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Testing interactions between above- and belowground ecosystem services

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Table of contents

Summary

Agriculture is the predominant form of land management worldwide. During the last fifty years production intensification dramatically impacted the environment and biodiversity harming the ability of ecosystems to provide services essential for human sustenance and well-being. Agricultural ecosystems receive a wide variety of supporting and regulating services crucial for crop production (e.g. soil fertility or pollination). The enhancement of ecosystem services (ecological intensification) has been proposed as a promising solution to meet the growing demand for agricultural goods, decreasing production dependence to external inputs and therefore limiting detrimental effects on environment. However, the implementation of ecological intensification into crop production systems is knowledge-intensive and it requires a comprehensive understanding of the relationships among multiple ecosystems services.

The aim of this thesis was to test for potential interactions between above- and belowground ecosystem services for major European crops and to identify innovative management options for ecological intensification in agricultural ecosystems. We explored the relationships between soil-based supporting services and pollination, biological control and an aboveground crop pest (disservice).

In Chapter 2, the interactions between pollination and nitrogen input for three different cultivars of oilseed rape (*Brassica napus*) were explored in a field experiment. Open pollinated cultivar largely depended on pollination, whereas hybrid cultivars did not. However, pollination benefits to yield increased with decreased nitrogen inputs, indicating that nitrogen use efficiency was probably enhanced by pollination. These findings show that the use of hybrid cultivars and the application of fertilizers can compensate degraded pollination service, whereas optimal pollination can potentially replace fertilization.

In Chapter 3 we tested potential interactions between soil fertility and pollination in sunflower (*Helianthus annuus*). To obtain different levels of soil fertility we used soils from a long-term trial with different input management histories. Pollination benefits to yield formation were observable only under good soil fertility conditions whereas they were canceled in more deteriorated soils: limited nutrient availability led to limited yield even at optimal pollination level.

In Chapter 4 we performed a greenhouse experiment to test the effects of soil fertility and nitrogen input on herbivore performance (*Sitobion avenae*) and crop growth (*Triticum aestivum*) . Increased fertilizer applications strongly enhanced aphid performance. Increased soil organic matter contents had similar but minor effects on pest growth, maintaining similar crop growth.

Soil management is known to affect the provision of multiple ecosystem services. In Chapter 5 and 6 the effects of different tillage practices indicated that soil managements that reduce soil disturbance in cereal crops enhance soil fertility, arthropod abundance, biological control and maintain levels of crop production similar to those observable under conventional tillage.

This study provides evidence for interactions between above- and belowground ecosystem services, highlighting the role of supporting services in shaping the effects of other services-disservices on crop production. Our results will provide recommendations to correctly valuate ecosystem services and to implement their management in agricultural landscapes. In particular it appears clear that several ecosystem services do interact and that their contribution to yield cannot be considered additive as often assumed in current economic evaluation and mapping exercises.

Riassunto

L'agricoltura è la forma predominante di gestione del suolo a livello globale. Negli ultimi cinquanta anni l'intensificazione della produzione agricola ha causato un generale deperimento della qualità dell'ambiente e un'allarmante perdita di biodiversità, compromettendo la capacità degli ecosistemi di fornire servizi essenziali al sostentamento ed al benessere umano. Gli ecosistemi agricoli sono condizionati da molteplici servizi di supporto e di regolazione fondamentali al processo di produzione (ad es. servizio di fertilità del suolo o di impollinazione). L'intensificazione ecofunzionale è una nuova strategia di gestione che si propone di raggiungere adeguati livelli di produttivà e di limitare gli impatti ambientali potenziando i servizi ecosistemici coinvolti nel processo di produzione. Ciò nonostante, l'attuazione di questa strategia necessita di una approfondita conoscenza dei meccanismi di interazione fra i diversi servizi ecosistemici.

Il presente lavoro di tesi si propone di esplorare possibili interazioni fra servizi ecosistemici ipogei ed epigei in alcune colture agrarie di importanza economica e di identificare innovative tecniche di gestione nell'ottica di un'intensificazione ecofunzionale. In particolare abbiamo testato le potenziali interazioni fra qualità del suolo, impollinazione e controllo biologico.

Nel Capitolo 2 si presentano i risultati di un esperimento di campo sulle relazioni fra impollinazione e fertilizzazione minerale in tre varietà di colza. Le tre varietà hanno prensentato diversa dipendenza dalla presenza di impollinazione entomofila. La varietà più produttiva ha incrementato la resa del 20% in presenza di insetti rispetto alle piante esposte solo all'azione del vento. L'effetto positivo dell'impollinazione era però presente solo nelle piante non sottoposte a fertilizzazione evidenziando un aumento nell'efficienza dell'uso dell'azoto dovuto all'azione di insetti impollinatori. Questi risultati suggeriscono che l'uso di fertilizzanti può compensare la riduzione di produzione dovuta alla mancanza di impollinatori.

Nel Capitolo 3 sono state studiate le relazioni fra servizio di impollinazione e servizio di fertilità del suolo nel girasole. Per ottenere livelli contrastanti di fertilità, sono stati utilizzati dei suoli provenienti da un esperimento di lungo termine sottoposti negli ultimi quaranta anni a diverse intensità di gestione. L'effetto positivo dell'impollinazione era evidente solo nelle piante cresciute nei suoli più fertili. La

limitata disponibilità di nutrienti ha infatti limitato la produzione indipendentemente dalla presenza di impollinatori. Questo risultato contrasta con quanto trovato per il colza indicando un'elevata complessità delle interazione fra impollinazione e qualità del suolo a seconda della coltura agraria considerata.

Nel Capitolo 4 si presentano gli effetti della fertilità del suolo e della fertilizzazione minerale sulla crescita degli afidi e sulla produzione di frumento. Alti livelli di fertilizzazione hanno fortemente accresciuto la performance degli afidi. L'effetto di un'alto contenuto di sostanza organica nel suolo è risultato simile ma di minore intensità, sostenendo una produzione paragonabile a quella raggiunta grazie alle fertilizzazioni.

Le lavorazioni del suolo influiscono grandemente sulla erogazione di servizi ecosistemici. Nel Capitolo 5 e 6 sono stati studiati gli effetti di diverse tecniche di lavorazione del suolo su diversi servizi ecosistemici: I campi gestiti con la tecnica di minima lavorazione del suolo hanno mostrato maggiori livelli di fertilità, una maggiore abbondanza di artropodi terricoli, un controllo biologico più efficiente e rese paragonabili a quelle ottenute con un'aratura convenzionale.

Il presente studio dimostra che servizi ecosistemici ipogei ed epigei interagiscono e che l'effetto dei servizi di regolazione come impollinazione e controllo biologico dipendono largamente dalla qualità dei servizi di supporto. I risultati di questa tesi forniscono delle linee guida per una corretta valutazione e gestione dei diversi servizi ecosistemici in agricoltura. Gli attuali modelli di valutazione economica dei servizi ecosistemici devono tenere in considerazione gli effetti non additivi dei singoli servizi. L'identità della coltura sembra avere un ruolo chiave nel modificare le interazioni osservate in questo studio.

Chapter 1

Introduction

Chapter 1

Ecosystem services

Humanity has always depended on nature for its survival and welfare. The Millennium Ecosystem Assessment (2005) defined ecosystem services as the benefits that people obtain from ecosystems and classified them into four main categories: supporting, provisioning, regulating and cultural services (Fig.1). Provisioning services are the products that people obtain from ecosystems, such as food, fuel, fiber, fresh water, and genetic resources. Regulating services are the benefits people obtain from the regulation of ecosystem processes, including air quality maintenance, climate regulation, erosion control and water purification. Supporting services are those that are necessary for the production of all other ecosystem services, such as primary production, production of oxygen, and soil formation. Cultural services are the nonmaterial benefits people obtain from ecosystems through spiritual enrichment, cognitive development, reflection, recreation, and aesthetic experiences.

Since ecosystems are complex sets of interacting abiotic and biotic components, biodiversity is strictly linked to the provision of services. Biodiversity is in fact an important regulator of fundamental ecosystem processes that underpin multiple ecosystem services (Mace et al. 2012). For example the biological control of pests in many cropping systems is determined by the composition of predator communities (Cardinale et al. 2003). The alarming biodiversity loss caused by human activities has been broadly shown to alter the functioning of ecosystems and their capacity to provide services (Hooper et al. 2005, Balvanera et al. 2006, Cardinale et al. 2012). The present study focused on the potential interactions occurring between provisioning, supporting and regulating services in agricultural ecosystems.

