominant are more productive as sole crops than mixed with a less competitive species, and are also better at providing weed control (MacLaren et al. 2019; Reiss & Drinkwater 2020). In terms of nitrogen capture, mixtures of legumes and non-legumes can be better than sole crop legumes, as they can acquire nitrogen efficiently from the soil and from N_2 fixation (Reiss & Drinkwater 2020, 2022) and the proportion of legumes and non-legumes is regulated by access to mineral nitrogen in the soil (De Notaris et al. 2021). Depletion of soil mineral nitrogen from non-legumes may also increase legume N_2 fixation (Blesh 2019; Plumhoff et al. 2022), but not at high soil mineral nitrogen levels (De Notaris et al. 2021). Moreover, mixing species that can cope with different environmental conditions can increase the resilience of the service crop to shocks and ensure that the function of the service crop, *e.g.* weed control, nutrient capture or soil protection, is not lost in extreme weather situations (Justes et al. 2021). Mixing legumes and non-legumes can also compensate for differences in nitrogen status in the soil, with legume biomass being high if soil nitrogen is low and non-legume biomass being high if soil nitrogen is high, leading to high service crop biomass and low nitrogen leaching in both cases (De Notaris et al. 2021).

If main crops other than oats and winter wheat are grown, other service crop growth dynamics might be required. Oats is a relatively tall-growing and competitive crop, so if *e.g.* barley or spring wheat were grown instead less competitive service crops would likely be required to avoid reducing main crop yield. Moreover, if the first crop is a legume, *e.g.* peas or beans, the service crop should be a non-legume or a legume/non-legume mixture, to capture the nitrogen released after main crop harvest (Plaza-Bonilla et al. 2015; De Notaris et al. 2021). If the second crop has higher nitrogen demand in autumn, *e.g.* oilseed rape (Sieling et al. 2017), the frost-sensitive annuals with their high biomass production might be suitable as they could provide the initial fertiliser dose, whereas winter wheat does not require fertiliser application in autumn. Hence, service crop species or species mixtures, and their management, need to be chosen with regard to the main crop grown.

The diversity of potential service crops and service crop-main crop combinations creates a challenge when modelling these systems. Model calibration is a time- and data-consuming task, and calibrating all potential service crops and service crop-main crop combinations would require great resources. Less precise and more generalised crop modules, representing different functional characteristics, *e.g.* growth rate, legume/non-legume, frost-sensitivity and rough morphological traits, might be a more realistic target for crop model development than specialised crop modules (such as those in APSIM) that can even distinguish slight differences between cultivars.

4.9 Role of leguminous service crops in increasing agricultural sustainability

In the field experiments and modelling work reported in this thesis, positive effects on subsequent crop yields, weed suppression and indications of increased organic carbon inputs and reduced nutrient losses were observed when service crop biomass production was high and the service crop recovered after oat harvest. However, efforts need to be made to retain and increase these positive effects and preferably also take into account management over a longer period after service crop termination.

Service crops will likely never be as efficient in providing nutrients and weed control as chemical inputs, as their nutrient delivery and competitive ability build up slowly, while mineral nutrients and herbicides are fast-acting (Cornelius & Bradley 2017; Guiducci et al. 2018; Toukabri et al. 2020). Instead, service crop cultivation has to be viewed more holistically, acknowledging that these crops provide multiple services. For example, apart from direct nutrition and weed control, they contribute to soil fertility, protect the soil from erosion and can potentially shift the weed species community, reducing the amount of the most competitive species over time. Service crops also have the advantage that they do not contribute to herbicide resistance (Peterson et al. 2018; Kumar et al. 2020). Leguminous service crops are most efficient in systems where fertiliser doses are reduced (Guiducci et al. 2018; Toukabri et al. 2020), or when grown after a nitrogen-demanding main crop that leaves little nitrogen behind (De Notaris et al. 2021). Thus, for leguminous service crops to contribute nitrogen to cropping systems, the use of mineral nitrogen needs to be reduced. Such less intensive agriculture, combined with changed consumption habits with less animal-based food, would greatly reduce the environmental burden of European agriculture (Tilman & Clark 2014; Billen et al. 2021). Compared with use of mineral fertilisers and herbicides, best management practices to ensure service provision and reduce disservices from the use of service crops are still under development. This is especially true for intercropped service crops that could allow service crop growth in an extended range of climates and in other parts of the crop rotation than is possible under current common management.

Combined with other measures to reduce the demands of resource-intense agricultural production, *e.g*. a change of diet (Billen et al. 2021) and reduced food waste (Houlton et al. 2019; Lopez Barrera & Hertel 2021), cropping systems based more on natural processes for their function, such as service crops, can play an important role in increasing agricultural sustainability.

5. Conclusions

Service crops can be grown to improve cropping system sustainability by providing multiple services, *e.g.* addition of organic material to improve soil structure and nutrient provision, protection of soil from erosion, and weed control. The services provided can affect the cropping system itself, the immediate environment and/or the global climate. The desired services, and the management practices required to obtain these services, will vary depending on local pedo-climatic conditions. Short growing season is a major constraint for service crop cultivation at high latitudes.

This thesis assessed the ability of different types of leguminous service crops, grouped according to their expected functional traits in terms of frost sensitivity and longevity, to deliver services and disservices, and examined how these were affected by establishment time in two regions of southern Sweden. The main conclusions were as follows:

- The novel intercropping system studied has the potential to provide the desired services, *i.e.* yield improvements in the subsequent crop, weed control and substantial addition of fresh organic carbon to the soil, while keeping main crop (oats) yield losses relatively low, if the service crop is well established in oats and recovers well after oat harvest.
- In the more north-east region, with slightly cooler and drier conditions, early establishment and fast growth was more important than in the most southerly part of Sweden.
- High biomass production of service crops early in the growing season of oats increase risk of grain yield loss, particularly with tall growing frost-tolerant annuals.
- High biomass production of service crops early in the growing season of oats combined with no recovery after harvest, as found for

frost-sensitive annuals sown early, increase the risk of nitrogen losses during autumn and winter.

- In oats, high competition from service crop and main crop reduced the occurrence of some of the most problematic weeds during the service crop establishment phase, although some of the favoured weeds are also problematic species.
- Mechanistic crop models have the potential to be a valuable tool in improving assessment of service crops, as they can be used to simulate more scenarios than would be realistic in field experiments. However, the APSIM model requires improvements in simulating early growth of service crops and main crops and decomposition rates of organic matter in cold temperate climates before being used for detailed management scenarios or long-term simulations.
- Simulations of the system using 30 different years of weather data, indicate positive effects on yield of subsequent winter wheat and input of organic material.
- Modelling indicate an increased risk of nitrogen losses in the fallow period after harvest of winter wheat with service crops than without, with large losses both as gases and by leaching, indicating that this period needs extra attention in research, policy and practice.

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Popular science summary

Modern agriculture is generally highly productive, thanks to successful plant breeding of crop cultivars that produces high yields of good quality, mineral fertilisers that provide the crop with the nutrients it needs at the right time, and chemical pesticides that reduce yield losses from insect or fungal outbreaks and competition with weeds. These highly productive agricultural systems have helped feed a growing global population since the second half of the 20th Century. However, the intensification of agriculture has come at a cost to the environment. Modern agriculture causes leaching of nutrients, which leads to eutrophication of waterways, lakes and oceans, affecting aquatic plants and other organisms, including humans through toxic algal blooms or nitrates in drinking water. Nitrogen not taken up by crops also risks being lost through gaseous emissions, thereby contributing to global warming and deteriorating air quality. Pesticide residues can have negative effects on non-target organisms. An increasing number of weeds have also developed resistance to herbicides, while other plant species are becoming extinct due to intensive control and a homogenous cultivated landscape. The homogeneous agricultural landscape, that is typical for many of the main production areas in the world, where crop cultivation and animal husbandry occur in different places, has a negative impact on the diversity of insects and birds and has also led to a decrease in the supply of animal manure to arable land in areas with specialisation on crop production, which in the long run reduces soil fertility.

The negative impacts of agriculture on the environment, the climate and its own foundation, *i.e.* the soil itself, have led to initiatives by farmers and scientists to find solutions. The concept of ecosystem services is a way of acknowledging, and in some cases even valuing, services other than the productive benefits that an ecosystem can contribute. In the case of agricultural systems, these include carbon sequestration, water purification and supporting pollinating insects.

Service crops, crops that are grown to supply various ecosystem services, have attracted great interest from farmers in recent years and are highlighted as an important environmental measure in the EU Common Agricultural Policy (CAP). Service crops is a collective term for additional crops to the main crop that can offer various services. They are often divided into cover crops for carbon sequestration and weed control, catch crops to reduce nitrogen leaching, and green manure crops to add organic material and thus nutrients to the cropping system. The positive effects of service crops are often linked to the amount of biomass they produce, with more positive effects if the biomass is high. At high latitudes, *e.g.* in Sweden, it is difficult to achieve sufficiently high biomass to obtain positive effects if the service crop is sown in late summer after harvesting the main crop. If the service crop is sown into the main crop, it has a longer time to grow and a better chance of producing a large amount of biomass.

I have studied a cropping system where a nitrogen-fixing service crop was sown together with or into spring-sown oats and allowed to grow after harvest. A few weeks later, winter wheat was sown into the service crop. From autumn to spring, the service crop provided ground cover to reduce erosion and weed competition. In spring, the service crop was terminated, making nutrients in its biomass available to the winter wheat crop in time to help meet its nutritional needs.

In my first article I investigated how different species mixtures affected yield of the two main crops (spring oats, winter wheat), weed control at harvest and soil nitrogen before and after winter. I found that the most productive service crops (early sown hairy vetch + crimson clover, persian clover + squarrose clover and red clover + white clover + black medic) reduced oat yield by 12-18% on average. If these service crops recovered after oat harvest (hairy vetch + crimson clover and red clover + white clover + black medic), they also showed the best potential to increase winter wheat yield, by on average 11% in a relatively normal year and 47% (hairy vetch + crimson clover only) in the dry year of 2018, although with great variation between experimental plots. There were also large differences between experimental sites and years, with higher biomass production in Skåne (farther south, warmer and with more rainfall) than in Östergötland, and with higher biomass production in 2017 (more rain) than in 2018. The highest producing service crops also reduced the amount of weeds in both crops and gave the lowest risk of nitrogen leaching.

In my second article, I investigated whether the service crop favoured or disfavoured certain types of weeds in oats, and found that if the service crop produced more than 200 kg of biomass per hectare during the summer, tall-growing weeds such as cleavers, common hemp-nettle and common nipplewort with a life strategy characterised as competitive-stress tolerant were particularly disfavoured. Instead, the weed flora consisted of more low-growing ruderal weeds such as field pansies, speedwell and field forget-me-not, but also some more problematic ruderal weeds such as lamb’s quarters, field mustard and scentless mayweed. Relatively high oat biomass was found to reduce the amount of perennial competitive weeds, such as couch grass, field thistle and field milkweed.

In the third and final study, I modelled the intercropping system using a cropping system model that I first calibrated against data from the field experiments. Next, a comparison between the intercropping system and the same main crop sequence without the service crop was done. Since service crops grow differently in different years due to weather conditions, the system was simulated with 30 different years of weather data. I found that, on average, winter wheat yield increased and the amount of fresh organic carbon added in spring through roots of the killed service crop was up to 70% higher than in a control scenario with no service crop. Losses of nitrogen and water were lower in the service crop scenario than in the control scenario when oats or winter wheat was grown, but during the fallow period after harvesting winter wheat until spring of the following year, losses were higher in the service crop scenario. Losses of nitrogen in particular increased sharply, both in the form of nitrous oxide and nitrogen leaching. To reduce the risk of nitrogen losses and their negative effect on the climate and environment, extra agronomic measures during the autumn and winter after the service crop has been killed may be needed, *e.g.* sowing a new, more nitrogen-demanding crop or a second autumn-sown crop.

The intercropping system studied in this thesis showed potential to provide multiple services e.g. weed control, both as reduction of total biomass and by reducing some of the most problematic species, yield improvements of subsequent crop, increase input of fresh organic carbon and reduce nitrogen losses, especially leaching. However, this was only achieved if large amounts of service crop biomass was produced and was also associated with yield losses in oats during the establishment year. The large amount of nitrogen added with service crop biomass also increased the risk for nitrogen losses, the first autumn/winter if the service crop did not recover after oat harvest (field study) and the second autumn/winter when the soil was left bare (modelling study). Moreover, the system is in need of some improvements to for better and more stable service provision. First, even establishment of the service crop is needed to ensure good soil cover and uniform biomass production. Second, service crop termination must be improved, to prevent service crop growth the second summer.

Populärvetenskaplig sammanfattning

Dagens moderna jordbruk har generellt en hög produktivitet som möjliggjorts av framgångsrik växtförädling som ger hög skörd av god kvalitet, mineralbaserade gödselmedel som ger grödan den näring den behöver vid rätt tidpunkt och kemiska bekämpningsmedel som minskar skördeförluster orsakade av insekter, svampar eller konkurrens med ogräs. Dessa högproduktiva jordbrukssystem har varit nödvändiga för att föda en växande befolkning under 1900-talets andra hälft. Detta har dock skett på bekostnad av vår miljö. Modernt jordbruk orsakar näringsläckage av framförallt kväve och fosfor som leder till övergödning av vattendrag, sjöar och hav. Detta i sin tur påverkar vattenlevande växter och andra organismer, även människan genom giftig algblomning eller nitrat i dricksvatten. Kväve som inte tagits upp av grödan riskerar också att gå förlorad i gasform och därmed bidra till den globala uppvärmningen och bidra till försämrad luftkvalitet. Bekämpningsmedelsrester kan också ha en negativ påverkan på andra organismer än dem de var tänkta att påverka. Fler och fler ogräs har också utvecklat resistens mot ogräsmedel, samtidigt som andra växtarter riskerar utrotas på grund av intensiv bekämpning och ett homogent odlingslandskap. Det homogena odlingslandskapet, där växtodling och djurhållning ofta sker på olika platser, har också negativ påverkan på mångfalden av insekter och fåglar. Uppdelningen mellan djurhållning och växtodling har också lett till att tillförseln av djurgödsel minskar på åkermarken, vilket på sikt minskar markens bördighet.

Problemen med jordbrukets negativa inverkan på miljö, klimat och på sin egen grundpelare – jorden själv, har lett till initiativ från så väl lantbrukare som forskare att hitta lösningar. Konceptet ekosystemtjänster är ett sätt att uppmärksamma, och i vissa fall även värdesätta, andra tjänster än den produktiva som ett ekosystem kan bidra med. I fallet odlingssystem kan relevanta ekosystemtjänster till exempel vara kolinbindning, vattenrening eller stödjande av pollinerande insekter.

Servicegrödor, grödor som odlas för att bidra med olika ekosystemtjänster, har de senaste åren väckt stort intresse hos lantbrukare och lyfts fram som en viktig miljöåtgärd i EU:s gemensamma jordbrukspolicy (CAP). Servicegrödor är ett samlingsnamn för grödor som kan erbjuda olika tjänster och delas ofta upp i mellangrödor, för kolinbindning och ogräskontroll, fånggrödor, för att minska näringsläckage (framförallt kväve), och gröngödslingsgrödor, för att få in organiskt material och därmed näring i odlingssystemet. I praktiken överlappar tjänsterna som de olika typerna av service grödor bidrar med varandra, där av användes termen servicegröda i avhandlingen. De positiva effekterna av servicegrödor är ofta kopplande till mängden biomassa de producerar, med mer positiva effekter om biomassan är stor. Långt norrut, som i Sverige, är det dock svårt att uppnå tillräckligt stor biomassa för att få positiva effekter om servicegrödan sås på sensommaren efter skörd av årets huvudgröda. Om servicegrödan sås in i huvudgrödan har den längre tid för att växa, och har bättre chans att producera en stor mängd biomassa.

I mitt doktorandprojekt har jag studerat ett odlingssystem där kvävefixerande servicegrödor sås tillsammans med eller in i vårsådd havre och får växa kvar efter skörd. Några veckor senare sås höstvete in i servicegrödan. Från höst till vår bidrar servicegrödan med marktäckning för att minska erosion och ogräskonkurrens. På våren hackas servicegrödan bort, med tanken att näringsämnena i dess biomassa ska bli tillgänglig för höstvetet och i tid för att bidra till att tillgodose dess näringsbehov. I min första artikel undersökte jag hur olika artblandningar påverkade skörden av de båda huvudgrödorna, ogräskontroll vid skörd och markkväve innan och efter vintern. Jag fann att de mest produktiva service grödorna (luddvicker + blodklöver, doftklöver + spärrklöver och rödklöver + vitklöver + humlelucern) minskade havreskörden med 12-18% i medel, men om de hämtade sig efter havreskörden var det också dessa som visade bäst potential att höja höstveteskörden (luddvicker + blodklöver och rödklöver + vitklöver + humlelucern), i medel 11% ett relativt normalt år och 47% (bara luddvicker + blodklöver) torråret 2018, dock med stor variation mellan försöksrutorna. De mest produktiva servicegrödorna minskade också ogräsmängden i båda grödorna och när de odlades var också risken för kväveläckage lägst. Det var dock en del skillnader mellan försöksplatser och mellan år, med generellt mer produktiv mellangröda i Skåne än i Östergötland och under 2017 (mer nederbörd) jämfört med 2018.

I min andra artikel undersökte jag om servicegrödan gynnade eller missgynnande vissa typer av ogräs i havre, och fann att med en väletablerad servicegröda missgynnades framförallt högväxande ogräs med en livsstrategi som karaktäriseras som konkurrenskraftig och stress-tolerant, så som snärjmåra, dån och harkål. Istället bestod ogräsfloran av mer lågväxande ruderala ogräs som åkerviol, veronikor och åkerförgätmigej, men också några mer problematiska ruderala ogräs som svinmålla, åkersenap och baldersbrå. En relativt hög havrebiomassa visade sig minska mängden perenna, konkurrenskraftiga ogräs som kvickrot, åkertistel och åkermolke.

I den tredje och sista studien modellerade jag odlingssystemet med hjälp av en odlingssystemsmodell (APSIM NG) som jag först kalibrerat med data från mina försök. Eftersom servicegrödor växer olika bra olika år på grund av väderförhållandena simulerade jag systemet med 30 olika väderdata. Jag fann att i genomsnitt ökade höstveteskördarna och mängden färskt organiskt kol som tillfördes på våren genom rötter från den avdödade servicegrödan var upp till 70 % högre än i kontroll scenariot utan servicegröda. Förluster av kväve och vatten var mindre från scenariot med servicegrödan än från kontrollscenariot när havre eller höstvete odlades, men under trädesperioden efter skörd av höstvete till våren nästkommande år var förlusterna högre i scenariot med servicegrödan. Speciellt förlusterna av kväve ökade kraftigt, både i form av lustgas och nitratläckage. För att minska risken för kväveförluster, och deras negativa effekt på klimat och miljö, kan extra odlingsåtgärder under hösten och vintern efter att servicegrödan avdödats behövas. Dessa åtgärder skulle kunna vara att så in en ny, mer kvävekrävande servicegröda, eller en andra höstsådd huvudgröda.

Det samodlingssystem som studerades i denna avhandling visade potential hos servicegrödorna att bidra med flera tjänster, t.ex. ogräsbekämpning (både minska den totala biomassan och minska några av de mest problematiska arterna), öka avkastningen av efterföljande gröda, öka tillförseln av färskt organiskt kol och minska kväveförlusterna, särskilt nitratläckage. Detta uppnåddes dock endast om stora mängder biomassa producerades och var också förknippat med skördeförluster i havre under etableringsåret. Den stora mängden kväve som tillfördes med hjälp av biomassa från servicegrödor ökade också risken för kväveförluster, den första hösten/vintern om servicegrödan inte återhämtade sig efter havreskörden (fältstudie) och den andra hösten/vintern när jorden lämnades bar (modellstudie). Dessutom är systemet i behov av vissa förbättringar för att få en bättre och mer stabil leverans av tjänster. För det första behövs en jämn etablering av servicegrödan för att säkerställa en bra marktäckning och en jämn biomassaproduktion. För det andra måste avdödningen av servicegrödan förbättras för att den inte ska ta över andra sommaren i höstvetet.

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Article

Temporal and Spatial Positioning of Service Crops in Cereals Affects Yield and Weed Control

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Abstract: Leguminous service crops (SCs) can provide multiple services to cropping systems, reducing the reliance on external resources if sufficient biomass is produced. However, rapid light and temperature reductions limit post-harvest cultivation of SCs in Northern Europe. A novel practice of intercropping SCs in two consecutive crops (spring–winter cereal) to extend the period of SCs growth, and hence improve yield and reduce weeds, was tested. Three spatial and temporal arrangements of SCs and cash crops were investigated, as well as three SC mixtures, characterized by their longevity and frost sensitivity. Compared to no SC, the best performing mixture, frost-tolerant annuals, increased grain and N yield of winter wheat by 10% and 19%, respectively, and reduced weed biomass by 15% and 26% in oats and winter wheat, respectively. These effects were attributed to high biomass production and winter survival. However, this SC reduced oat yields by 15% compared to no SC. Furthermore, SC growth and service provision varied largely between experiments, driven by the weather conditions. Extending the SC's growth period by intercropping in two consecutive cereal crops has potential, but locally adapted species choices and establishment strategies are needed to ensure SC vitality until termination.

Keywords: cropping systems; innovation; relay intercropping; legume service crops; yield; nitrogen dynamics; weeds

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1. Introduction

Including legume crops as living ground cover can improve the performance of cropping systems by providing additional ecosystem services [1]. Among the services are, maintaining soil biological activity [2], suppressing weeds [3], protecting the soil from wind and water erosion [4], retaining nutrients [5,6], increasing soil organic carbon content [7], and the addition of nutrients via dinitrogen (N_2) fixation [8] or nutrient mining from deeper soil layers [9]. It has also been shown that several desired services can be provided simultaneously [10], indicating the great potential of using these crops as a tool for more sustainable agriculture. Crops that are managed as living ground cover may have different names, depending on their main function and how they are used in the cropping system [11]. In this paper, we use the term *service crops* (SCs) for these crops to emphasize that their role is to provide one or more services supporting the cropping system.

Biomass production has been shown to correlate well with several target functions of service crops, including ground cover, weed suppression, and reduced nitrate leaching [12], as well as the yield of the subsequent crop. For example, under northern European conditions, grass/clover under-sown into spring barley can produce about 2 t dry matter ha^{-1}

of biomass until late in autumn if conditions are suitable [13]. This led to a yield increase in subsequent spring barley by 1–2 t ha^{-1} when the grass/clover crop had been ploughed under in late autumn. Similar amounts of service crop biomass have, in other studies in northern Europe, been shown to also reduce N leaching by about 70% [14]. Achieving sufficient service crop biomass can, however, be difficult in northern Europe if the service crop is sown after cash crop harvest due to low daily incoming solar radiation and temperatures [15], meaning that SCs are mainly used prior to a spring-sown cash crop. Spring-sown cash crops, however, generally yield lower than autumn sown cash crops, and when autumn tillage is required, as on clay soils, the soil will be left bare for several months. Bare soils are prone to erosion as well as weed infestation, and SCs grown during autumn–spring can serve an important role in reducing both [16,17]. Extending the growth period of SCs, their establishment as well as termination time, into periods when the cash crops are growing but not using all available resources is a potential way to increase their biomass production and the related services.

In intercropping, two or more species are grown together to have more efficient use of resources, compensate poor performance of the other species, and/or cooperate via modifications of the environment [18]. Intercropping the SCs with a cash crop can also have undesired consequences, sometimes called “disservices”. The most obvious and important for many farmers is competition between the SC and the cash crop, resulting in cash crop yield reduction [18,19]. However, it is possible to mitigate these undesired consequences to some extent through cropping system design and management [19]. Finding suitable combinations of cash crop and SC species according to the desired services, as well as designing the system to minimize competition with the cash crop, are clearly priorities and need to be done, taking local conditions into consideration. Understanding how to manage the different species to utilize resources in a complementary way in space and time is one of the keys to successful intercropping [18].

In this study, our aim was to evaluate how different functional groups of leguminous SCs affect cereal yield and weed biomass when intercropped in a spring oats-winter wheat cropping sequence under organic management. The SCs comprised three mixtures differing in longevity and frost sensitivity. This affected their period of main biomass production, which was either in summer or autumn/spring or continuously throughout the experimental period. Three spatial arrangements of the crops (SCs and cash crops) were also tested, with associated differences in SC growth and crop management.

We hypothesized that the SC mixtures, with their ability to fix atmospheric nitrogen and the additional biomass they provide, would (1) increase winter wheat grain yield, (2) suppress weeds, and (3) not jeopardize the yields of oats. The hypotheses were tested in four field experiments conducted in two regions of southern Sweden.

2. Materials and Methods

2.1. Experimental Sites

The four experiments were located on organic farms in two cereal-dominated regions of Sweden, the provinces Skåne (SK) and Östergötland (OG). In each region, two experiments were set up in 2017 (SK1, 55°40″ N 13°13″ E and OG1, 58°26″ N 15°18″ E) and 2018 (SK2, 56°13″ N 12°54″ E and OG2, 58°26″ N 15°18″ E). The 30-year means for annual temperatures are 9.0, 8.4, and 7.1 °C, and for annual precipitation are 676, 755, and 565 mm at SK1, SK2, and the two OG experimental sites, respectively. During the study, weather conditions differed much between experiments and years (Figure 1). Water surpluses were generally greater in SK than in OG, but precipitation was extremely low during summer at all sites in 2018.

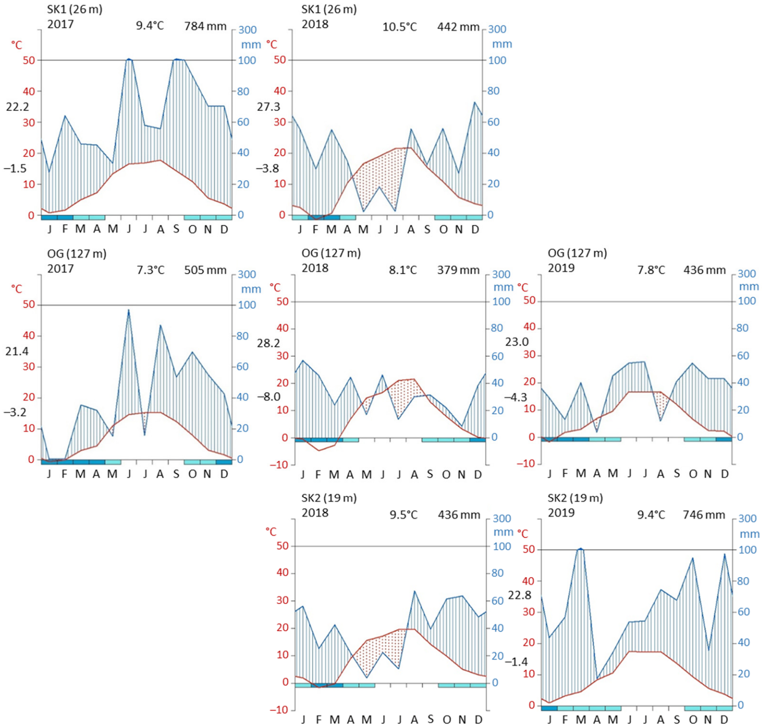

Figure 1. Meteorological data from the weather station closest to the experiments in SK and OG expressed in Walter–Leith graphs [20] for the three experimental years: 2017 at the top, 2018 in the middle, and 2019 at the bottom. The blue thick solid lines represent monthly precipitation, and the red solid lines represent average monthly temperature. The blue striped areas indicate excess water, and the red dotted areas indicate periods of (likely) water deficit, i.e., periods with precipitation less than two times the average monthly temperature. The number in parentheses after the site name is the altitude at the site. The numbers at the top are mean temperature of the year (**middle**) and accumulated precipitation during the year (**right**). The numbers to the left of the y-axes are the mean temperature of the warmest (**top**) and coldest (**bottom**) month. The dark turquoise boxes at the bottom indicate months when frost temperatures are common, and the light turquoise boxes indicate when frost could occur.