Ecosystem services and disservices in agriculture

Agricultural ecosystems cover more than one third of the global land area (FAO 2009), roughly half of the land not classified as desert, rock or permafrost. Hence, agriculture is the principal form of land management worldwide. Agricultural ecosystems are mainly managed to maximize the provisioning services (e.g. food, fuel, fiber), but they also receive and provide a wider variety of ecosystem services (and disservices) that are, for the most part, underestimated, unmanaged and unvalued

(Swinton et al. 2007). However, agricultural productivity depends on many supporting and regulating services (e.g. soil fertility or pollination) that directly or indirectly sustain and shape the ability of cropping systems to provide goods. Agricultural ecosystems also receive disservices (e.g. damage by insect pests and pathogens) that can limit productivity or increase production costs. In addition to provisioning services, cropping systems can provide a range of supporting and regulating services such as the maintenance of soil fertility, water quality control and disease regulation (Power et al. 2010).
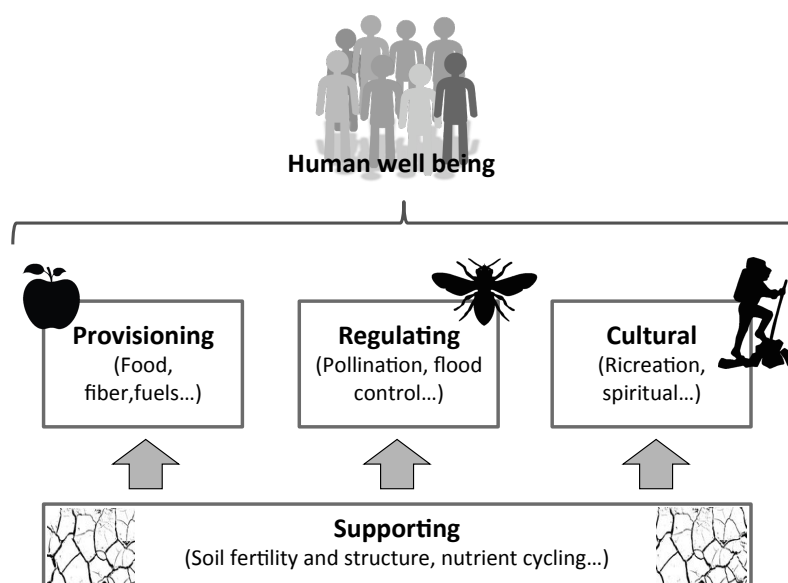

Fig. 1. Classification of ecosystem services from the Millennium Ecosystem Assessment (adapted from Zhang et al. 2007).

Management practices greatly influence the services and disservices flowing to and from agricultural ecosystems. The extensive land-use changes and the increased external inputs of energy and agrochemicals typical of modern agriculture are recognized as the main drivers of global biodiversity loss and related degradation of ecosystem services (e.g. Daily 1997, Schröter et al. 2005, Hooper et al. 2005). For example landscape simplification and fragmentation have been shown to harm pollination service (Klein et al. 2007), or high applications of nitrogen fertilizers to negatively affect soil biota activity (Tilman et al. 2001, Guo et al. 2010). However, agricultural management can also ameliorate many of the negative impacts of agriculture adopting, for example, conservation tillage in order to reduce soil disturbance (e.g. Holland 2004) enhancing floral resources and nesting sites to

promote pollinators (e.g. Carvell et al. 2011). The next paragraphs briefly describe the ecosystem services and disservices to agriculture that have been considered in the present study and their response to management practices.

Soil services

Soil is the foundation for all terrestrial ecosystems and it is defined as a multicomponent and multifunctional system (Kibblewhite 2008) characterized by interrelated physical, chemical and biological properties (e.g. texture, cation exchange capacity, microbial biomass and activity respectively). Fundamental soil services that sustain crop production include soil fertility and soil structure that influence the availability of nutrients to plants, water retention and erosion control (Zhang et al. 2007, Power et al. 2010). Soil biota plays a key role in the provision of these services. The activity of micro- and macro-invertebrates, fungi and bacteria affects soil porosity and aggregation enhancing water retention and it contributes to the degradation of animal and plant residues. Microorganisms support nutrient cycling through the decomposition of detrital organic matter and through nitrogen fixation (Barrios 2007). Intensive management practices such as powered tillage, repetitive harvesting of crops and inadequate nutrients replacement have been shown to degrade soil structure, fertility and the functioning of soil biota communities (Oldeman 1994, Giller et al. 1997, Postma-Blaauw et al. 2010). Moreover, the negative effects of agricultural intensification on soil ecosystem are demonstrated by the general decline in soil organic matter (Paul et al. 1996). Soil organic matter is often used as proxy for soil services (Magdoff and Weil 2004) because strongly related to good soil structure and porosity, water retention and nutrient availability (Bot and Benites 2005). However, management practices can also maintain and restore soil fertility and structure: the addition of organic inputs that enhances soil organic matter, the inclusion of perennial grasses and legumes in the crop rotation, the adoption of cover crops that limits soil nutrient runoff are examples of practices that preserve soil-based ecosystem services.

Insect pollination

Pollination is considered a key ecosystem service to agriculture supporting yield formation in 75% of all crop species (Klein et al. 2007). The global economic value of this service was estimated in 2005 to be US$215 billion representing the 9.5% of the global food production value (Gallai et al. 2009). Furthermore, many insect-pollinated crops are essential source of vitamins and micronutrients for human diet (Eilers et al. 2011). Although the honey bee (*Apis mellifera* L.) is the principal pollinator species used in cropping systems worldwide (Free 1993) wild insects have been shown to significantly contribute to crop pollination (Greenleaf et al. 2006, Garibaldi et al. 2013). Moreover, despite the strong increase in honeybee colonies worldwide, the demand for pollination service in agriculture is increasing owing to the rapid expansion of pollinator-dependent crop cultivation (Aizen and Harder 2009).

During the last decades a growing body of literature reported evidences for a global decline of both domesticated and wild pollinators (e.g. Hayes et al. 2008, Potts et al. 2010, Cameron et al. 2011). Among different potential drivers of pollinator decline (climate change, pest and pathogens, alien species; see Potts et al. 2010), agricultural intensification has been recognized as one of the most important (Brow and Paxton 2009). Habitat loss and fragmentation caused by land-use conversion to agriculture, negatively affect pollinator abundance and diversity, reducing forage sources and nesting sites (Ricketts et al. 2009, Steffan-Dewenter and Schiele 2010, Winfree et al. 2011). Furthermore, the increased use of pesticides in intensive cultivated crops has been shown to harm pollinators causing mortality by direct intoxication and behavioral changes by sub-lethal exposure to insecticides (Brittain et al. 2010, Cresswell 2011, Gill et al. 2012, Palmer et al. 2013). Several studies linked a higher proportion of natural and semi-natural habitats in the landscape to increased visitation rate, fruit-set, yield and stability of pollination service for many important crops (e.g. Greenleaf et al. 2006, Garibaldi et al. 2011, Klein et al. 2012). Local management strategies that maintain healthy pollinator communities include provisioning of additional nectar resources (e.g. flower stripes), reduced tillage and cultivation of mass flowering crops (Westphal et al. 2003, Williams et al. 2010, Carvalheiro et al. 2012)

Crop pests and biological control

Crop pests such as weeds, pathogens and herbivores, are recognized as one of the most important limiting factor to crop production. The yield loss to animal pests alone is estimated to be approximately the 10% of the total crop production worldwide. The application of chemical pesticides is the most common form of pest control and, although it has been increased 15-20 folds during the last 50 years, the overall yield losses are not decreasing (Oerke 2006). Moreover, the strong dependence on pesticide use had serious secondary consequences such as environmental pollution, human health issues, impact on non-target species, insecticide resistance and uneconomic crop production (Thomas 1999).

Natural pest control is an important ecosystem service supporting agricultural production, estimated to have an annual minimum value of US$400 billion per year worldwide (Costanza et al. 1998). Natural enemies such as predators, parasitoids and pathogens can effectively reduce pest damage (Losey and Vaughan 2006). For example insect natural enemies have been estimated to be responsible for the 50-90% of the pest control occurring in crop fields (Pimentel 2005) saving $4.5 billion per year in agricultural crops in the United States alone (Losey & Vaughan 2006, Power 2010). The provision of biological control service in agricultural ecosystems needs to be considered at both the field and the landscape scale. Semi-natural habitats in the landscape provide alternative food, hosts and winter refuges for a wide range of predators and parasitoids (Tscharntke et al. 2007). Several studies found that complex landscapes support more abundant and diverse communities of natural enemies (Bianchi et al. 2006, Chaplin-Kramer et al. 2011) and, in some cases, higher control of pest populations (Thies et al. 2005, Gardiner et al. 2009, Letourneau et al. 2009, Chaplin-Kramer et al. 2012). At the field scale, organic farming and crop rotation have been shown to enhance local biological control (Crowder et al. 2010, Rusch et al. 2013). Thus, landscape simplification, high pesticide use and intensive monoculture systems have the potential to constrain biological control service.

Ecological intensification

During the last 50 years modern agriculture greatly raised global crop production through land-use conversion, management intensification, increase of energy and agrochemical inputs and introduction of high-yielding and pest resistant crop varieties. Although agricultural intensification significantly contributed to feed the growing human population, it also dramatically impacted the environment and biodiversity harming the ability of ecosystems to provide services contributing to human sustenance and well-being (MA 2005). Moreover, evidence shows an arrest in the agricultural productivity increase for some important crops in several countries (Cassman et al. 2010). Nevertheless, global demand for food is steadily increasing and it is forecasted to double by 2050 (Tillman et al. 2011). Future agriculture needs to be more productive while preserving environment health (Godfray et al. 2010).