The topsoil was classified as loam at SK1, clay loam at SK2, silty clay loam at OG1, and silty clay at OG2 (USDA, 2022), with a general increase in the finer particle sizes in the subsoil (Table S1.1). Topsoil pH (H_2O) ranged from 6.8 to 7.1 between sites and in the subsoil between 7.2 and 7.8. Soil mineral nitrogen was about 0.5 mg 100 g^{-1} in the topsoil prior to the experiment's start, except for SK2, where it was about half as much. Soil carbon was generally higher in the OG experiments than in the SK experiments (Table S1.1).

2.2. Crop Sequence and Management

The first cash crop was spring oats (*Avena sativa* L.), followed by direct-seeded winter wheat (*Triticum aestivum* L.). Service crops (SCs) were sown into oats and were intercropped with both oats and winter wheat until they were terminated in the spring of the second year (Figure 2a). Due to wet conditions in autumn 2017, it was not possible to sow winter wheat in experiment OG1. In this experiment, winter wheat was replaced with spring wheat, and therefore this experiment was excluded from the analyses in its second year, 2019.

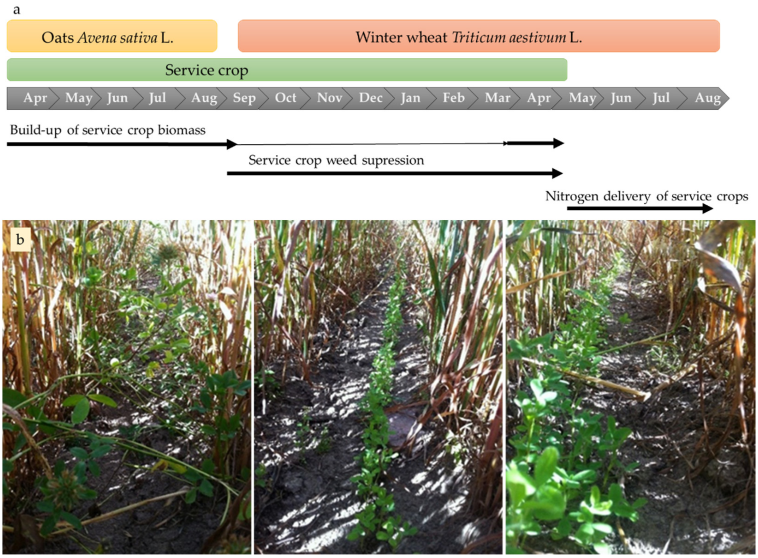

Figure 2. Visualization of the study system. (**a**) Timeline of crops grown in the field and main expected service delivery. In oats, the service crop mainly builds up biomass for later service provision. (**b**) Visualization of the placement of service crops depending on system strategy. From the left: Early Intra (service crop sown in crop row), Late Inter (service crop sown in inter-row centres at first row hoeing), and Late Adjacent (service crops sown adjacent to crop row at first row hoeing). All pictures are of the frost-sensitive annual service crop mixtures, *Trifolium resupinatum* and *T. squarrosum*. Photos by E. Lagerquist, experiment OG1, July 2017.

The experiments in OG were conducted with experimental equipment in small plots, 3.1 m × 36 m, while the experiments in SK were managed by the farmers with their own machinery; hence, larger plot sizes were used (8 m and 9 m × 50 m in SK1 and SK2, respectively). The smaller plots in OG allowed for more treatments (see Section 2.3 for a detailed description), and a total of 9 treatments were included in the study. At SK, the experimental design was reduced to include only 6 treatments (Table 1). Oat was sown in 7 cm bands with inter-row distances of 33 cm in the OG experiments, 25 cm in experiment SK1, and 32 cm in experiment SK2 due to different machines. Winter wheat was direct drilled with straight coulters leaving approximately 2 cm-wide rows. For information on the timing of field operations, see Table S1.3.

2.3. Experimental Design

The two experimental factors, service crop mixture and system strategy (Table 1), were arranged in randomized complete blocks with four replicates (five at experiment OG2, for data from cash crop harvest). Six treatment combinations were tested in all experiments. In the OG experiments, there was one additional level for each factor, and hence twelve treatment combinations (Table 1).

Table 1. Main characteristics of the different levels of the two treatment factors; service crop mixture and system strategy. The two columns to the far right indicate at which experimental regions, SK = Skåne or OG = Östergötland, each factor level is present. For visualization of the sowing of service crops in the different system strategies, see Figure 2b.

Service Crop Mixture	**Speed of Growth**	**Frost Sensitivity**		**SK**	**OG**
Frost-sensitive annual *	Fast	High		X	X
Perennial **	Slow	Low		X	X
Frost-tolerant annual ***	Fast	Relatively low			X
Control, no service crop				X	X
System strategy	**Sowing of service crops**	**Number of row hoeing events**	**Sowing of winter wheat**	**SK**	**OG**
Early Intra	Same time as oats, in oat rows	2	Between oat rows	X	X
Late Inter	At first row hoeing, in inter-row centers	1	In oat stubble	X	X
Late Adjacent	At first row hoeing, adjacent to oat row	2	Between oat rows		X

* Squarrose clover (*Trifolium squarrosum*) and Persian clover (*T. resupinatum*). ** Red clover (*T. pratense*), white clover (*T. repens*), and the short-lived perennial black medic (*Medicago lupulina*). *** Crimson clover (*T. incarnatum*) and hairy vetch (*Vicia villosa*).

2.3.1. Service Crop Mixtures

The SCs were grouped into mixtures according to the functional traits of frost sensitivity and longevity. The groups were (i) frost-sensitive annuals, which included squarrose clover (*Trifolium squarrosum* L.) and Persian clover (*T. resupinatum*), (ii) perennials, which included red clover (*T. pratense*), white clover (*T. repens*) and the short-lived perennial black medic (*Medicago lupulina* L.), and (iii) frost-tolerant annuals, which included crimson clover (*T. incarnatum*) and hairy vetch (*Vicia villosa* Roth). Frost-tolerant annual SCs were only present in the experiments in OG. Sowing density for annual SCs was 250 viable seeds m^{-2} per species, except for *V. villosa*, which, due to its vigorous growth, was sown with 30 viable seeds m^{-2}, and for perennial species, the rates were 167 and 95 viable seeds m^{-2} per species in the SK and OG experiments, respectively (Table S1.2). For each system strategy (see below), there was also control without SCs.

2.3.2. System Strategy

The system strategies differed in their spatial and temporal arrangement of cash crops and SCs (Figure 2b), which also affected row hoeing intensity (Table 1). In the first system strategy, Early Intra, SCs were sown at the same time and in the same rows as oats. The inter-row space in Early Intra was crop free during the whole oat cropping sequence and was hoed twice. Winter wheat was direct drilled in the inter-row space after oat harvest. In system strategies, Late SCs were sown between the rows of oat approximately one month after the sowing of oats. The sowing with both strategies was performed in conjunction with row hoeing. In Late Inter, the SCs were sown in oat inter-row centres, and the SCs, therefore, prevented later row hoeing. Winter wheat was direct drilled in the row of oat stubble. In Late Adjacent (only in the OG experiments), SCs were sown adjacent to the oat, thus allowing for a second-row hoeing if needed (not done), but with a smaller hoe than in Early Intra. Winter wheat was direct drilled into the inter-row space of SCs. In winter wheat, row hoeing was conducted twice with all system strategies.

2.4. Data Collection

Destructive sampling was restricted to the two ends of the experimental plots, saving the plot centre for crop harvest (Figure 3a). Sampling of SC and weed data was performed from an area covering two cash crop rows and two inter-row spaces (i.e., 50 cm or wider) and 50 cm long (Figure 3b). The different sizes of the areas were accounted for in later calculations. Samples from each frame were pooled into one sample per plot. For detailed timing of data collection, see Table S1.4.

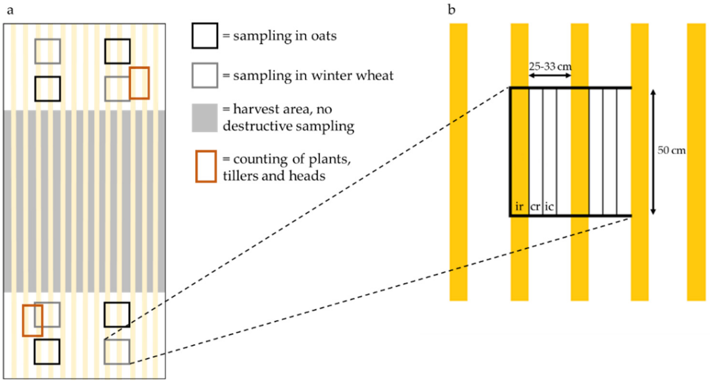

Figure 3. (**a**) Sketch over a plot and where data were collected for the different measurements and crops. For the data collection in oats (black squares) in OG1, only three frames were used. In this case, one frame was used instead of two at one end of the plot and located in the centre of the sampling area. The yellow stripes represent the cereal rows to demonstrate how samples were collected in relation to these. (**b**) Subsections for weed biomass sampling in both oats and winter wheat. Abbreviations: ir = in crop row, cr = close to crop row, ic = inter-row centres.

2.4.1. Service Crop Performance

Service crop performance was evaluated by measures of (i) plant density, (ii) biomass production until oat harvest, (iii) autumn soil cover, and (iv) percent N derived from the atmosphere (%Ndfa). Data on SC performance were only collected from system strategies Early Intra and Late Inter. For all variables except %Ndfa, the data were collected according to the above description. Biomass was collected at the species level and cut at ground level when the cash crop approached maturity. Percent soil cover was assessed before winter. For the %Ndfa analysis, above-ground biomass was collected from 10 randomly selected plants from each SC species within each plot in late autumn. In 2017, SC samples were only collected from treatments with system strategy Late Inter because there was almost no biomass present in treatments with system strategy Early Intra. All biomass samples were dried at 60 °C for 48 h before weighing or further sample treatment (see below).

Nitrogen Isotope Analysis

Plants were thoroughly washed before drying. The dried plant samples were ball milled and analysed with an Elemental Analyzer (Flash EA 2000, Thermo Fisher Scientific, Bremen, Germany) and Isotope Ratio Mass Spectrometer (EA-IRMS, DeltaV, Thermo Fisher Scientific, Bremen, Germany) according to [21] for $\delta^{15}N$ isotope profiles. Calibrated samples with wheat and maize flour were used as working standards.

The N_2 fixation of each SC species was estimated by calculating the percentage of N derived from the atmosphere (%Ndfa) according to [22]:

$$\%\text{Ndfa} = (\delta^{15}\text{N}_{\text{reference crop}} - \delta^{15}\text{N}_{\text{SC}})/(\delta^{15}\text{N}_{\text{reference crop}} - \text{B}) \times 100 \quad (1)$$

with the reference crop being winter wheat plants collected in plots without SCs or from the area outside of the experiment, which had the same soil properties and received the same amount of fertilizer. The B-value represents the δ15N profile of each SC species when N_2 is the only N source. B-values could not be estimated in this experiment; hence literature values were used. B-values used for the calculation of %Ndfa for *T. resupinatum* (−0.81), *V. villosa* (−0.35), and *T. incarnatum* (−0.67) were derived from [8], while those for *T. pratense* (−1.3), *T. repens* (−1.7), and *M. lupulina* (−1.01) were derived from [23]. For *T. squarrosum*, no B-value was found in the literature, so the lowest value measured in the field (−0.35, measured in experiment SK2) was used [24].

No inoculation of SCs was performed since both clovers and lucerne are part of the crop rotations on the farms included in the study.

2.4.2. Cash Crop Performance

Cash crop performance was evaluated by measures of (i) grain yield, (ii) nitrogen (N) concentration in winter wheat kernels, (iii) winter wheat N yield, (iv) number of plants and heads, (v) heads plant^{-1}, kernels head^{-1} and thousand kernel weight (tkw). All data are reported in dry weight (DW). Grain was harvested using a plot combined in the centre of each plot (Figure 3). The size of the harvested area was 26–54 m^2, depending on the plot size. A subsample of 400–500 g was taken from continuous flow from the combine. These samples were cleaned, and the moisture was measured before analysing for tkw and grain N concentration. Grain N concentration was analysed with the near-infrared transmittance (NIT) method (InfratecTM 1241 Grain Analyzer, Foss, Denmark) and used to calculate N yields. Counting of plants and heads was performed in two parallel one-meter rows on two fixed locations in each plot (Figure 3) and converted to number per m^2.

2.4.3. Weed Biomass Assessment

Initial weed density was counted about two weeks after the sowing of oats. The weed counting was performed at the same locations where the biomass samples were later taken (Figure 3a). Weed biomass was sampled when oats or winter wheat approached maturity. Sampling was performed separately in three equally sized subsections of the sampled areas: in cash crop row, close to cash crop row, and in inter-row centres (Figure 3b), and were dried at 60 °C for 48 h before weighing. The analysis was performed both across the whole area and split into the three subsections. For the analyses, including the subsections, the data were standardised to cover an equally large area since the subsections covered areas of different sizes.

2.4.4. Soil Mineral Nitrogen

Soil mineral nitrogen was measured in late autumn (all experiments) and in the subsequent spring (experiments SK2 and OG2). Soil samples were collected from 0–30, 30–60, and 60–90 cm depths, except for in experiment SK2 in autumn 2018, where the deepest layer was excluded due to a high density of stones. In each plot, subsamples were pooled into one sample per plot and depth (8 subsamples per plot in the OG experiments and 10 subsamples per plot in the SK experiments due to the difference in plot size). The samples were kept cool in the field, frozen the same day, and analysed within 1–2 weeks. Soil mineral nitrogen was determined using FOSS TECATOR FIAstar 5000 Analyzer (FOSS GmbH, Hamburg, Germany) after extraction with 2 M KCl.

2.5. Statistical Analysis

The statistical analyses, both in oats and winter wheat, were performed in two steps. First, all experiments were analysed for the effect of intercropping with frost-sensitive annual SCs, perennial SCs, and no SC, as well as system strategy Early Intra and Late Inter. All results presenting differences between these treatments or factors come from these analyses. Secondly, the experiment(s) in OG were analysed, comparing the treatments from the first analysis to frost-tolerant annual SCs and, when relevant, system strategy Late Adjacent.

The factors SC mixture, system strategy, experiment, and their interactions were set as fixed factors, whereas block was set as a random factor and nested within the experiment. The initial weed number was set as a covariate in all analyses of data collected in oats. All data were tested for normality and homogeneity of variance by plotting residuals versus fitted, normal Q-Q, and by running a box-cox test. When the assumptions of normality and homogeneity of variance were not met, the data were log- or square root-transformed. Variation in data is visualized by using 95% confidence intervals. All analyses were conducted in R Studio with R version 4.1.1 [25] using linear mixed models, with the function *lmer()* from the lme4 package [26] and analysis of variance performed with the *Anova()* function from the car package [27]. A pairwise comparison was performed with the *emmeans()* function from the emmeans package [28] with Tukey HSD to adjust p-values. Visualisation was performed with the standard *barplot()* function or by the *ggplot()* function from the ggplot2 package [29].

3. Results

3.1. Effect of Local Conditions on the System

The growing conditions varied widely between experimental sites and years (Figure 1). In 2018 there was an extreme drought event with both low precipitation and high temperatures, affecting all experiments. In 2017 and 2019, the OG region was drier than the SK region. The factor "experiment" and interactions with the experiment were significant for most of the variables (Table S2.1–S2.9). Therefore, results from the different experiments are presented separately in figures and text. However, some effects of other factors or factor combinations could be generalised, and when they are, these generalisations are mentioned in the text. Unless otherwise stated, presented p-values come from pairwise comparisons of means.

3.2. Service Crop Performance

3.2.1. Establishment and Productivity of Service Crop Mixtures

Service crop (SC) plant numbers were significantly higher for frost-sensitive annual SCs than for perennial SCs at OG1 and OG2 ($p < 0.001$ for both experiments, Table S3.1), while the opposite was shown at SK2 ($p = 0.01$). At SK1, plant numbers did not differ significantly between SC mixtures. Furthermore, in 2017 plant numbers were significantly higher with the system strategy Late Inter than Early Intra ($p = 0.005$ and 0.003 for SK1 and OG1, respectively, Table S3.1), while the opposite was shown in 2018 ($p < 0.001$ for both experiments SK2 and OG2).

Biomass production in oats was generally higher for both frost-sensitive (*T. resupinatum* and *T. squarrosum*, including all experiments) and frost-tolerant (*V. villosa* and *T. incarnatum*, including only OG experiments) annual SCs than for perennial (*T. pratense*, *T. repens*, *M. lupulina*) SCs ($p < 0.001$). Moreover, SCs produced more biomass with system strategy Early Intra than with Late Inter ($p < 0.001$). With system strategy Early Intra, both frost-sensitive and -tolerant annual SCs were clearly more productive than perennial SCs (Figure 4a–d, $p < 0.001$ in experiments OG1, OG2, and SK2, $p = 0.002$ in experiment SK1 for frost-sensitive annual SCs, and $p < 0.001$ for frost-tolerant annual SCs in both experiments OG1 and OG2). In 2018 frost-tolerant annual SCs produced more biomass than any of the other mixtures with system strategy Early Intra ($p < 0.001$). With system strategy Late Inter, no significant differences in SC biomass were observed.

3.2.2. Service Crop Soil Cover in Late Autumn

Perennial SCs had greater soil cover in late autumn than frost-sensitive annual SCs ($p < 0.001$, Figure 4e–h) in all experiments except OG2, where the opposite was observed ($p = 0.03$). Frost-tolerant annuals generally had a more extensive soil cover than both frost-sensitive annuals and perennials, regardless of the system strategy and experiment ($p < 0.001$ and $p = 0.003$ compared to frost-sensitive annuals and perennials, respectively, Figure 4g,h, and Table S2.1).

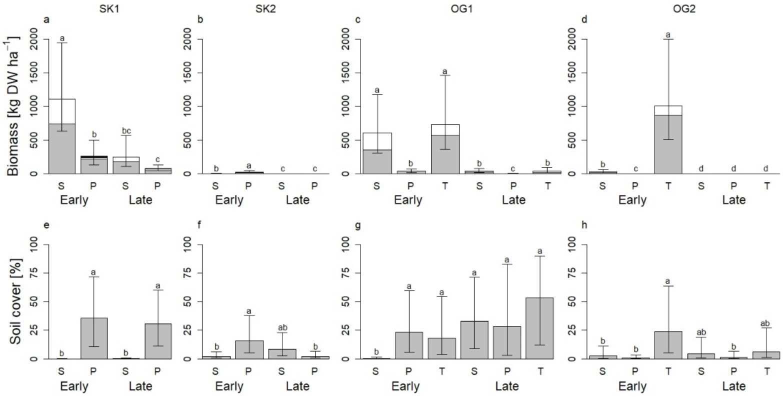

Figure 4. Service crop biomass before oat harvest (**a**–**d**) and soil cover in autumn (**e**–**h**) at the four experimental sites. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, C = no service crop, S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. Biomass values are back-transformed from log-transformed model estimates. In biomass plots the grey scale indicates the species in the mixtures: gray = *Trifolium squarrosum* (S), *T. pratense* (P) and *Vicia villosa* (T), white = *T. resupinatum* (S), *T. repens* (P), and *T. incarnatum* (T), black = *Medicago lupulina* (P). Early and Late indicate system strategy. See Materials and Methods and Table 1 for detailed information. Error bars indicate the 95% confidence interval for the total biomass and soil cover, respectively. The letters indicate significant differences between treatments within each experiment.

3.2.3. Service Crop Dinitrogen Fixation

Annual SCs generally had a slightly higher percentage of nitrogen derived from the atmosphere (%Ndfa) in above-ground tissues than perennial SCs (Figure 5; Table S2.2). The highest %Ndfa was found in *T. squarrosum* and *V. villosa*, and it was clearly lowest in *M. lupulina*. The pattern was similar in all experiments, although the absolute differences between species varied. In experiment OG2, %Ndfa was generally lower than in the other experiments.

3.3. Delivery of Services and Disservices by Service Crops to the System

Effects of Service Crops on Winter Wheat Grain Yield and Nitrogen Content in Kernels

The effect of SCs on winter wheat grain yield, nitrogen (N) concentration, and N yield could not be generalised because of differences in crop performance between experiments (Figure 6). With perennial SCs, grain yield was increased compared to no SCs by an average of 0.5 t ha^{-1} in experiment SK1 ($p = 0.05$), while it was reduced in experiment SK2 compared to frost-sensitive annual and no SCs ($p = 0.012$ and 0.02, respectively. Figure 6b). System strategy Early Intra yielded more (3.9 t ha^{-1}) than Late Inter (3.5 t ha^{-1}) in experiment OG2 ($p < 0.001$).

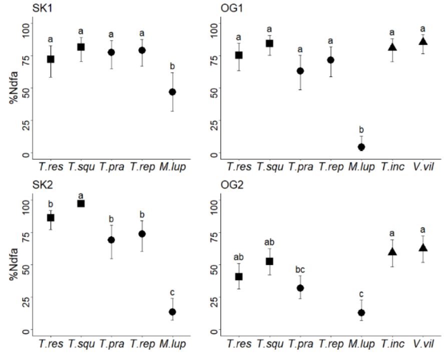

Figure 5. Percent of nitrogen derived from the atmosphere [%Ndfa] before winter in the forage legumes at the four experiments. Abbreviations: SK and OG stand for the two experimental regions, 1 = starting 2017, 2 = starting 2018. Point shapes indicate mixture: frost-sensitive annuals (squares), perennials (circles), and frost-tolerant annuals (triangles). T.res = *Trifolium resupinatum*, T.squ = *T. squarrosum*, M.lup = *Medicago lupulina*, T.pra = *T. pratense*, T.rep = *T. repens*, V.vil = *Vicia villosa,* and T.inc = *T. incarnatum*. Error bars indicate the 95% confidence interval, and the letters indicate significant differences between treatments within each experiment.

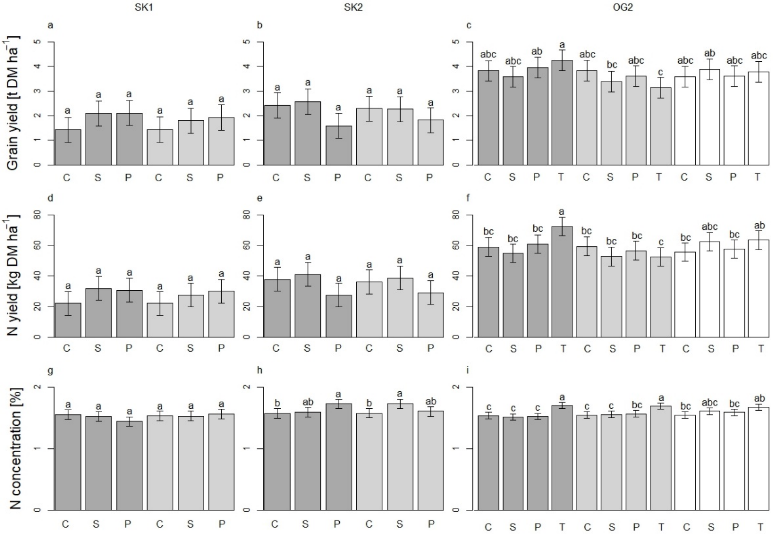

Figure 6. Dry matter (DM) grain yield (**a**–**c**), nitrogen yield (**d**–**f**), and nitrogen concentration in kernels (**g**–**i**) of winter wheat in the three experiments. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, C = no service crop, S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. No data are presented for winter wheat in the OG1 experiment since spring wheat was grown here this year. Colour coding indicating system strategy: dark grey = Early Intra, light grey = Late Inter, white = Late Adjacent. See Materials and Methods and Table 1 for information on treatments. Error bars indicate the 95% confidence interval, and the letters indicate significant differences between treatments within each experiment.

Nitrogen concentration in winter wheat kernels was higher with frost-sensitive annual SCs in system strategy Late Inter and with perennial SCs in system strategy Early Intra than with no SC in experiment SK2 (Figure 6h, $p = 0.04$ and 0.03, respectively). In experiment OG2, N concentration was higher with frost-tolerant annual SCs than with other SCs and no SCs ($p < 0.001$, for all comparisons. Figure 6i). In experiment SK1, there was no difference between treatments. Nitrogen yields were largely determined by grain yield, and these two variables thus followed the same pattern (Figure 6d–f). However, with frost-tolerant annual SCs, the effect of a higher N concentration clearly improved crop performance with regard to N yield. This was seen as a relatively larger difference in N yield compared to grain yield in treatments with frost-tolerant SCs compared to other treatments.

Intercropping with SCs affected the winter wheat stand and its yield components. Winter wheat had fewer heads m^{-2} in treatments with perennial SCs than with frost-sensitive annual and no SCs in SK1 and SK2 (Table S2.4, $p < 0.001$ and $p = 0.018$, respectively). However, in experiment SK1, this was compensated for by more kernels $head^{-1}$ with perennial SCs than with frost-sensitive annual and no SCs (Table S2.4, $p = 0.014$ and 0.0026, respectively). No difference in winter wheat plant numbers was observed when established in frost-sensitive annual, perennial, or no SCs. By contrast, when established in frost-tolerant SCs, the number of winter wheat plants was reduced compared to perennial and frost-sensitive annual SCs (Table S2.4, $p = 0.04$ and 0.06, respectively), especially with system strategy Early Intra ($p_{\text{Mixture:System}} = 0.03$). Despite fewer plants established with frost-sensitive annual SCs, the grain yields were highest with winter annual SCs and system strategy Early Intra (Figure 6c). During the growing season, the winter wheat crop compensated for the smaller number of plants by producing more heads per plant than in all other treatments (Table S2.4, $p = 0.03$, 0.014, and 0.011 for frost-sensitive annual, perennial, and no SCs, respectively), and significantly higher thousand kernel weight than in treatments with frost-sensitive annual SCs (Table S2.4 and Table S3.2, $p = 0.009$).