Several authors proposed ecological intensification as a promising strategy to meet the demand for agricultural goods, decreasing production dependence to external inputs and therefore limiting negative effects on environment (e.g. Bommarco et al. 2013). Ecological intensification aims to maximize yield formation through the management of those ecosystem services involved in crop production (Fig. 2). The enhancement of eroded services could potentially result in a yield increase in several agricultural ecosystems (ecological enhancement; e.g. soil fertility restoration in highly degraded soils). Alternatively there might be the potential for the replacement of external inputs with ecosystem services (ecological replacement; e.g. biological control partially replacing pesticide use). The two processes can be also combined to augment yield. The integration of the management of ecosystem services delivered by biodiversity into crop production systems is knowledge-intensive and it requires a comprehensive understanding of the relationships among multiple ecosystem services.
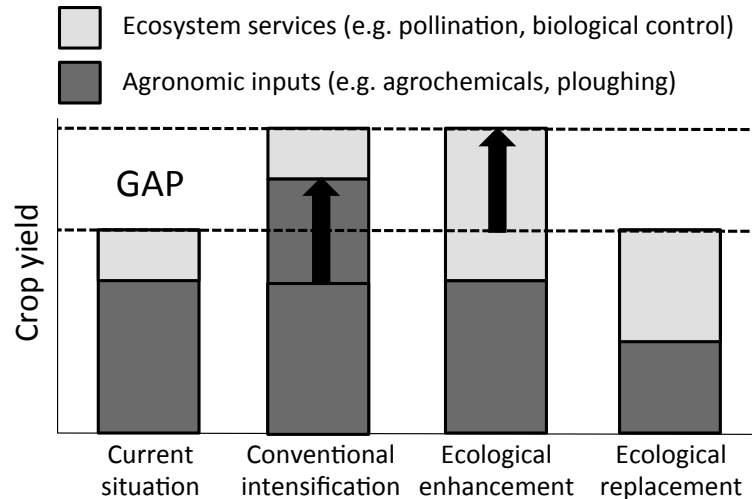
Fig. 2. Ecological intensification. Ecosystem services can be managed to increase crop production (ecological enhancement), or to replace agronomic inputs maintaining the productivity unchanged (adapted from Bommarco et al. 2013).

Interacting ecosystem services

Yield formation is sustained and regulated by bundles of ecosystem services delivered by above- and belowground biodiversity. Bennet et al. (2009) identified two types of relationships occurring between ecosystem services: response to common drivers and actual interactions. Management practices (drivers) can simultaneously influence different services. For example cultivation of perennial grasslands was found to enhance both pollination and biological control service (Werling et al. 2014). Furthermore, ecosystems services can also interact: the enhancement or the deterioration of the first service influences the provision of the second, positively or negatively. For instance, Lundin et al. (2013) found that increased pest control enhances pollination benefit to yield in red clover. Nevertheless, studies exploring direct interactions between ecosystem services in agricultural systems are still scarce (Seppelt et al. 2011).

Interactions between ecological processes occurring above and below ground are poorly explored (Wardle et al. 2004) although above- and belowground ecosystems are known to influence each other (e.g. Bezemer et al. 2005) and supporting ecosystem services are expected to strongly affect regulating services (MA 2005). Only recently scientists investigated this topic. For example water availability was found to influence pollination benefits in almond (Klein et al. 2014). Ignoring

potential interacting mechanisms occurring between different ecosystem services may lead to unwanted tradeoffs and synergies among ecosystem services-disservices, resulting in unexpected outcomes when turned into management practices (Seppelt et al. 2011)

Research objectives and thesis structure

The general objective of this thesis is to identify potential interactions between above- and belowground ecosystem services for important European crops and to identify innovative management options for ecological intensification in agricultural ecosystems. The thesis is divided into two parts: in Part I we explored the relationships between soil-based supporting services and pollination. In Part II we examined the relationships between soil-based supporting services and an above ground crop pest and biological control.

Part I: Pollination

Chapter 2 explored the interactions occurring between pollination and nitrogen availability for three different cultivars of oilseed rape (*Brassica napus* L.). In a field experiment we combined extreme high and low levels of inorganic nitrogen, and of insect pollinators visiting the crop by adding honeybees and excluding them from parts of the crop with cages.

Chapter 3 tested potential interactions between soil fertility and pollination in sunflower crop (*Helianthus annuus* L.). We used soils from a long-term trial with different input management histories in order to recreate plausible levels of soil fertility resulting from poor and good soil management. Pollinator exclusion was used as a proxy for highly eroded pollination service.

Part II: Crop pests and Biological control

Chapter 4 explored interactions between soil fertility, drought, fertilization level and an above ground herbivore in spring wheat (*Triticum aestivum* L.). In a greenhouse experiment we exposed wheat plants infested with the grain aphid (*Sitobion avenae* F.*)* to contrasting levels of SOM, nitrogen fertilizer and water availability.

Chapter 5 investigated interactions between soil habitat quality, landscape complexity and biological control in cereal crops. In a field exclusion experiment, we examined the relative importance of tillage management and landscape complexity on the biological control of aphids (*S. avenae*) provided by three different guilds of natural enemies in winter cereal crops: birds, flying insects/vegetation-dwelling predators and ground-dwelling predators.

Chapter 6 evaluated potential interactions between management intensity, landscape complexity and multiple ecosystem services in cereal crops. In a field experiment we explored the effects of tillage management, nitrogen input and landscape complexity on yield formation, weed and aphid biological control and soil quality.

Chapter 7 provides a summarizing discussion on the fundamental and applied research findings.

Chapter 2

Crop management modifies the benefits of insect pollination in oilseed rape

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Chapter 2

Abstract

Ecological intensification through the promotion of biodiversity and associated ecosystem services that directly support crop yield has been proposed as a promising approach to maintain or enhance agricultural productivity, while minimizing environmental impacts. We tested for an interaction in the benefit to yield characteristics of two resources in winter oilseed rape: availability of insect pollinators and nitrogen. In a factorial field plot experiment we combined high and low levels of inorganic nitrogen, and of insect pollinators visiting the crop by excluding them with cages. We also included a third factor, testing whether hybrid heterosis might buffer poor pollination and/or nutrient availability compared to a traditional open-pollinated cultivar. We found that insect pollination is required to reach high yield and seed quality in OSR. Final benefits of pollination service were, however, greatly modified by cultivar, where the seed yield of the open pollinated cultivar largely depended on pollination whereas the two hybrid cultivars did not. We also found a near significant interaction between nitrogen inputs and insect pollination. Benefits to crop yield from insect pollination appeared to increase with decreased nitrogen levels. The differential response of the three cultivars suggested opportunities to use plant material that is less dependent on pollination in landscapes where the service is more deteriorated. Increased access of nitrogen seems to partly compensate yield losses from poor insect pollination and pollination appears to enhance nitrogen use efficiency.

Introduction

Demand of agricultural products is predicted to double by year 2050 as a result of a growing world population and economic development (MEA, 2005). An outstanding challenge is that future crop production systems will have to provide high yields, while increasing resource use efficiency, and minimizing negative impacts on the environment (Power, 2010). Ecological intensification through the promotion of biodiversity and associated ecosystem services that directly support crop yields (e.g., through crop pollination, or biological pest control), has been proposed as a promising approach to handle this delicate balance (Cunningham et al., 2013). Much remains to

be done to implement this framework in mainstream agriculture. Bundles of ecosystem services delivered to the crop from below and above ground biodiversity need to be efficiently combined with external inputs such as fertilizers, pesticides, machinery and breeding in modern crop production systems. The adoption of ecological intensification is knowledge-intensive and requires interdisciplinary efforts to identify concrete options to replace external inputs with ecosystem services, and enhance services that support yield characteristics (Bommarco et al., 2013).

A factor that is often overlooked in the currently rapidly expanding research on ecosystem services linked to agriculture, is that different resources delivered to the crop plant, either as an ecosystem service provided by biodiversity (e.g., crop pollination, or nutrient cycling), or as an external input applied by the farmer (e.g., chemical fertilizer), might interact in their relative contribution to crop yield (Klein et al., 2014). Instead, each single service is often studied in isolation, and its benefit to crop yield is most often implicitly considered as independent from the presence of other services, level of external inputs, and environmental conditions (Bennett et al., 2009). This assumption risks leading to double counting or underestimation of service benefits, and unrealistic management strategies in contrasting cropping systems (Seppelt et al., 2011). Context dependencies in general, and interactive benefits of multiple service in particular, remain poorly studied but are probably common (Boreux et al., 2013). A recent study demonstrates, for instance, that the benefit to crop yield from animal-mediated pollination depends on pest control levels (Lundin et al., 2013).

Interactions between processes that occur below and above ground are particularly poorly investigated (Wardle et al., 2004). Nutrient inputs and soil fertility have long been studied by agricultural researchers and their direct effect on yield is well understood (e.g., Rathke et al., 2006). Above ground, the importance of insect-mediated pollination for crop production is becoming increasingly acknowledged worldwide (Klein et al., 2007; Garibaldi et al., 2013). Although a deteriorated pollination service can lead to significant yield losses in many crops, this effect can vary considerably due to a number of abiotic and biotic factors (Bos et al., 2007; Hoover et al., 2012; Boreux et al., 2013). Hence, the availability of both soil and pollination resources are main direct determinants of reproduction in many flowering plants, but the degree to which each factor is limiting and how they can interact in

their impact on plant reproduction is unknown for many species (Burkle and Irwin, 2009). The reproduction of many plants depends, for instance, directly on nitrogen availability that determines how many ovules a plant will produce. Nutrients can, however, also strongly affect floral traits such as flower number or size, nectar production, and quality, which are important for attracting pollinating insects (Munoz et al., 2005; Burkle and Irwin, 2010). Research on these lines has been performed in wild plant communities, but we are aware of only a very few studies (e.g. Klein et al., 2014) assessing the existence of potential interactions in the combined benefits of soil nutrients and insect pollination in agricultural crops.

The dependence of animal pollination for yield varies not only among crop species, but might also differ among cultivars of the same crop. The importance of assessing cultivar-specific responses to insect pollination has been highlighted for some time, but few studies have addressed this (Klein et al., 2007; Hudewenz et al., 2013). So far, breeding programmes have rarely aimed at changing, or even measuring pollination dependence. There is therefore a lack of basic information on plant reproductive biology such as dependence on insect pollination for seed set, and nectar and pollen production for cultivars of most crops (Allen-Wardell et al., 1998). The development of genetic modifications and hybridization techniques in crop breeding has stimulated an interest on how traditional open-pollinated cultivars, novel genetically modified and hybrid cultivars differ in their interaction with the environment and available resources (Morandin and Winston, 2005), but large knowledge gaps still remain. Variation in the dependence of animal-mediated pollination among crop genotypes deserves more attention also because deterioration of pollination services delivered both by wild pollinators and managed honey bees is being observed in important agro-ecosystems (Potts et al., 2010).

In the present study, we tested for an interaction in the benefit to yield characteristics of two resources in winter oilseed rape (OSR, *Brassica napus* L. var. *oleifera*): availability of insect pollination and of nitrogen. In a factorial field plot experiment we combined high and low levels of inorganic nitrogen and of insect pollinators visiting the crop by adding honey bees and excluding them from parts of the crop with cages. These are both resources managed by man, but can be considered as proxies for ecosystem services, where a high level of nitrogen reflects a fertile soil and where saturation of honey bees reflects a situation with excellent availability of

pollinating insects (Jauker et al., 2012). With this set-up we tested the hypothesis that abundant availability of nitrogen can compensate yield losses due to poor insect pollination and *vice versa*. If crop yield does not respond to such extreme conditions of high or low pollination and nutrient availability, yield will not be likely to respond to varying delivery of services linked to these resources in real agricultural fields.

OSR yield has been shown to increase considerably when receiving insect pollination, often by 20% or more (Free and Nuttal, 1968; Manning and Wallis, 2005; Sabbahi et al., 2005; Bommarco et al., 2012). However, there are also reports when no clear benefit has been found (Williams et al. 1987; Mesquida et al., 1988; Hayter and Cresswell, 2006). A possible explanation is a difference in yield response to pollination among cultivars (Hudewenz et al., 2013). The results will reflect potential differences among cultivars in resource dependency and resource use efficiency which is a poorly studied area.

Our study provides a test of potential interactions in the combined benefits of pollination and nutrient availability for contrasting crop genotypes in an important annual crop. The existence of interactions among these factors has several practical implications (Klein et al., 2014). It will inform us about the level of yield increases that we can expect when promoting ecosystem services that enhance availability of nutrients or pollination to the crop. It will also indicate the potential to buffer the impact of a deteriorated ecosystem service by promoting another service. The direction of interactions will further assist to identify options for ecological intensification through replacement, *i.e.* where external inputs are replaced with ecosystem services in resource efficient cropping systems.

Materials and Methods

Plant material

The experiment was performed on three cultivars of OSR. The first cultivar (Catalina, Dekalb, Italy) was selected among the traditional open pollinated cultivars. Cultivars of this type were the only ones used in Europe until late 1990s. Recently, new cultivars have been developed that exploit hybrid heterosis in the first offspring (F_1)

from two crossed inbred lines. Amongst the hybrids we selected one normal size (Excalibur; Dekalb, Italy) and one semi-dwarf type (PR45D01; Pioneer, Italy). Both hybrids had the male fertility restored. Both traditional pedegree selection and hybridization aim at improving yield, but not at changing pollination dependency. However, hybrid heterosis may be expected to compensate yield loss in a situation of lack of insect pollinators through increased plant vigour and number of flowers. These cultivars were commonly used in South Europe and were selected to represent current market alternatives to farmers.

Experimental design

The experiment was performed during the 2012 growing season in a field located at the Experimental farm of the University of Padova (Legnaro, Agripolis, elevation: 8 m, WGS84 N45 20'42'', E 11 56'60''). Soil characteristics of the experimental field were measured in 2011 (soil organic matter: 2.52%, total P: 822 mg P_2O_5 kg^{-1}, C/N ratio: 15.5, pH: 8.38). Preceding crop was winter wheat in 2010/2011. The crop was sown on the 24-Sep-2011 applying a seed density of 63 m^{-2} by means of an inter-row distance of 45 cm and seed distance within the row of 3.5 cm.

A factorial split-plot design was adopted with three crossed factors: cultivar, N and pollinator treatment. The experimental field was composed of three blocks. Within each block we created three long crop strips (75 m x 15 m), one for each of the three cultivars. Each cultivar strip was split into two plots treated with two nitrogen levels (NO: control and N170: 170 kg N ha^{-1}) (Fig. 1). The N x cultivar parcels were separated by large corridor of bare ground (4 m wide) to allow harvest machinery passage. The pollination treatment was done by installing two pairs of cages one at each end of the parcel for a total of 72 cages. Each pair was composed of two adjoining cubic cages made of metallic tubes (each cage was 2 x 2 x 2 m). The cage pairs were placed on the vegetation in the early spring and covered four crop rows. Within each pair of cages we regulated crop density by removing plants in early spring to obtain an equal number of plants in each of the two neighbouring cages. On 21-Mar-2012, well before the onset of flowering, we sprayed the entire experimental field with Lambda-cyhalothrin (9.48%) with a dose of 100 g l-1 (Karate Zeon, Syngenta) to suppress an attack of the pollen beetle (*Meligethes aeneus* F.). At the onset of flowering between the 31-Mar and the 2-Apr-2012, we closed one of the two

cages with a plastic transparent net with a mesh of approximately 1 x 1 mm to exclude insect pollinators. Several studies indicated that this type of net only slightly changes microclimatic conditions (e.g. Martin et al., 2013). Preliminary tests indicated that the temperature did not differ between close and open cage. We found a significant difference in night minimum temperature where the open cage has c. 1°C lower temperature than the close cage for c. 2 hours (one week of observation). To minimise micro-climatic differences between the open and the close cage we modified the open cage by closing with the same net the roof. The position of the open cages was alternated among plots between east and west relative to the closed cage to evaluate possible effects of differences in sun exposures (Fig. 1). Preliminary analyses showed that compass direction did not affect any of our response variables. Once the flowering ended between 27-Apr and 28-Apr-2012, all nets were removed and the crop was left open to ripen under the same environmental conditions. During ripening, both cages were protected with an anti-bird net with a mesh size of 1 x 1 cm to support the plants against lodging in the wind and to protect the pods from bird predation.
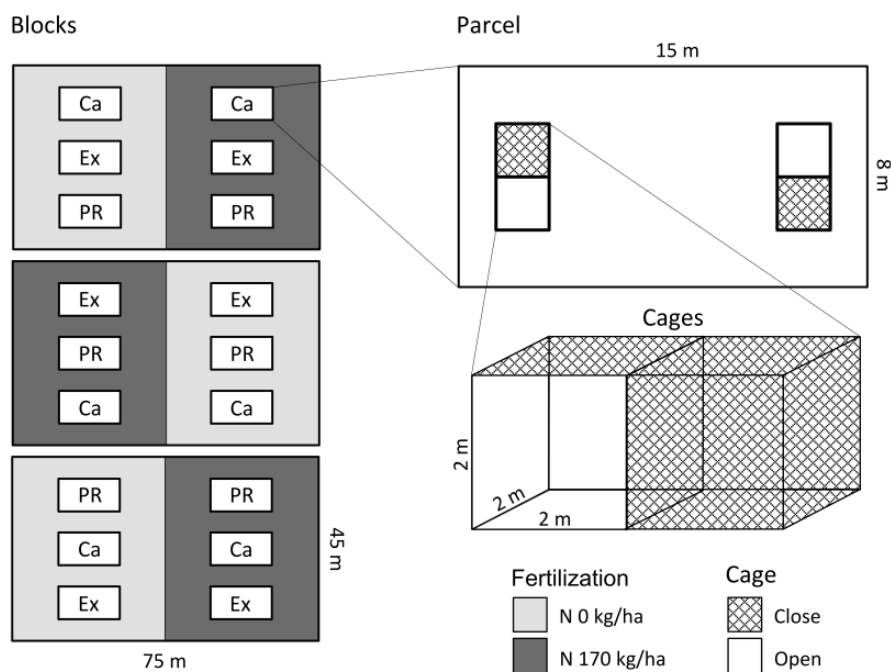

Fig. 1 Scheme of the experimental design. Each of the three blocks were split into two plots treated with two N levels N0: 0 kg N ha^{-1} and N170: 170 kg N ha^{-1}). The three cultivars Ca: Catalina, Ex: Excalibur, PR: PR45D01) were randomly sown in strips within each block. Two pairs of cages were placed within each parcel for the pollination treatment.