3.4. Oat Yields

Intercropping with the most productive SCs, both frost-sensitive annual and perennial SCs in SK1 and frost-tolerant annual SCs at OG1 and OG2, led to reductions in oat yields (Figure 7). In experiment SK1, frost-sensitive annual SCs reduced oat yields by 0.5 t ha^{-1}, 11%, compared to no SC ($p = 0.003$). In the OG experiments, frost-tolerant annual SCs in system strategy Early Intra reduced oat yields by 0.3–0.4 t ha^{-1} compared to all other treatments (Figure 7). No effects of SCs on the number of plants or heads were observed.

In experiment SK1 system strategy Early Intra, oats yielded 8% less than Late Inter ($p = 0.002$). In addition, head numbers tended to be fewer in system strategy Early Intra than in Late Inter (Table S2.5, $p = 0.09$). The number of oat plants was not significantly affected by system strategy in any experiment.

Intercropping with SCs did not affect heads $plant^{-1}$ or kernels $head^{-1}$ in oats. Thousand kernel weight had a tendency to be higher in treatments with system strategy Late Inter than with Early Intra ($p = 0.06$) and was significantly lower in 2018 than in 2017 ($p < 0.001$, Table S2.5).

3.5. Weed Biomass

In winter wheat in experiment SK1, weed biomass was lower with perennial SCs than with frost-sensitive annual and no SCs ($p = 0.005$ and 0.04, respectively). Weed biomass was especially low with perennial SCs in system strategy Late Inter (Figure 8). In experiment OG2, frost-tolerant annual SCs reduced weed biomass by 55% compared to frost-sensitive annual SCs ($p = 0.03$) over both system strategies. Moreover, it was clear that the lower weed biomass with perennial SCs, compared to frost-sensitive annual and no SCs, was due to the reduction in weed biomass in the winter wheat row (0.001 g m^{-2}, vs. 0.14 and 0.71 g m^{-2} for frost-sensitive annuals and no SCs, respectively, $p = 0.001$). Furthermore, in experiment SK1, weed biomass in winter wheat rows was lower with system strategy Early Intra than Late Inter ($p = 0.014$).

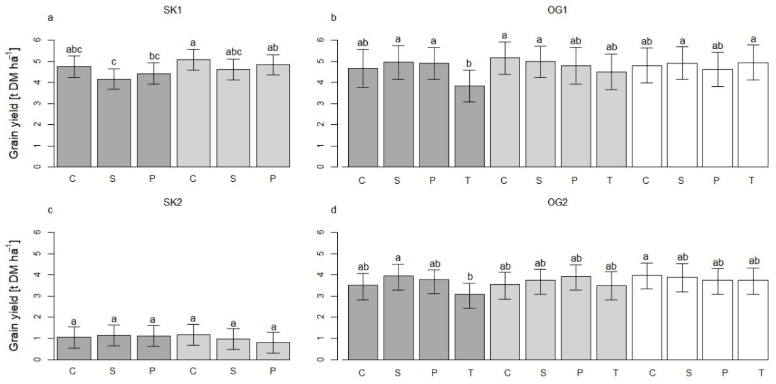

Figure 7. Oat dry matter (DM) grain yield in the four experiments (**a**–**d**). Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, C = no service crop, S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. Colour coding: dark grey = Early Intra, light grey = Late Inter, white = Late Adjacent. See Materials and Methods and Table 1 for detailed information. Error bars indicate the 95% confidence interval, and the letters indicate significant differences between treatments within each experiment.

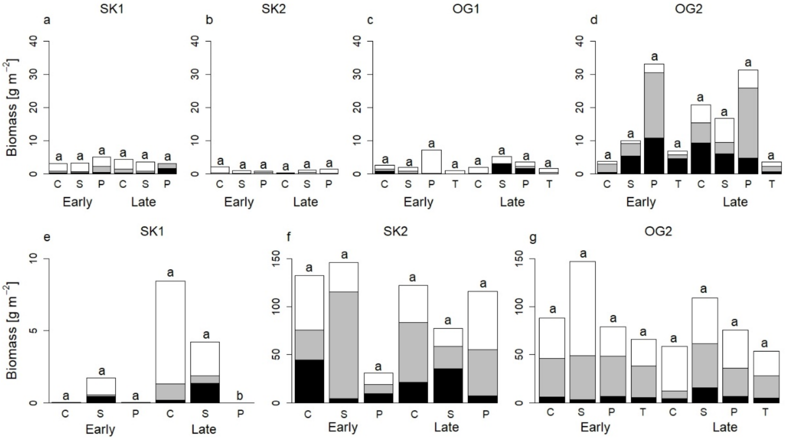

Figure 8. Weed biomass in oats (**a**–**d**) and winter wheat (**e**–**g**) before cash crop harvest. Biomass values are back-transformed from log-transformed model estimates. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, SC = service crop, S = frost-sensitive annuals, P = perennials, T = frost-tolerant annuals. No data are presented for winter wheat in the OG1 experiment since spring wheat was grown here this year. The colour coding shows the biomass in the different subsections of the sampled areas: white = in crop row, grey = close to crop row, black = inter-row centres. The letters indicate significant differences in total biomass, error bars for the 95% CI were not included in the figure since the wide CIs made the mean values and proportions difficult to read. Confidence intervals can be found in Table S3.4. See Section 2 for detailed treatment explanations and Table 1 for treatment overview. Observe that y-axis values differ between crops, and that of SK1 in winter wheat differs from the two others.

In oats, frost-tolerant annual SCs reduced weed biomass by 80% compared to perennial SCs (p = 0.03), both in experiments OG1 and OG2 (Figure 8). However, due to the large variation in weed biomass between plots, no significant differences between specific treatments were observed. Moreover, weed biomass in inter-row centres was lower with system strategy Early Intra (0.037 g m^{-2}) than Late Inter (0.14 g m^{-2}, p = 0.06).

3.6. Soil Mineral Nitrogen

No general effects of SC or system strategy on profile SMN (0–60 and 0–90 cm) in autumn were observed, but there was an interaction between SC mixture and system strategy (Table S2.8). Frost-sensitive annual SCs resulted in the highest amount of SMN when sown early, but among the late-sown treatments, it had the lowest SMN (p = 0.017, Figure 9). The same pattern could also be observed in the topsoil (0–30 cm) but not in the other soil layers (Table S2.8). Frost-tolerant annual SCs did not affect SMN significantly compared to other SCs and the control, and SMN was at an intermediate level compared to them, both with system strategy Early Intra and Late Inter (data not shown).

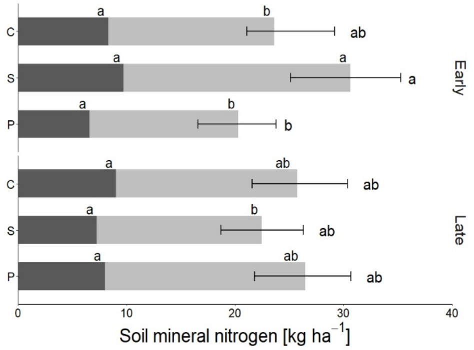

Figure 9. Autumn soil mineral nitrogen (SMN) at 0–90 cm as treatment averages over all experiments. Colour coding indicates the amounts in each measured layer: 0–30, 30–60, and 60–90, going from light to dark grey. C = control (no service crop, SC), S = frost-sensitive annual SCs, P = perennial SCs. Error bars show the 95% confidence interval whole profile SMN. Significance letters show the statistical difference between treatments in each soil layer and should not be compared between layers.

In spring 2019, in experiment SK2, SMN was slightly higher with system strategy Early Intra (27 kg N ha^{-1}) than with Late Inter (23 kg N ha^{-1}, p = 0.017). This was mainly due to the difference in the 0–30 cm soil layer (19 kg N ha^{-1} with system strategy Early Intra and 15 kg N ha^{-1} with Late Inter). At 60–90 cm depth over both experiments, treatments with perennial SCs had lower SMN than those with no SC (p = 0.03; 4.8 kg N ha^{-1} and 6.5 kg N ha^{-1}, respectively). There was much more SMN in experiment OG2, 49 kg ha^{-1}, than in experiment SK2, 25 kg ha^{-1}, but no differences between treatments in experiment OG2 (Table S2.9).

4. Discussion

The frost-tolerant annual service crop (SC), the mixture with *Vicia villosa* and *Trifolium incarnatum*, sown early, showed the best overall service provision. This treatment resulted in the highest grain and nitrogen (N) yields, N concentrations (Figure 6), and among the lowest weed biomass (Figure 8). The drawback was the vigorous early growth of this SC, which reduced the yield of oats (Figure 7).

In the following discussion, we will address the different aspects of the system and how service delivery can be enhanced and disservices avoided by crop management in terms of species choice and system strategy.

4.1. Nitrogen Provision for Yield and Grain Nitrogen Content in Winter Wheat

Only treatments with early sown frost-tolerant annual SCs showed a significant increase in both grain yield and N concentration for winter wheat (Figure 6). In experiment SK1, all SC treatments increased grain yield by 0.5 t ha^{-1} compared to those with no SCs, despite varying SC growth, maybe because the drought caused generally low yields this year, lowering crop nutrient demand. Moreover, at SK1, SCs did not affect grain N concentration, which could be due to low access to soil mineral nitrogen (SMN) during this dry summer.

The timing of N availability from SC residues is an important aspect of this system. Winter wheat does not take up much N until the beginning of stem-elongation in late spring, so N released following early decay of senescing or frost-killed SCs is at risk of being lost. In our experiment, early sown frost-sensitive annuals left behind more SMN in autumn than all other treatments, probably due to the combination of high biomass production in oats and decomposition of SC material after oat harvest. Little effect of *T. resupinatum*, one of our frost-sensitive annual SCs, on the subsequent crop was observed in another study, which was attributed to winter wilting of the legume and subsequent N leaching [30]. However, when managed appropriately according to the conditions in the field, even leguminous SCs can reduce N leaching [31]. In our experiments, SMN was not increased by SC mixtures, which remained vigorous throughout autumn. Letting SCs remain throughout the winter instead of ploughing them down in autumn is a method for keeping SMN low [32]. Finding a suitable termination time must, however, take into consideration other factors affecting nutrient availability, such as the chemical composition of the SCs [33–35] and weather conditions [36], as well as the practicality for farmers. Under expected wet and mild conditions, termination should occur relatively late since the risk of leaching and gaseous emissions increases with increased precipitation [33,37,38], while when precipitation is low, N provision to the subsequent crop is improved by early termination [36]. Furthermore, traffic with heavy machines on wet soils increases the risk of soil compaction [39]. Since weather cannot be reliably predicted over longer periods and farmers must adapt their management to soil conditions, knowledge of typical local weather patterns must be used to decide termination time. Furthermore, in our experiments, the reduced tillage probably affected N availability as well. Soils under reduced and no tillage tend to warm up slower compared to ploughed [40], which can delay or slow down decomposition and mineralization. The low soil disturbance desired in these systems hence risks affecting soil temperature and associated N availability in a way that is negative to crop growth and development.

4.2. Weed Suppression

The most consistent weed suppression was observed with frost-tolerant annual SCs, in which the dominating species *V. villosa* (Figure 4c,d) probably made the main contribution. With this mixture, weeds were suppressed in both oats and winter wheat, with both early and late sowing (Figure 8c,d). A similar effect of *V. villosa* has been observed by others and was correlated to fast establishment, high biomass productivity, and large amounts of SC residues [41]. Perennial SCs, which provided relatively suitable soil cover in autumn, reduced weed biomass in winter wheat but did not reduce weed biomass in oats (Figure 8), in which they generally had small biomass production (Figure 4a–d).

Scaling down the weed biomass assessment to subsections of the plots showed that in oats, higher row hoeing intensity decreased weed biomass in inter-row centres. This was expected and confirmed other findings [42]. However, close to the cash crop rows, row hoeing intensity did not matter, which could be because the hoe did not reach all the way to the crop row. It was also observed that weeds were not always cut off by the hoe but pushed to the side. Plants uprooted by mechanical control have been shown to have about a 50% survival rate; moist soil conditions increase the chance [43]. The main effect of mechanical weed control is that it reduces weed biomass and increases crop yield [44], but sometimes it can also affect the crop negatively. In SK1, we could observe damages on oats after the second-row hoeing, which was reflected in the yields. Hence, some care needs to be taken with the mechanical weeding, although going in close proximity to the crop row is important for effective weed control. In addition, in winter wheat in SK2 and OG2, better weed control would have been needed.

The smaller weed biomass in winter wheat with perennial SCs was only observed in the crop rows. This is of particular interest since it is more difficult to manage weeds within than between cash crop rows, where row hoeing can efficiently remove weeds [44–46]. However, the perennial SCs were difficult to terminate in the second year and hence continued to grow in or close to the winter wheat row (data not shown), contributing to weed suppression in winter wheat, which was not seen for the other treatments in this experiment. This points to the importance of continuous competition for weed suppression in this system. However, having a vigorous SC providing this competition involves a high risk of negatively affecting the crop, as was also observed in our study.

The highest weed biomass was observed in treatments with frost-sensitive annual SCs. This was probably due to a combination of increased SMN and the lack of competition after oat harvest with this SC. The weed community composition is formed by agronomic management, and many plants that have become agricultural weeds have a high affiliation to N [47,48]. Our results show that early termination of a highly productive SC may give weeds an advantage over winter wheat when mechanical and chemical weed control is low.

4.3. Oats–Service Crop Interaction

In the majority of the treatments, oat yields were not reduced by the SCs (Figure 7). This goes in line with other studies on relay intercropping with legumes and other crops [13,31,49,50]. In these studies, the SCs are, however, under-sown at a later stage in the cash crop's development, which gives the cash crop a greater advantage. In our experiments, late sowing was the safest way to establish the SCs to prevent competition. It has been shown that if the SC biomass is 20% or more of the cash crop biomass, cash crop grain protein content and, to a lesser extent, cash crop yield can be reduced [51]. However, high SC biomass alone does not explain the yield reduction. Frost-sensitive annual SCs in system strategy Early Intra in experiment OG1 did not decrease oat yields, despite reaching the same biomass as the frost-tolerant annual SCs. Furthermore, in experiment SK1, less productive SCs reduced oat yields compared to the control. The negative effect of the frost-tolerant annuals could be because they grew through the oat canopy and competed for light during grain filling, while the frost-sensitive annuals did not grow out of the oat canopy in OG. In experiment SK1, the reduction in oat yield even with less productive SCs could partly be due to a narrower row spacing in experiment SK1 (25 cm compared to 33 cm in the OG experiments and 32 cm in experiment SK2), increasing the competitive impact of SCs on oats in SK1.

4.4. Service Crop Performance and Species Choice

Early sowing of SCs resulted in larger biomass production than with late sowing for all SC mixtures (Figure 4a–d). This was expected since they had a longer growth period, and the oat crop did not have much of an advantage over the SC in acquiring resources in terms of light, water, and nutrients, in contrast with late-sown SCs. The difference was, however, larger for the annual than for the perennial SCs, for which sowing time was of less importance. The relatively vigorous growth of late-sown SCs in experiment SK1 indicates that water was the most limiting factor for SC growth in our experiments. This experiment received a more even distribution of precipitation in spring and summer in the establishment year compared to all other experiments (Figure 1). The performance of oats was similar in the two experiments (Figure 7). Furthermore, a trade-off between biomass production during summer and soil cover in autumn was observed for most SC mixtures. The exception was early sown frost-tolerant annual SCs, which provided both.

The analysis showed that high biomass production of SC was essential to increase grain and N yield of winter wheat and reduce weed biomass, although stronger and more consistent effects are desirable. Earlier studies have shown that an SC biomass around 2 t ha^{-1} significantly increases the grain and N yield of the subsequent crop and reduces weed biomass [13,52]. However, because of the low soil cover by the SCs that provided the most biomass during summer, combined with the reduction in yield of oats (already at 1 t SC biomass ha^{-1}), a high SC biomass production during summer should probably not be the target in this system. Increasing the soil cover in autumn is hence a better target for service delivery in these types of systems, especially for weed control [16]. Under similar climatic conditions to ours, a 6-fold increase in biomass of under-sown SCs from cash crop harvest to November was observed [13]. However, a 6-fold increase in biomass of late-sown SCs in OG1 and of all SCs, except the frost-tolerant annual, in 2018 with both system strategies and in both experiments would still result in low final biomass before winter. Species choice and sowing time need to be adapted to local conditions to ensure sufficient soil cover in autumn. Increasing the SC sowing density is a way of increasing biomass production and soil cover. More studies are needed to gain a better understanding of how SC biomass production can be managed to provide the desired services without suppressing the cash crop by choice of species and cultivars, as well as by adjusting the time and density of sowing under different pedo-climatic conditions.

In our experiments, most SCs gained 60–85% of their N from the atmosphere, similar to other observations [8]. The exception was *M. lupulina*. Forming symbioses with natural rhizobial communities has shown to be more difficult for *Medicago* species compared to *Trifolium* species, probably because of a lower presence of bacterial strains that form a symbiosis with *Medicago* species in agricultural soils [53]. Generally, perennial SCs had slightly lower %Ndfa than annual SCs, except in SK1. The %Ndfa is often correlated with biomass production [54]. In our study, perennial SCs in experiment SK1 showed both higher biomass and %Ndfa than perennials in all other experiments, which supports the positive correlation between legume biomass and %Ndfa. Water availability is another factor influencing the ability of N_2 fixation [55,56], and the greater fixation found in SK1 could also be explained by greater water availability than in the other experiments. The perennials grew slower than the annuals at the beginning of the season, but at SK1, with an excess of water, perennial and frost-sensitive annuals showed similar %Ndfa. In 2018, %Ndfa was greatly reduced in OG2, which could be due to the poor growth and lack of water; this was, however, not expected to have differed much compared to the conditions in SK2. The higher soil organic matter content at OG2 than at SK2, indicating a greater potential for N mineralization from the OG2 soil, combined with lower phosphorus (P) content, could have disfavoured N_2 fixation at OG2 compared to SK2 since the high availability of N and low availability of P constrain biological N_2 fixation [57]. Hence, many factors influence the ability of legumes to provide the cropping system with nitrogen. Although legume biomass production is the main factor influencing N input [56,58,59], legume species choice and crop management need to be carefully chosen if the cropping system should rely less on external inputs.

The severe drought in 2018 provided insights into the tolerance of the different species to the combination of water and heat stress. The only SC that showed significant growth in 2018 was *V. villosa*. Its fast establishment and growth were probably beneficial in this dry year. *Vicia villosa* also has hairs covering the leaves, which is known to reduce transpiration [60,61]. These extreme weather events are expected to become more common in the future [62]; hence, drought tolerance of SCs will be another trait to consider. However, access to irrigation is probably necessary to ensure the high and stable growth of SCs under variable weather and soil conditions.

4.5. Developing (Relay) Intercropping Systems with Forage Legumes

In this study, SC species mixtures were grouped according to longevity and frost sensitivity to distinguish between different growth dynamics. Different establishment strategies of under-sowing in a spring cereal were tested under northern European growth conditions. Early sown frost-tolerant annuals showed to be the most favourable for improving yields and providing weed control due to high productivity in summer, recovery after oat harvest, and winter hardiness. Service crops showing other growth dynamics failed in service provision, at least partly, or even caused disservices. When the SCs established slowly, they generally did not reduce weed biomass and had too little biomass in late summer for sufficient growth in autumn, and did not increase the yield of the subsequent winter wheat. When the SCs grew fast in the beginning but did not recover after oat harvest, they increased SMN in autumn, which could be leached out of the field or fertilize weeds. Species combinations that mix groups of species might provide similar positive effects as the frost-tolerant annuals if the above-mentioned combination is achieved and could even improve this dynamic. Mixing species allows the complementarity in species traits to be exploited [18,63] or to guarantee that at least one species will do well [18,64]. Having identified promising trait combinations for service delivery in our system, future studies should explore how more complex mixtures can be composed in this or similar systems.

Biomass production and hence the potential service provision of SCs varied between years. This has also been observed in other studies (e.g., [41,65]) and calls for a better understanding of how variations in environmental conditions affect different SC species and how cropping system design, including SC choice and crop management, might enhance SC productivity and delivery of different services. Mixtures with both diversity and redundancy in traits have been shown to better cope with stressful environments [64]. However, the more species that are included, the harder it will be to evaluate the effect of the specific species on the overall performance and hence understand how the mixture will perform in other environments.

Many services could be desired from an agricultural system, and it is difficult to avoid trade-offs [66,67]. In our system, the clearest trade-off risk occurs from the biomass produced for weed suppression and nutrient accumulation and the competition with oats. However, if other services were to be taken into account, additional trade-offs might become evident.

Finally, we have identified some technical aspects of the system in need of improvement to make the system more robust and farmer friendly. These are to ensure a more even establishment of both cash crops and SCs in this intercropped and direct-seeded system and better removal of both weeds and SCs.

5. Conclusions

There are many aspects to consider when designing and managing SCs in intercropping systems. The positive effects on grain and nitrogen yield and weed suppression show that there is potential in this system, but the varying SC growth and following effects on winter wheat yields and weed control indicate that the system needs further optimization. This optimization should aim at ensuring suitable SC establishment before oat harvest so that the SC can fill the empty space and, by doing so, suppress weeds and retain nutrients to become available the following spring. The best performing SC mixture in our study was with frost-tolerant annuals in system strategy Early Intra. This treatment combination resulted in a large SC biomass in oats, which recovered after the harvest of oats and stayed alive during winter. Hence, high productivity of the SC seems to be key to producing the desired services but also increases the risk of potential disservices such as competition and nitrogen leaching. Other species, or combinations of species, probably have the potential to do the same if the aforementioned growth dynamics are achieved.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12091398/s1, Supplementary Material S1:

S1_Materials_methods; Supplementary Material S2: S2_ANOVA_tables; Supplementary Material S3: S3_Result_tables.

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S1. Supplementary material to materials and methods

Table S1.1. Soil chemical and physical properties in the four experiments. Total carbon (C), mineral nitrogen (N), phosphorus (P) and potassium (K) are reported in mg kg^{-1} dried soil*. Clay, silt and sand content are reported in % of air dried soil

Experiment	**Depth**	**Total C**	**N**	**P**	**K**	**pH****	**Clay**	**Silt**	**Sand**
SK1	0-30	0.11	4.5	68	110	6.9	21	32	47
	30-60	0.04	6.3	17	110	7.7	31	30	39
	60-100			46	91		24	33	43
SK2	0-30	0.15	2.8	52	98	7.0	29	42	29
	30-60	0.03	1.6	44	120	7.8	43	41	16
	60-100			86	120		48	40	12
OG1	0-30	0.17	6	67	150	7.1	34	56	10
	30-60	0.05	10	110	73	7.4	31	61	8
	60-100		4.6***	160	160		72	27	1
OG2	0-30	0.25	-	36	130	6.8	41	49	10
	30-60	0.07	-	80	140	7.23	69	29	2
	60-100		-	170	110		49	31	20

*N was extracted with 2 M KCl and determined on a FIAstar 5000 Analyzer (Foss Tecator, Hilleröd, Denmark) and P and K was measured with the ammonium lactate extraction method, and analysed with ICP/OES (Avio200, Perkin Elmer, USA)

**analysed with MeterLab®, Radiometer Analytical SAS, Lyon, France
***taken from 60-90 cm.

Table S1.2. Sowing densities of the different legume species in the three mixtures tested

Mixture	**Legume species (variety)**	**Sowing density kg ha^{-1}**	
		SK	**OG**
Summer annuals	*Trifolium squarrosum* (?)	10.3	10.3
	T. resupinatum (Maral)	3.7	3.7
Perennials	*T. repens* (Klondike)	1.2	0.7
	T. pratense (Taifun)	3.5	2
	Medicago lupulina (Virgo Pajberg)	2.8	1.6
Winter annuals	*T. incarnatum* (Kardinal)	-	9.5
	Vicia villosa (Villana)	-	15

Table S1.3. Timing and details on field operations, in chronological order, in the four experiments. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018

Field operation	**SK1**	**SK2**	**OG1**	**OG2**
Soil cultivation	2017-04-27	2018-04-03	2017-05-02	2017-05-08
Fertiliser*, oats	2017-04-22	2018-04-03	2017-04-05	2018-05-18
	80 kg N ha^{-1} (Biofer) 10-3-1 (% N, P and K)	130 kg N ha^{-1} chicken manure	60 kg N ha^{-1} (Ekoväx) 8-3-5-3 (% N. P, K and S)	60 kg N ha^{-1} (Ekoväx) 8-3-5-3 (% N. P, K and S)
Roller	-	2018-04-10	2017-05-07	2017-05-21
Sowing, oats	2017-04-28	2018-04-14	2017-05-05	2018-05-21
Sowing density, cultivar	160 kg ha^{-1}, Nike	180 kg ha^{-1}, Haga	131 kg ha^{-1}, Galant (SW 051020)	200 kg ha^{-1}, Galant (SW 051020)
Sowing, SC in oat rows	2017-04-28	2018-04-14	2017-05-05	2018-05-21
Sowing, SC between and adjacent to oat rows	2017-06-02	2018-05-23	2017-06-19	2018-06-02
Row hoeing 1	2017-06-02	2018-05-23	2017-06-19	2018-06-02
Row hoeing 2	2017-06-27	-**	2017-07-07	2018-06-29
Harvest, oats	2017-09-03	2018-07-26	2017-08-24	2018-08-21
Straw removal	2017-09-06	-	2017-08-26	-
Mowing of SC	2017-09-25	2018-09-16	-	Just before harvest
Sowing, winter wheat, row hoeing 3	2017-09-28	2018-09-17	***	2018-09-21
Sowing density, cultivar	220 kg ha^{-1}, Stava	220 kg ha^{-1}, Stava		221 kg ha^{-1}, Stava
Mechanical termination of SC, row hoeing 4	2018-05-09	2019-04-15		2019-04-30
Fertiliser, winter wheat	2018-04-28	2019-04-15		2019-04-26
	130 kg N ha^{-1} Pig manure, liquid	70 kg N ha^{-1} (Biofer) 10-3-1 (%N, P and K)		100 kg N ha^{-1} Ekoväx, 8-3-5-3 (% N. P, K and S)
Row hoeing 5	-**	2019-05-07		2019-05-31
Row hoeing 6	-**	2019-05-29		-
Harvest, winter wheat	2018-07-31	2019-08-26		2019-08-26

*Biofer: pelleted chicken manure and meat flour, Ekoväx: meat flour and yeast residues

**In 2018 the second row hoeing was excluded due to high risk of affecting the main crop negatively and very few weeds.

***In 2017 it was too wet in autumn to sow winter wheat in no-till plots, instead spring wheat was sown in 2018.

Table S1.4. Dates for collection of data in the four field experiments. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018