Visitation rate

Five honey bee hives were placed at 500 m from the experimental field to guarantee large visitation rates. Between 17-Apr and 21-Apr-2012 we measured insect visitation rates in two occasions at the peak of blossom by counting the number of flower visits in the open cages. Insect visits were observed for 5 minutes. We made our observations between 10.30 and 17.00 under sunny weather conditions with temperature above 17°C.

Plant measurements

Once the crop was ripe (between 18-Jun and 20-Jun-2012) we sampled 1 m^2 in the middle of each cage. The entire above ground parts of the plants were harvested by cutting each plant 5 cm above the soil surface. Plants from each cage were placed in an open bag and placed in a ventilated greenhouse to dry. After that the plants were dried at 65°C for 24 hours after which plants were threshed. Seed yield and total biomass were measured. We measured seed weight by weighing 10 random samples of 100 seeds each taken from the total yield sample. We also measured oil content using the Twisselmann extraction method (instrument FOSS, SOXTEC 1042) (Zanetti et al., 2009). We further assessed the average number of seeds per pod by randomly sampling 5 plants per cage. From each plant we collected 20 pods starting from the tip of the main inflorescence and we counted the number of seeds in each pod. We further estimated the number of pods per m^2 by dividing the total seed yield by the average individual seed weight, and dividing this quantity by the number of seeds per pod and finally by the cage area. This was the only parameter that was not directly measured due to the very large samples that would have had to be needed. The number of pods was estimated using the number of seeds per pod from the main inflorescence and was therefore not representative of the whole plant. However, it can be considered a standardized relative measure that was useful to test for difference between the treatments.

Data analysis

We tested the effect of cultivar, N and pollination treatment on the yield parameters described above by using linear mixed model. The models included the three treatments and all the interactions as fixed effects while the random effects reflected

the split-plot design (cage pair/N plot/cultivar plot/block). The model was estimated using the REML estimation method in the nlme package (Pinherio et al., 2009) for R (R Core Team 2012). The total seed and oil yield were log-transformed before analyses. Just after the end of the flowering period a storm event damaged a part of the experimental plots. We had to remove data from all the cages with the Excalibur cultivar from one block (n=8) and two other cages (i. Catalina, N: 170 kg, no pollination and ii. Excalibur, N: 170 kg, open pollination). The analyses were thus based on data from 62 cages. For testing the effect of N and cultivar and their interaction on pollinator visitation rate we performed the same model as described above but without the pollination treatment. In total we had data from the 36 open cages in two occasions.

Results

The honey bee was the most common pollinator species with approximately the 70% of the total visits. Wild bees (mostly *Andrena* spp. and *Lasioglossum* spp.) accounted for 18%, while hover flies accounted for 7%. The other visits (~5%) were made mainly by lepidopterans, coleopterans and dipterans. The mixed models showed no effect of cultivar or N on the visitation rate considering both the whole pollinator community and the single pollinator groups (Table 1). Only the N treatment had a marginally significant effect on wild bees (0.077), where the high N treatment received relatively larger number of visits than the low N treatment.

Table 1 Results of the linear mixed model testing the effect of N and cultivar on pollinator visitation rate. Insect visits were recorded in two occasions at the peak of the blossom between 17-Apr and 21-Apr-2012). Analyses were performed separately for the total pollinator community, honey bee, wild bees, and hover flies. The models included N and cultivar and their interaction as fixed effects, while the random effects reflected the split-plot design cage pair/N plot /cultivar plot/block/date).

Pollinator group	Cultivar			N			N x Cultivar		
	d.f.	F	P	d.f.	F	P	d.f.	F	P
Honey bee	2, 1	2.74	0.112	1, 14	0.00	0.968	2, 14	1.40	0.278
Wild bees	2, 1	0.91	0.431	1, 14	3.62	0.077	2, 14	1.23	0.321
Hover flies	2, 1	2.75	0.111	1, 14	0.16	0.693	2, 14	1.34	0.292
Total	2, 1	2.81	0.107	1, 14	1.09	0.314	2, 14	1.24	0.317

There was a positive main effect of insect pollination on both seed yield and oil content (Table 2). There was, in addition, a significant cultivar x pollination interaction indicating that the pollination effect differed among cultivars for seed yield but not for oil content. Insect pollination increased seed yield only for the open pollinated line cultivar (Catalina), while for the yield of the two hybrid cultivars did not increase with the presence of insects (Fig. 2a). The open pollinated plants of Catalina produced on average 19% more seeds compared to the plants inside the cages. In contrast the effect of pollination on oil content (%) was not affected by the cultivar (Fig. 2b). We also found a marginal significant interaction on seed yield between N and pollination where fertilization partly compensated the lack of insect pollinators (Fig. 2a). The same interaction was not found for oil content (Fig. 2b). We found only a tendency for a positive effect of N on seed yield (Table 2).

Table 2 Results of the linear mixed model testing the effect of N, cultivar and pollination on the main yield parameters of OSR. Seed yield and oil yield were log-transformed.

	d.f.	Seed yield F	Seed yield P	Oil %) F	Oil %) P	Seed weight F	Seed weight P	Seed per pod F	Seed per pod P	Pods m^{-2}* F	Pods m^{-2}* P
N	1, 5	4.32	0.092	1.21	0.322	0.19	0.682	0.11	0.758	8.27	0.035
Cultivar	2, 3	0.80	0.528	0.94	0.480	89.13	0.002	128.97	0.001	19.25	0.019
Pollination	1, 24	6.97	0.014	6.64	0.016	364.43	<0.001	239.39	<0.001	5.64	0.026
N x Cultivar	2, 5	0.40	0.687	0.55	0.617	1.66	0.279	0.36	0.712	2.10	0.218
N x Pollination	1, 24	3.64	0.069	0.08	0.776	0.78	0.385	4.58	0.043	11.25	0.003
Cultivar x Pollination	1, 24	6.24	0.007	1.21	0.316	24.66	<0.001	20.56	<0.001	7.28	0.003
N x Cultivar x Pollination	1, 24	0.89	0.425	2.93	0.073	2.37	0.115	0.34	0.713	2.41	0.112

* This parameter was not directly measured but it was estimated using the following formula: number of pods=seed yield/seed weight/average number of seed per pod from the main inflorescence.
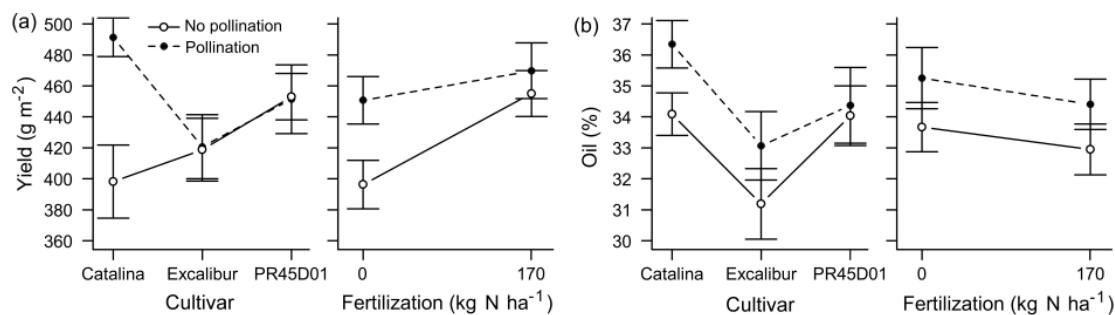
Fig. 2 Interaction plot between cultivar and pollination treatment, and between N and pollination on a) seed yield, and b) oil content %). The plots show mean ± SE. Significances of the effects are reported in Table 1.

Although seed yield of the hybrid cultivars was not affected by pollination, several yield components were strongly affected by pollination in all the cultivars. We found an interaction between pollination and cultivar for seed weight, number of seed per pod and estimated number of pods m^{-2}. Insect pollination created smaller seeds in all cultivars, but the effect was stronger for the open pollinated line (Catalina) than for the two hybrids (Fig. 3a). The number of seeds per pod was also enhanced by insect pollination in all cultivars, but the effect was stronger for the semi-dwarf hybrid (PR45D01) (Fig. 3b). The estimated pod number increased by insect pollination in the open pollinated cultivar and decreased in the two hybrids (Fig. 3c). The estimated number of pods increased by N in case of no insect pollination, but was not affected by N in the presence of insect pollinators (Fig. 4).
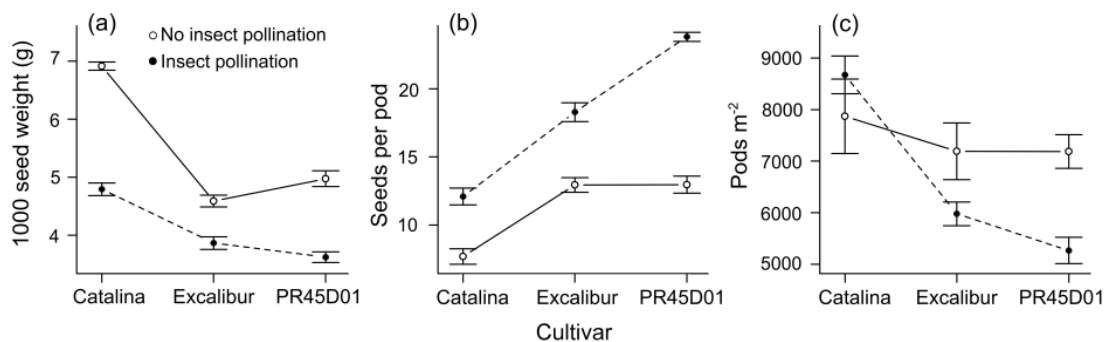