Data collected	**SK1**	**SK2**	**OG1**	**OG2**
Plant counts oats				
Plants	2017-05-19	2018-05-11	2017-06-01	2018-06-18
Tillers	2017-06-15	2018-05-30	-	2018-06-29
Heads	2017-07-27	2018-06-18	2017-07-25	2018-07-27
Weed and SC$^{\pm}$ counts	2017-05-22/23 2017-05-17/19$^{\pm}$	2018-05-15/16 2018-07-25$^{\pm}$	2017-06-05/06 2017-08-09/10$^{\pm}$	2018-06-04/06 2018-08-08$^{\pm}$
Biomass harvest 1*	2017-07-17/19	2018-07-25	2017-08-09/10	2018-08-08
Oat harvest	2017-09-03	2018-07-26	2017-08-23	2018-08-21
δ^{15}N sampling	2017-10-31	2018-10-28	2017-11-05/08	2018-10-22/23
SC soil cover	2017-10-31	2018-10-28	2017-11-05/08	2018-10-22/23
Soil mineral nitrogen				
Autumn	2017-11-22	2018-10-31	2017-11-08	2018-12-18
Spring	-	2019-04-12	-	2019-04-29
Plant counts wheat				
Plants	2017-10-19	2018-10-02	2018-06-05	2018-11-23
Tillers	2018-05-02	2019-05-02	2018-06-30	2019-05-20
Heads	2018-06-04	2019-07-01	2018-07-13	2019-05-20
Weed counts	2018-07-26	2019-07-29	2018-08-07/08	2019-08-05
Biomass harvest 2**	2018-07-26	2019-07-29	2018-08-07 – 2018-08-08	2019-08-05
Wheat harvest	2018-07-31	2019-08-26	10-08-2018	2019-08-26

*Main crop, service crop and weeds

**Main crop and weeds

S2. Supplementary material to results – ANOVA tables

Table S2.1. ANOVA table for the analyses of service crop (SC) establishment, biomass in oats and soil cover in autumn. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy Early Intra and Late Inter, and all three experiments (SK1, SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs and system strategy Late Adjacent. Here only the OG2 experiment was analysed. See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF**	**DF**	**Establishment**		**Biomass**		**Soil cover**	
	S &P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**
SC mixture	1	2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
System strategy	1	1	< 0.001	0.0081	< 0.001	< 0.001	< 0.001	0.0013
Experiment	3	1	< 0.001	0.17	< 0.001	< 0.001	< 0.001	0.16
Mixture*System	1	2	0.91	0.63	0.62	< 0.001	< 0.001	0.0022
Mixture*Experiment	3	2	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
System*Experiment	3	1	< 0.001	< 0.001	< 0.001	< 0.001	0.0030	< 0.001
Mixture*System*Experument	3	2	< 0.001	< 0.001	< 0.001	< 0.001	0.020	0.011

Table S2.2. ANOVA table for the analyses % nitrogen derived from air in the service crop (SC) species. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy Early Intra and Late Inter, and all three experiments (SK1, SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs and system strategy Late Adjacent. Here only the OG2 experiment was analysed. See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF**	**DF**	**Species**		**DF**	**DF**	**Species and system strategy**	
	S & P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**
Species	4	6	< 0.001	< 0.001	4	5	< 0.001	< 0.001
System	-	-	-	-	1	1	0.62	0.67
Experiment§	3	1	< 0.001	< 0.001	1	-	< 0.001	-
Species*System	-	-	-	-	4	5	0.23	< 0.001
Species*Experiment	11	5	< 0.001	0.010	3	-	< 0.001	-
System*Experiment	-	-	-	-	1	-	0.58	-
Species*System*Experiment	-	-	-	-	3	-	0.012	-

§For the analysis of both species and system strategy only data from the experiments SK2 and OG2 were used.

Table S2.3. ANOVA table of winter wheat grain yield, nitrogen yield and nitrogen concentration. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy Early Intra and Late Inter, and all three experiments (SK1, SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs and system strategy Late Adjacent. Here only the OG2 experiment was analysed. See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF**	**DF**	**Grain yield**		**Nitrogen yield**		**Nitrogen concentration**	
	S &P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**
SC mixture	2	3	0.068	0.72	0.57	0.0060	0.52	< 0.001
System strategy	1	2	0.98	< 0.001	0.37	< 0.001	0.15	0.16
Experiment	2	-	< 0.001	-	< 0.001	-	< 0.001	-
Mixture*System	2	6	0.81	< 0.001	0.82	< 0.001	0.25	0.38
Mixture*Experimet	4	-	0.0035	-	< 0.001	-	0.068	-
System*Experiment	2	-	0.94	-	0.96	-	0.76	-
Mixture*System*Experiment	4	-	0.80	-	0.93	-	0.0019	-

Table S2.4. ANOVA table of winter wheat plants, tillers, heads, heads plant^{-1}, kernels head^{-1} and thousand kernel weight^{-1}. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy Early Intra and Late Inter, and all three experiments (SK1, SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs and system strategy Late Adjacent. Here only the OG2 experiment was analysed. See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF**	**DF**	**Plants**		**Tillers**		**Heads**		**Heads plant^{-1}**		**Kernels head^{-1}**		**Thousand kernel weight**	
	S &P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**
SC mixture	2	3	0.42	0.018	0.55	0.027	< 0.001	0.18	0.17	0.0022	0.34	0.30	0.51	0.0062
System strategy	1	2	0.58	0.083	0.86	0.89	0.54	0.85	0.81	0.22	0.89	0.28	0.77	0.84
Experiment	2	-	< 0.001	-	< 0.001	-	< 0.001	-	< 0.001	-	< 0.001	-	< 0.001	-
Mixture*System	2	6	0.77	0.028	0.83	0.36	0.90	0.43	0.72	0.56	0.52	0.16	0.13	0.082
Mixture*Experiment	4	-	0.19	-	0.25	-	< 0.001	-	0.60	-	0.0067	-	0.72	-
System*Experiment	2	-	0.48	-	0.78	-	0.51	-	0.81	-	0.64	-	0.38	-
Mixture*System*Experiment	4	-	0.39	-	0.46	-	0.57	-	0.60	-	0.70	-	0.45	-

Table S2.5. ANOVA table of oat yield, plants, tillers, heads, heads plant^{-1}, kernels head^{-1} and thousand kernel weight^{-1}. The analysis was divided into two steps (here reported as S & P, and S, P & W). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy Early Intra and Late Inter, and all three experiments (SK1, SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs and system strategy Late Adjacent. See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF**	**DF**	**Yield**		**Plants**		**Tillers**		**Heads**		**Heads plant^{-1}**		**Kernels head^{-1}**		**Thousand kernel weight**	
	S &P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**
SC mixture	2	3	0.83	< 0.001	0.34	0.12	0.14	0.97	0.99	0.79	0.64	0.41	0.78	0.14	0.76	0.85
System strategy	1	2	0.10	0.26	0.72	0.81	0.59	0.91	0.74	0.84	0.77	0.91	0.98	0.40	0.06	0.16
Experiment	3	1	< 0.001	< 0.001	< 0.001	0.47	< 0.001	-§	< 0.001	0.89	< 0.001	0.33	< 0.001	0.20	< 0.001	< 0.001
Mixture*System	2	6	0.47	0.0057	0.76	0.54	0.35	0.68	0.56	0.34	0.74	0.22	0.73	0.58	0.36	0.38
Mixture*Experiment	6	3	0.0030	0.73	0.46	0.16	0.44	-	0.62	0.79	0.98	0.42	0.47	0.89	0.69	0.73
System*Experiment	4	2	0.018	0.071	0.21	0.37	0.33	-	0.069	0.44	0.34	0.20	0.32	0.28	0.60	0.72
Mixture*System*Experiment	6	6	0.65	0.33	0.58	0.88	0.77	-	0.59	0.70	0.65	0.89	0.64	0.92	0.031	0.37

§this data was only available from OG2

Table S2.6. ANOVA table of weed biomass in oats and winter wheat. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) service crops (SCs) and no SC as SC mixture levels and system strategy Early Intra and Late Inter, in oats with all four experiments. S, P & T also included treatments with frost tolerant annual (T) SCs which was present only in OG. See Materials and method for and Table 1 for treatment explanations

Effect	**DF** S & P	**DF** S, P & T	**In oats**		**In winter wheat**	
			S & P	**S, P & T**	**S & P**	**S, P & T**
SC mixture	2	3	0.072	0.027	0.0022	0.018
System strategy	1	1	0.61	0.53	0.97	0.19
Experiment	3 (2)§	1 (-)**	< 0.001	0.28	< 0.001	-
Mixture*System	2	3	0.75	0.70	0.16	0.90
Mixture*Experiment	6 (4)	3 (-)	0.74	0.96	0.0023	-
System*Experiment	3 (2)	1 (-)	0.41	0.73	0.96	-
Mixture*System*Experiment	6 (4)	3 (-)	0.26	0.52	0.011	-

§In winter wheat only SK1, SK2 and OG2 was included in the analysis.

**In winter wheat only OG2 was included in the analysis.

Table S2.7. ANOVA table of weed biomass in different subsections of the oats and winter wheat plots. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) service crops (SCs) and no SC as SC mixture levels and system strategy Early Intra and Late Inter, in oats with all four experiments. S, P & T also included treatments with frost tolerant annual (T) SCs which was present only in OG. See Materials and method for and Table 1 for treatment explanations

In oats	**DF**	**DF**	**S & P**			**S, P & T**		
Effect	**S & P**	**S, P & T**	**ir**	**cr**	**ic**	**ir**	**cr**	**ic**
SC mixture	2	3	0.4	0.1	0.8	0.4	0.07	0.05
System strategy	1	1	0.9	0.6	0.001	0.9	0.9	0.4
Experiment	3	1	0.05	0.003	< 0.001	0.9	0.3	0.04
Mixture*System	2	3	0.4	0.5	0.2	0.2	0.6	0.2
Mixture*Experiment	6	3	0.5	0.7	0.9	0.6	0.8	0.6
System*Experiment	3	1	0.3	0.9	0.02	0.2	0.6	0.2
Mixture*System*Experiment	6	3	0.2	0.4	0.009	0.4	0.2	0.05

In winter wheat§	**DF**	**DF**	**S & P**			**S, P & T**		
Effect	**S & P**	**S, P & T**	**ir**	**cr**	**ic**	**ir**	**cr**	**ic**
SC mixture	2	3	0.004	0.08	0.8	0.05	0.1	0.7
System strategy	1	1	0.08	0.7	0.3	0.7	0.08	0.2
Experiment	2	-	< 0.001	< 0.001	< 0.001			
Mixture*System	2	3	0.1	0.8	0.2	0.3	0.5	0.4
Mixture*Experiment	4	-	< 0.001	0.2	0.4			
System*Experiment	2	-	0.005	0.8	0.5			
Mixture*System*Experiment	4	-	0.01	0.06	0.9			

§In winter wheat only SK1, SK2 and OG2 was included in the analysis for S & P, and only OG2 for S, P and T.

Table S2.8. ANOVA table of soil mineral nitrogen (SMN) in autumn at three different depths (0-30, 30-60 and 60-90 cm) and in the whole soil profile. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy Early Intra and Late Inter, and all three experiments (SK1, SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs. See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF**	**DF**	**0-30 cm**		**30-60 cm**		**60-90 cm**		**0-60 cm§**	**0-90 cm**	**0-90 cm**
	S & P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S & P**	**S, P & T**
SC mixture	2	3	0.22	0.44	0.16	0.89	0.18	0.30	0.21	0.21	0.55
System strategy	1	1	0.69	0.33	0.94	0.57	0.98	0.40	0.99	0.74	0.44
Experiment	3 (2)**	1	0.0024	< 0.001	0.033	0.026	0.45	0.18	< 0.001	< 0.001	< 0.001
Mixture*System	2	3	< 0.001	< 0.001	0.040	0.028	0.52	0.66	0.0019	0.0024	0.0083
Mixture*Experiment	6 (4)	3	0.81	0.67	0.12	0.49	0.046	0.35	0.46	0.099	0.55
System*Experiment	3 (2)	1	0.28	0.49	0.76	0.65	0.76	0.28	0.42	0.45	0.15
Mixture*System*Experiment	6 (4)	3	0.58	0.19	0.70	0.69	0.26	0.22	0.59	0.57	0.63

§60-90 cm could not be sampled at SK2 due to a large amount of stones and hence only the two first layers were included in this analysis.