Fig. 3 Interaction between cultivar and pollination on a) 1000 seed weight, b) number of seeds per pod, and c) estimated number of pods. The number of pods was estimated using the following formula: number of pods=seed yield/seed weight/number of seed per pod from the main inflorescence. The plots show the mean ± SE. Significances of the effects are reported in Table 1.
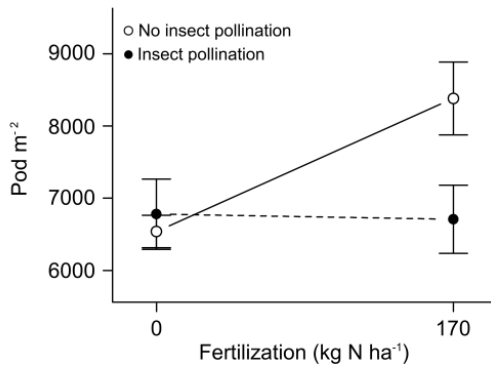
Fig. 4 Interaction between N and pollination on the estimated number of pods per m^2. The number of pods was estimated using the following formula: number of pods=seed yield/seed weight/number of seed per pod from the main inflorescence. The plot shows the mean ± SE.

Discussion

Although the pollination biology of important crops is becoming increasingly understood, the context dependence of pollination benefits remains poorly explored (Bos et al., 2007). Our study is one of the first that test potential interactions between benefits of pollination service and nutrient resource availability in an important crop. We found a near significant interaction between nitrogen inputs and insect pollination, where benefits to crop yield from insect pollination appeared to increase with decreased nitrogen levels. It is an interesting observation that pollination service might increase nutrient resource use efficiency and such interactions clearly need more attention. The average yield difference of c. 600 kg ha^{-1} between pollinated and not pollinated plants at a low nitrogen level is an interesting contrast for farmers. Our results show promise for identifying options for ecological replacement between ecosystems services and agronomic inputs emphasising the economic importance of insect pollination for modern agriculture (Garibaldi et al. 2013). It also shows that nutrient status and, by extension, access to other vital resources (e.g., water), or removal of resources (e.g., through pest attacks) (Lundin et al. 2013), would need to be considered when estimating pollination benefits to seed set and yield (Klein et al., 2014).

Our study shows that insect pollination is required to reach high yield in OSR. Although oilseed rape is generally considered self fertile (Free and Nuttal 1968), cross pollination is probably required to maximize yield and economic return (Bommarco et

al., 2012). Several plant and yield quality characteristics were affected by pollination, cultivar and nutrient availability partly indicating how formation for the overall yield differed among treatments. Insect pollination, in particular, affected the way the plants modified yield characteristics. The insect pollinated plants gave smaller seeds and increased the number of seeds per pod in all cultivars. This is in line with predictions and observations in several other plants, where poorly pollinated plants invest more into the fewer seeds that are set from self- or wind-pollination in lack of insect pollination (Jauker et al., 2012; Klein et al., 2014).

Final benefits of pollination service were, however, greatly moderated by cultivar, where the seed yield of the open pollinated cultivar depended largely on pollination whereas the two hybrid cultivars did not. This is line with a recent study comparing insect pollination dependence in different cultivars of oil seed rape (Hudewenz et al., 2013). The pollinated line cultivar provided the overall highest yields. On the other hand, quality was enhanced by insect pollination in all the investigated cultivars. In the current study, we were only able to include three common cultivars available on the market, but a more complete screening of cultivar differences in nutrient use efficiency and pollination dependence would be needed in collaboration with crop breeders (Klein et al., 2007; Hudewenz et al., 2013). The observation of pollinator visits indicated that cultivar did not affect the visitation rate suggesting that the three cultivars presented similar attractiveness to pollinators. A possible explanation for the contrasting result between line and hybrid cultivars may therefore relate to hybrid heterosis. Hybrids are expected to produce a larger number of flowers that may compensate for poor cross pollination. This is partly indicated by the increase in the number of estimated number of pods in absence of insect pollination that was detected only in the two hybrid cultivars. An alternative explanation is that individuals in a field of hybrid plants are more genetically similar compared to open pollinated plant individuals and therefore benefit less from cross pollination. Due to the low number of tested cultivars, no generalization on the differential response between line and open pollinated cultivars can be made at this stage and more research is needed to shed light of the mechanisms underpinning this pattern. For instance a recent study did not find the same response of line and hybrid cultivars (Hudewenz et al., 2013).

We also found an almost significant interaction between pollination and nitrogen on seed yield. Inorganic nitrogen and insect pollinators used in the current experiment are both resources managed by man, but can also be considered as proxies for ecosystem services provided by biodiversity. Nitrogen availability to the plants is greatly affected by soil organisms and can be considered a proxy for soil services linked to nutrient cycling, where a high level of nitrogen reflects a fertile soil and, hence, the return we can expect in terms of yield if we invest in soil measures that enhance nitrogen efficiency use (Power 2010). Honey bees, that were the main flower visiting insects in the experiment, can similarly be used as proxy for different levels of pollination services. In real field situations, wild pollinators contribute substantially to crop yields across the globe (Garibaldi et al., 2013) and a saturation of honey bees reflects a situation with excellent availability of pollinating insects. The experiment indicates that pollination can, at least partly, enhance resources use efficiency of nitrogen, providing interesting options to farmers to increase or maintain yield in field with low nutrient inputs. The few studies testing interactions between nitrogen and pollination have found weak or no significant interaction between the two variables in wild plants (Burkle and Irwin 2010). We found a trend for slightly higher visitation rates in the plots with high nitrogen inputs. As we found a positive pollination effect only in the poorly fertilized plots, the interaction between nitrogen and pollination cannot be explained by pollinator behavior.

A great challenge for ecologists and conservation biologists in recent years has been to understand the impact of established and novel agricultural systems on biodiversity and associated ecosystem services. The next step will be to understand the potential negative feedback of biodiversity loss to agricultural production and to develop cost-effective mitigation measures through alternative management options (Cardinale et al., 2012). A close integration between conservation, environmental and agronomic sciences is now crucial to meet increasing food demand. Our results suggest alternatives for such integration in the development and implementation of strategies for management of resources needed for crop production. The results outline alternative options for farmers, agribusiness and society in deciding how to invest in management of ecosystem services, breeding, or external inputs such as chemical fertilizers. Increased access of nitrogen appears only to partly compensate yield losses from poor insect pollination, and to secure continued high yields we can

certainly choose to add more inorganic fertilizer in areas where pollination is degraded (Potts et al., 2010; Garibaldi et al., 2011). However, nitrogen is a major production cost to the farmers. Up to 40% of the energy consumed to produce a crop is invested into inorganic nitrogen and this energy use contributes to climate change (Cameron et al., 2013), and the cost is expected to increase with energy prices. Furthermore, only about 40-50% of the applied nitrogen is actually harvested in many crops (Connor et al., 2011). Instead, nitrogen leakage to ground water, with ensuing eutrophication of streams, lakes and seas, has become a huge environmental problem worldwide (Compton et al., 2011). Much nitrogen is also lost by volatilization where arable soils emit nitrogenous gases that contribute to climate change and nitrogen deposition (Bobbink et al., 2010). Enhanced nitrogen use efficiency is therefore high on the policy agenda, both to decrease costs privately for farmers and for society (e.g., Jones et al., 2005).

Choice of cultivar together with enhanced pollination appears to be an alternative path to strive towards high yield targets. The differential response of the three cultivars suggested opportunities to use plant material that is less dependent on pollination in landscapes where the service is more deteriorated. Pollination can be provided by honey bees with a cost to the farmer or through the enhancement of wild pollinators that have been shown to be more efficient than previously thought (Garibaldi et al., 2013). More options are needed to be developed for this. Importance of landscape complexity (Morandin and Winston, 2006; Arthur et al., 2010; Klein et al., 2012), diversified crop systems (Kremen et al., 2004) and on-farm options such as flower strips have been demonstrated to contribute improving pollination service (Carvalheiro et al., 2012). A complementary option is to enhance soil fertility and nitrogen retention. Instead of replacing degraded pollination with inorganic nitrogen, a more appealing option would be to combine investments in increased soil fertility with interventions that enhance pollination by wild insects.