**Numbers in parenthesis are DF for the depth 60-90 cm where there is only data from SK1, OG1 and OG2.

Table S2.9. ANOVA table of soil mineral nitrogen (SMN) in spring at three different depths (0-30, 30-60 and 60-90 cm) and in the whole soil profile. Only the SK2 and OG2 experiment were included in the analysis. The analysis was divided into two steps (here reported as S & P, and S, P & T). S & P included treatments with frost sensitive annual (S), perennial (P) SCs and no SC as SC mixture levels and system strategy A and B, and both experiments (SK2 and OG2). S, P & T also included treatments with frost tolerant annual (T) SCs (only OG2). See Materials and method for detailed treatment explanations and table 1 for treatment overview

Effect	**DF** S & P	**DF** S, P & T	**0-30 cm**		**30-60 cm**		**60-90 cm**		**0-90 cm**	
			S & P	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**	**S & P**	**S, P & T**
SC mixture	2	3	0.19	0.40	0.98	0.71	0.039	0.39	0.13	0.52
System strategy	1	1	0.48	0.54	0.95	0.53	0.92	0.87	0.57	0.45
Experiment	1	-	0.020	-	< 0.001	-	< 0.001	-	< 0.001	-
Mixture*System	2	3	0.60	0.37	0.046	0.33	0.34	0.32	0.67	0.20
Mixture*Experiment	2	-	0.82	-	0.66	-	0.85	-	0.96	-
System*Experiment	1	-	0.042	-	0.20	-	0.35	-	0.010	-
Mixture*System*Experiment	2	-	0.81	-	0.94	-	0.35	-	0.40	-

S3. Supplementary material to results – result tables

Table S3.1. Service crop establishment [plants m^{-2}] for the different treatments in the four experiments. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, S = frost sensitive annuals, P = perennials, T = frost tolerant annuals. S = frost sensitive annual service crops (SCs), P = perennial SCs, T = frost tolerant annual SCs. Early Intra = early sowing of SCs in crop rows and Late Inter = late sowing of SCs in inter row centres. See Materials and method for detailed treatment explanations and table 1 for treatment overview. Means are back-transformed from square-root model estimates, CI denotes the 95% confidence interval and significance letters indicate the significances between treatments within experiments

System strategy	**Service crop**	**SK1**			**OG1**		
		Mean	**CI**	**Sign**	**Mean**	**CI**	**Sign**
Early Intra	*S*	139	102-182	b	65	33-107	ab
	P	160	114-215	ab	31	11-59	b
	T				49	22-86	b
Late Inter	*S*	216	154-287	a	127	83-182	a
	P	190	147-237	ab	31	5-79	b
	T				75	34-132	ab
		SK2			**OG2**		
		Mean	**CI**	**Sign**	**Mean**	**CI**	**Sign**
Early Intra	*S*	14	5-28	b	83	48-129	a
	P	37	20-59	a	16	3-39	bc
	T				89	50-138	a
Late Inter	*S*	0*	0*	c	5	0-20	c
	P	0*	0*	c	6	0-22	bc
	T				31	10-62	b

*In 2018 the emergence of frost sensitive annuals and perennials was delayed in both experiments due to the dry summer. At the time of the last plant counting very few plants had emerged. However, in august after rain had come the SC plants emerged also in these treatments.

Table S3.2. Stand development (number of plants, tillers and heads) and harvest components (heads plant^{-1}, kernels head^{-1} and thousand kernel weight), mean and 95% confidence interval (CI), in oats in the four experiments. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, S = frost sensitive annuals, P = perennials, T = frost tolerant annuals. S = frost sensitive annual service crops (SCs), P = perennial SCs, T = frost tolerant annual SCs. Early Intra = early sowing of SCs in crop rows, Late Inter = late sowing of SCs in inter row centres and Late Adjacent = late sowing of SCs close to crop row. See materials and methods and table 1 for information on treatments

		Plants								**Tillers**								**Heads**							
System strategy	**Service crop**	**SK1**		**SK2**		**OG1**		**OG2**		**SK1**		**SK2**		**OG1**		**OG2**		**SK1**		**SK2**		**OG1**		**OG2**	
		Mean	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**
Early Intra	*None*	274	±32	163	±32	229	±28	218	±25	402	±44	294	±25			290	±47	325	±36	111	±35	305	±41	281	±37
	S	268	±30	166	±30	222	±28	228	±25	383	±42	275	±24			284	±47	296	±34	143	±33	300	±41	313	±37
	P	258	±32	164	±30	227	±28	221	±25	386	±43	288	±24			301	±47	308	±36	138	±33	320	±41	298	±37
	T					237	±28	261	±25							278	±47					295	±41	289	±37
Late Inter	*None*	317	±31	172	±30	237	±28	209	±25	427	±42	293	±24			283	±47	335	±35	141	±33	305	±41	296	±37
	S	269	±31	163	±30	230	±28	224	±25	399	±42	282	±24			283	±47	338	±35	131	±33	297	±41	291	±37
	P	260	±30	167	±30	233	±28	216	±25	369	±41	271	±24			270	±47	315	±33	137	±33	262	±41	295	±37
	T					238	±28	243	±25							299	±47					298	±41	320	±37
Late Adjacent	*None*					228	±28	214	±25							287	±47					313	±41	308	±37
	S					219	±28	231	±25							280	±47					284	±41	285	±37
	P					251	±28	220	±25							289	±47					291	±41	297	±37
	T					231	±28	219	±25							285	±47					317	±46	290	±37

		Heads plant^{-1}								**Kernels head^{-1}**								**Thousand kernel weight**							
		SK1		**SK2**		**OG1**		**OG2**		**SK1**		**SK2**		**OG1**		**OG2**		**SK1**		**SK2**		**OG1**		**OG2**	
		Mean	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**
Early Intra	*None*	1,2	±0,193	0,707	±0,19	1,34	±0,2	1,33	±0,18	46,4	±8,7	33,1	±8,5	47	±7,9	46	±7,1	32	±1,5	26,9	±1,5	34,2	±2	28,5	±1,8
	S	1,11	±0,183	0,859	±0,18	1,35	±0,2	1,39	±0,18	45,4	±8,2	29,9	±8,1	50,1	±7,9	44,5	±7,1	31,1	±1,4	26,9	±1,4	34	±2	28,6	±1,8
	P	1,209	±0,192	0,851	±0,18	1,42	±0,2	1,35	±0,18	45,5	±8,7	30,5	±8,1	43,2	±7,9	44,3	±7,1	31,7	±1,4	26,6	±1,4	35,9	±2	28,2	±1,8
	T					1,25	±0,2	1,1	±0,18					40,3	±7,9	40,1	±7,1					34,4	±2	27,2	±1,8
Late Inter	*None*	1,075	±0,187	0,816	±0,18	1,29	±0,2	1,43	±0,18	47,4	±8,5	31,5	±8,1	47,7	±7,9	42,8	±7,1	31,9	±1,4	26,7	±1,4	36	±2	28,7	±1,8
	S	1,279	±0,187	0,795	±0,18	1,29	±0,2	1,36	±0,18	42,7	±8,4	28,8	±8,1	49,3	±7,9	45,3	±7,1	32,1	±1,4	27,3	±1,4	34,5	±2	28,5	±1,8
	P	1,235	±0,181	0,82	±0,18	1,15	±0,2	1,38	±0,18	47,7	±8,1	22,3	±8,1	54,8	±7,9	46,8	±7,1	32,3	±1,4	26,6	±1,4	34,9	±2	28,7	±1,8
	T					1,26	±0,2	1,31	±0,18					47,5	±7,9	39,2	±7,1					34,4	±2	29,3	±1,8
Late Adjacent	*None*					1,37	±0,2	1,44	±0,18					44,4	±7,9	46,1	±7,1					35,5	±2	28,5	±1,8
	S					1,3	±0,2	1,24	±0,18					49,8	±7,9	45,9	±7,9					35,6	±2	29,1	±1,8
	P					1,16	±0,2	1,37	±0,18					48,4	±7,9	44,6	±7,1					34,6	±2	28,4	±1,8
	T					1,4	±0,2	1,34	±0,18					45,5	±9,1	45,9	±7,1					35,4	±2	28,7	±1,8

Table S3.3. Stand development (number of plants, tillers and heads) and harvest components (heads plant^{-1}, kernels head^{-1} and thousand kernel weight), mean and 95% confidence interval (CI), in oats in the four experiments. Abbreviations: SK and OG stands for the two experimental regions, 1 = starting 2017, 2 = starting 2018, S = frost sensitive annuals, P = perennials, T = frost tolerant annuals. S = frost sensitive annual service crops (SCs), P = perennial SCs, T = frost tolerant annual SCs. Early Intra = early sowing of SCs in crop rows, Late Inter = late sowing of SCs in inter row centres and Late Adjacent = late sowing of SCs close to crop row. See materials and methods and table 1 for information on treatments

		Plants						**Tillers**						**Heads**					
		SK1		**SK2**		**OG2**		**SK1**		**SK2**		**OG2**		**SK1**		**SK2**		**OG2**	
System strategy	**Service crop**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**
Early Intra	*None*	217	±25	179	±25	151	±17	132	±46	182	±46	270	±34	268	±45	166	±45	270	±35
	S	233	±25	178	±26	152	±17	134	±46	182	±46	255	±35	314	±45	154	±45	286	±35
	P	211	±25	168	±26	163	±17	125	±46	160	±46	292	±35	184	±45	131	±45	301	±35
	T					122	±17					231	±35					285	±35
Late Inter	*None*	223	±25	176	±26	148	±17	138	±46	180	±46	279	±35	277	±45	144	±45	282	±35
	S	245	±25	166	±26	150	±17	147	±46	160	±46	244	±34	305	±45	152	±45	266	±35
	P	196	±25	189	±26	138	±17	116	±46	186	±46	269	±35	213	±45	123	±45	261	±35
	T					154	±17					246	±35					307	±35
Late Adjacent	*None*					161	±17					266	±35					262	±35
	S					165	±17					294	±34					290	±35
	P					168	±17					267	±35					278	±35
	T					139	±17					235	±35					309	±35

		Heads plant^{-1}						**Kernels head^{-1}**						**Thousand kernel weight**					
		SK1		**SK2**		**OG2**		**SK1**		**SK2**		**OG2**		**SK1**		**SK2**		**OG2**	
		Mean	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**	**Mean**	**CI**
Early Intra	*None*	1,281	±325	0,928	±325	1,8	±34	17,1	±10,1	44,8	±10,1	31	±4,8	30,9	±1,6	35	±1,6	40,2	±0,9
	S	1,338	±325	0,882	±325	2	±34	22,4	±10,1	49,6	±10,1	27,8	±4,8	31,2	±1,6	35,1	±1,6	38,9	±0,9
	P	0,857	±325	0,813	±325	1,88	±34	36,4	±10,1	35,7	±10,1	28,7	±4,8	32,2	±1,7	35	±1,6	40,1	±0,9
	T					2,4	±34					31,8	±4,8					41	±0,9
Late Inter	*None*	1,243	±325	0,821	±325	1,92	±34	17,3	±10,1	49,4	±10,1	29,2	±4,8	31,2	±1,6	33,4	±1,6	40,5	±0,9
	S	1,246	±325	0,924	±325	1,78	±34	18	±10,1	43,5	±10,1	28,8	±4,8	32,4	±1,6	35,9	±1,6	39	±0,9
	P	1,123	±325	0,672	±325	1,9	±34	33,5	±10,1	46,7	±10,1	29,9	±4,8	30,2	±1,6	34	±1,6	40,3	±0,9
	T					1,99	±34					22,2	±4,8					39,6	±0,9
Late Adjacent	*None*					1,67	±34					29,6	±4,8					39,7	±0,9
	S					1,77	±34					29,4	±4,8					39,8	±0,9
	P					1,65	±34					28,8	±4,8					39,5	±0,9
	T					2,24	±34					26,5	±4,8					40,6	±0,9

Table S3.4. Weed biomass [g m^{-2}] in oats and winter wheat before cash crop harvest in each experiment. Abbreviations: SK and OG stands for the two experimental locations, SC = service crop, S = summer annuals = P perennials, W = winter annuals. No data is presented for winter wheat in OG 2018 since spring wheat was grown here this year. See Materials and method for detailed treatment explanations and table 1 for treatment overview. Variation is given by the 95% confidence interval (CI)

In oats		**SK1**			**SK2**			**OG1**			**OG2**		
System strategy	**Service crop**	**Mean**	**CI**	**Sign**	**Mean**	**CI**	**Sign**	**Mean**	**CI**	**Sign**	**Mean**	**CI**	**Sign**
Early Intra	*No SC*	3.1	0.49-19	a	2.2	0.54-8.6	a	2.6	0.23-30	a	3.7	0.44-32	a
	S	3.3	0.60-18	a	0.93	0.24-3.7	a	1.9	0.22-16	a	10	1.4-74	a
	P	5.1	0.84-31	a	0.84	0.21-3.4	a	7.3	0.96-55	a	33	4.2-260	a
	W						a	1.0	0.12-9.2	a	6.9	0.84-56	a
Late Inter	*No SC*	4.5	0.84-24	a	0.35	0.086-1.4	a	2.0	0.22-18	a	21	2.6-170	a
	S	3.6	0.42-32	a	1.1	0.28-4.6	a	5.2	0.66-41	a	17	2.4-120	a
	P	3.2	0.78-13	b	1.4	0.36-5.8	a	3.6	1.6-80	a	31	4.4-220	a
	W							1.6	1.2-22	a	3.6	0.43-29	a

In winter wheat		**SK1**			**SK2**			**OG1**			**OG2**		
System strategy	**Service crop**	**Mean**	**CI**	**Sign**	**Mean**	**CI**	**Sign**				**Mean**	**CI**	**Sign**
Early Intra	*No SC*	0.029	0.0013-0.62	a	130	6.1-290	a				88	46-140	a
	S	1.7	0.080-37	a	150	6.8-320	a				150	91-220	a
	P	0.031	0.0014-0.67	a	31	1.4-67	a				79	40-130	a
	W						a				66	31-120	a
Late Inter	*No SC*	8.4	0.39-180	a	120	5.7-260	a				59	26-110	a
	S	4.2	0.20-91	a	78	3.6-170	a				110	62-170	a
	P	0.000040	0-0.00090	a	120	5.4-250	a				76	37-130	a
	W										54	22-99	a

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Cropping systems have to rely less on external nutrients and intensive weed management, and ensure ecosystem services that support long-term soil functioning. Including leguminous service crops in cropping systems has potential to contribute to these goals if enough biomass is produced. At high latitudes short growing seasons makes this difficult, if the proportion of main crops should be maintained. In this thesis the impacts of intercropped service crops and main crops on main crop performance and other services were assessed.

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