Acknowledgements

Chapter 3

Decreasing soil fertility cancels pollination benefits to sunflower yield

Tamburini G, Morari F, Berti A, Marini L
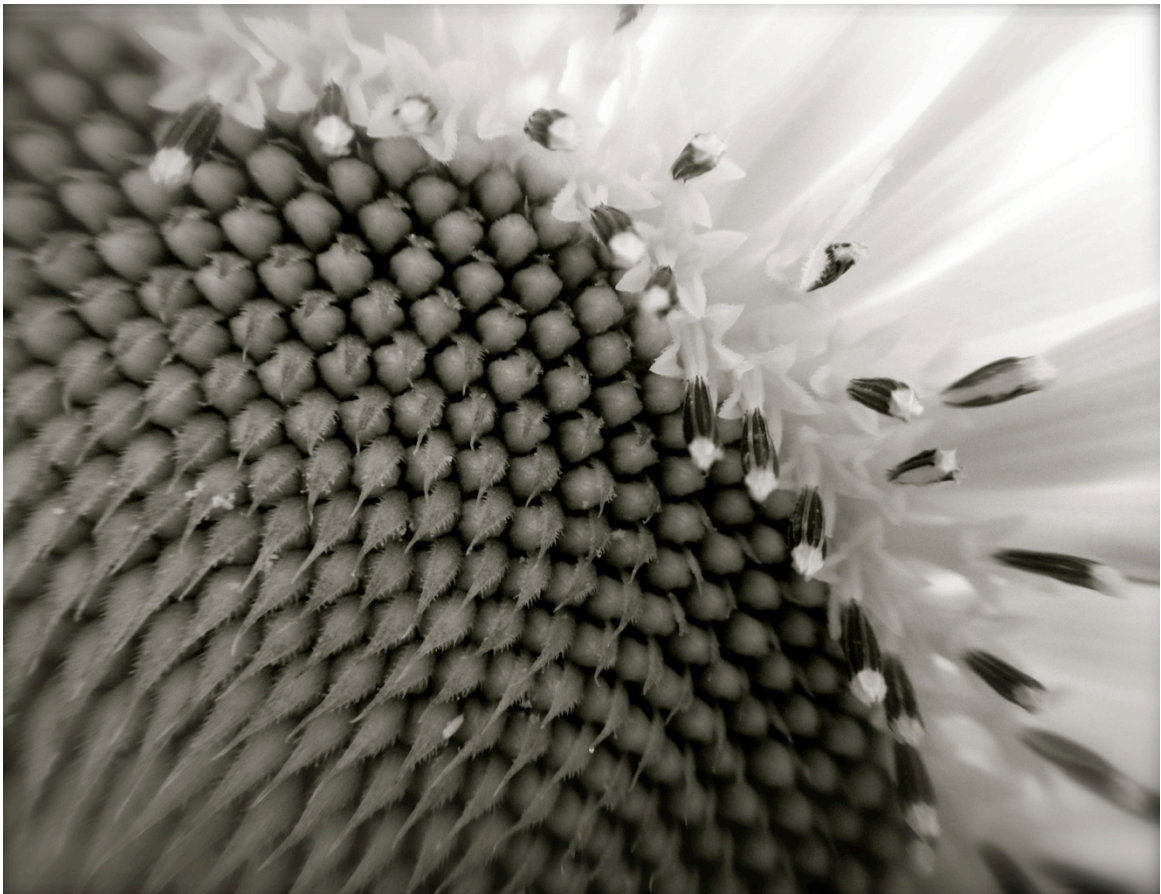
Chapter 3

Abstract

Pollination and soil fertility are important ecosystem services supporting crop production. These services have been usually studied in isolation, while their possible interactions are little understood. In a pot experiment we explored the combine effects of pollination and soil fertility in sunflower. We used soils from a long-term trial characterized by different input management histories in order to recreate plausible levels of soil fertility resulting from poor and good soil management. Pollinator exclusion was used as a proxy for highly eroded pollination service. We found that pollination benefit to yield depended on soil fertility, i.e. insect pollination enhanced fruit set and yield only under higher soil fertility conditions indicating that limited nutrient availability can constrain pollination benefits irrespective of pollinator presence. Our study provides evidence for interactions between above- and belowground ecosystem services highlighting the key role of soil in supporting multiple services. A comprehensive knowledge about service tradeoffs and synergies is essential for a correct evaluation and management of ecosystem services in agricultural landscapes.

Introduction

To meet growing global food demands, modern agricultural practices have greatly increased crop production. However, agricultural intensification has dramatically impacted the environment, harming the ability of ecosystems to provide services essential to human well being (Power et al. 2010), including those directly linked to crop production (e.g. soil fertility or pollination). Global food demand is forecasted to double by 2050 (Godfray et al. 2010). Therefore, future agriculture has to maintain high yield levels while preserving environment health and biodiversity. The enhancement of ecosystem services that support yield has been proposed as a promising solution to maximize agricultural production limiting the detrimental effects on the environment (Bommarco 2013). The translation of this strategy into management practices still lacks a comprehensive understanding of the relationships among multiple ecosystem services.

Although it is well known that crop production is sustained and regulated by a suite of different ecosystem services, the majority of studies have focused so far on the relative contribution to yield provided by single services (Seppelt et al. 2011) or on the responses of multiple ecosystem services to common environmental drivers (e.g. effects of landscape simplification on pollination and pest control; Bennet et al. 2009, Werling et al. 2014). This approach, even if essential for a basic knowledge about ecological processes, may lead to disregard possible tradeoffs and synergies among ecosystem services, resulting in unexpected outcomes when turned into management strategies (Seppelt et al. 2011). Studies about interactions between ecosystem services in agricultural systems are still scarce (but see Bureux et al. 2013, Lundin et al. 2013) and the occurrence of above-belowground interactions has been rarely demonstrated (e.g. pollination-water availability, Klein et al. 2014).

Insect pollination is considered a key ecosystem service to agriculture supporting yield formation in 75% of all crops (Klein et al. 2007, Garibaldi et al. 2013). Agricultural intensification with the increased use of agrochemicals and landscape simplification has been recognized as a major threat to pollination service owing to the associated decline in pollinator abundance and diversity (Potts et al. 2010). Nevertheless a number of recent studies indicated interacting effects between pollination and other ecosystem services on fruit set and yield formation (e.g. pest control; Classen et al. 2014, Motzke et al. 2014). Because of its intrinsic relation to plant physiology, pollination service and its contribution to yield are likely to be influenced by factors linked to plant quality (Bos et al. 2007). Water availability for example, has been shown to increase pollination benefit to yield (Bureux et al. 2013, Klein et al. 2014), whereas nutrient limitation to strongly affect floral traits (e.g. flower size and number) or early fruit abortion (Burkle and Irwin 2009, Bos et al. 2007). Soil properties and associated belowground ecosystem services are therefore expected to influence pollination aboveground.

Soil fertility is another important ecosystem service supporting crop production (Zhang et al. 2007, Power 2010). The availability of nutrients for plant growth is mediated by soil biota that provides essential biological processes (Wall 2004). Agricultural intensification together with poor soil management have led to widespread degradation of soils (European Commission 2002). However, agricultural practices characterized by addition of animal manure, incorporation of crop residues,

together with an accurate use of chemical fertilizers have been shown to restore and sustain soil fertility (Watson et al. 2002).

Although above- and belowground ecological processes have been largely shown to influence each other (e.g. Bezemer et al. 2005), interactions between ecosystem services occurring above- and belowground are still poorly explored (Wardle et al. 2004). To our knowledge there are no studies looking at interactions between soil fertility and pollination and their potential consequences on crop yield. In a highly replicated pot experiment we tested the interaction between an above- and a belowground ecosystem service by combining two levels of insect pollination with two levels of soil fertility in sunflower. We used soils from a long-term trial characterized by different input management histories in order to recreate plausible levels of soil fertility resulting from poor and good soil management. Pollinator exclusion was used as a proxy for highly eroded pollination service. The results of our experiment will elucidate the potential consequences of soil degradation on pollination benefits in sunflower. Moreover, our study will provide useful information for a more accurate assessment of pollination service in agricultural landscapes.

Material and methods

Study system

Sunflower (*Helianthus annuus*) is a worldwide economically important crop for oilseed and food production (FAO, 2014). Hybrid varieties generally replaced traditional lines and have been selected to increase yield, resistance to environmental stresses and self-compatibility. Nevertheless hybrid sunflowers have been proved to largely depend on insect pollination to transfer pollen from the male-fertile to male-sterile line (Free 1993, Greenleaf and Kremer 2006). Honey bees are usually the most abundant insects visiting sunflower inflorescences (flower heads) whereas wild bees are the most efficient (Parker 1981). Hybrid sunflowers typically produce one flower head per plant formed by hundreds of florets. Each head flowers for about 6-10 days. If pollination occurs, florets produce one seed each (full seed) whereas when it fails florets produce empty fruits (Free 1993). The experiment was performed on the hybrid variety of sunflower *Belmonte* (Strube, Italy).

Soil

In order to achieve two levels of soil fertility, the study was conducted using soils from the long-term trial located at the Experimental Farm of the University of Padova (Veneto Region, NE Italy 45 21° N; 11 58° E; 6 m a.s.l.) established in the early 1960s with the purpose of investigating the evolution of soil under different crop rotation, nutrient and management intensity variables (for more details about the complete experiment set up see Giardini 2004, Morari et al. 2006). Among different treatments, we selected soils managed under two different levels of input intensity expected to influence soil fertility (now on: soil input; HI, high input and LI, low input). HI management included the application of organic fertilizers, almost absent in LI management, whereas both managements received the same amount of inorganic fertilizers (see Supplementary Information). Morari et al. (2008) showed that after 40 years HI management resulted in higher available phosphorus (P_{av}), exchangeable potassium and magnesium concentrations (K_{ex}, Mg_{ex}) than the LI treatment. Moreover, cultivation systems using organic fertilizers (HI) showed increased soil quality leading to higher crop yield.

In February 2013 the top soil from HI and LI treatments was collected and stored under the same environmental conditions in large plastic boxes. Proceeding crop for all the selected soils was maize in 2012. The main chemical parameters of the collected soils are reported in Table 1 (Morari et al. 2008). Soils were then sieved through 1.0 cm mesh and set in 192 pots (6 liters, 20cm diameter, 9.2 kg of soil per pot).

Table 1. Averaged values (± SD) of the soil parameters for the two different input intensity management levels. Analyses were performed in 2000 (Morari et al. 2008).

Input intensity level	Soil parameters								
	pH	P_{av}	K_{ex}	Mg_{ex}	SOC	CEC	AWC	WFP	SQI
High input (HI)	7.85 (±0.16)	63.5 (±11.0)	82.0 (±12.7)	129.0 (±16.5)	0.80 (±0.06)	12.51 (±2.24)	0.2 (±0.01)	0.75 (±0.01)	0.55 (±0.06)
Low input (LI)	7.84 (± 0.33)	32.8 (± 10.8)	65.6 (± 14.0)	110.6 (± 19.0)	0.70 (± 0.05)	13.51 (±1.81)	0.2 (±0.01)	0.75 (±0.01)	0.46 (±0.06)

P_{av}, available phosphorus (mg kg^{-1}); K_{ex}, exchangeable potassium (mg kg^{-1}); Mg_{ex}, exchangeable magnesium (mg kg^{-1}); SOC, soil organic carbon (mg cm^{-3} cm^{-3}); CEC, cation exchange capacity (meq. 100 g^{-1}); AWC, available water content (m^3 m^{-3}); WFP, water filled porosity; SQI, soil quality index (ranging from 0, poor quality, to 1, high quality) developed by Morari et al. (2008) based on the previous soil parameters (see SI).

Experimental design

The pot experiment was performed during the 2013 growing season at the Experimental farm of the University of Padova. In May sunflower seeds were sown in plug trays (52 x 28, 32 round cells per sheet of 120 cubic cm each) in a cold greenhouse. After 20 days two V4 stage sunflowers (second leaf pair unfolded, Schneiter and Miller 1981) were transplanted into each pot filled with the soil taken from the long-term trial. One week later the best performing plant was selected and the other one clipped and removed. All plants were watered three times per weeks throughout the experiment.

A highly replicated randomized block design was adopted with two crossed factors: soil input (HI vs. LI) and pollination treatment (open pollination vs. pollinator exclusion). Pots were arranged into six outdoor cages made of metallic tubes (2 x 2 x 2 m, 32 pots per cage) in order to have in each cage the same number of replicates per treatment combination. Cages were covered by a plastic roof and closed by polyester fine net (mesh size: 1 mm) to protect the plants from extreme weather events and to exclude herbivores.

Just before the onset of flowering, the polyester net was removed from all the cages to allow pollinator access. The pollination treatment was done by placing nylon net bags over 16 sunflower heads per cage to prevent insect visitation. Each pollination level had a total of 96 replicates, 48 per soil input level. Three honey bee hives and one *Bombus terrestris* hive were placed 30 m from the experiment location to guarantee an optimal pollination service. Once flowering period ended plastic bags were removed. At physiological maturity (R9 stage; Schneiter and Miller, 1981), flower heads were harvested and put in paper bags to dry. Each inflorescence was manually inspected to count the total number of fruits (both filled with a seed and empty) and to calculate the proportion of full seeds (seed set; full seeds/total number of fruits). After that, seeds were dried at 65°C and total seed weight was measured. We further estimated the 1000 seed weight using the average seed weight calculated for each plant (seed yield/number of full seeds * 1000). At flowering peak, in a subset of open pollinated plants (32 replicates) we carried out observations on visiting rate. Total number of bees on each inflorescence irrespective of visit duration was visually recorded by an observer who spent three minutes for each plant. We recorded

data on both *A. mellifera* and native pollinators. We found no significant difference in visitation rate between the two levels of soil input management (HI vs. LI) (one way ANOVA, P>0.05). We did not carry out more thoroughly observations on visitation rate as pollinators clearly moved from one plant to the other irrespective of the soil treatment probably due to the interspersed spatial arrangement of the pots and the very high density of insects around the experiment (three bee hives, one bumblebee hive and abundant wild bee populations).

Statistical analyses

Data were analyzed using linear mixed model with REML estimation method in nlme package (Pinherio et al. 2013). We analyzed the fixed effect of soil input (HI vs. LI) and pollination treatment (open pollination vs. pollinator exclusion) and their interaction on the mean seed yield, seed set, number of fruits and the 1000 seed weight per plant. The seed set was square transformed before the analyses to achieve normal distribution of model residuals. To account for non-independence in the study design cage (block) was included as random factor in the analyses. We first built full models and then we simplified them by removing non-significant interactions. Tukey multiple comparison test was applied to determine significant differences among treatments. The analyses were performed using the R statistical language, version 3.1.1 (R Development Core Team 2013).

Results

The number of fruits per head increased with high level of soil input ($F = 26.55$, $p = < 0.0001$), whereas we did not find any effect of pollination ($F = 0.24$, $p = < 0.62$; figure 1*a*). The 1000 seed weight was not affected by both treatments (soil input: $F = 0.09$, $p = 0.75$; pollination: $F = 0.27$, $p = 0.60$). Seed set was positively affected by both soil input ($F = 17.45$, $p = 0.0001$) and pollination ($F = 19.61$, $p = < 0.0001$). There was, however, a significant interaction between the treatments ($F = 9.73$, $p = 0.0023$; figure 1*b*): the gain in seed set owing to insect pollination was observable only at high levels of soil input. There were no differences in seed set between soil input levels when pollinators were excluded (Tukey multiple comparison test, $p=0.87$). Effects of treatments on yield reflected those observed on seed set: we found

positive main effect of soil input ($F = 45.93$, $p =$ <0.0001) and pollination ($F = 13.17$, $p = 0.0004$) and a significant interaction between the two ($F = 9.61$, $p = 0.0024$; figure 1c): insect pollination increased yield only under high soil input conditions. Under pollinator exclusion, higher soil input led to a yield increment of 14.5% (Tukey multiple comparison test, $p=0.05$).
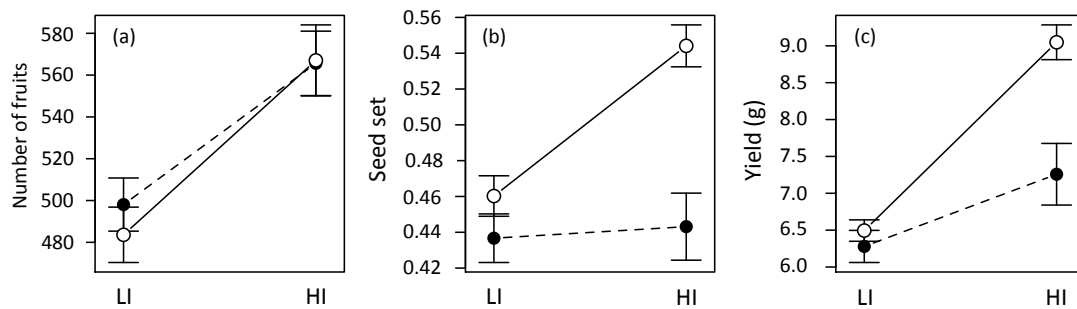

Fig. 1. Mean number of flowers (a), seed set (%) (b) and seed yield (c) in relation to soil input and pollination treatment. Error bars show ±1 SE. LI, low soil input; HI, high soil input; solid line and white dots, open pollination; dashed line and black dots, pollinator exclusion.

Discussion

Our study explored the combined effect of pollination and soil fertility on yield formation in sunflower. We found that insect pollination enhanced yield production only under higher soil fertility conditions. Our results are in line with recent findings, showing that pollination service is context-dependent and its contribution to crop production interplays with other environmental factors and services (e.g. Classen et al. 2014). These findings are also relevant for the management of ecosystem services in agricultural landscapes. Valuation of ecosystem services, essential for land-use decisions, is primarily based on land-cover mapping. Pollination is usually linked to the presence of semi-natural habitats able to sustain pollinator communities and to their distance to crops (Martínez-Harms and Balvanera 2012). Our study suggests that an evaluation solely based on the potential pollinator presence could lead to an overestimation of the service, especially in landscapes characterized by high soil degradation. Expanding the knowledge about service tradeoffs and making it explicit in decision making will improve the efficacy of sustainable management strategies both at local and regional scale (Goldstein et al. 2012).

As expected, high levels of soil input were found to enhance yield respect to soils with low organic input. Considering that the seed weight did not change under different soil input levels, the gain in yield under high soil fertility conditions was attributable to the increased number of fruits produced. As mentioned before, the application of organic inputs is a widespread farming practice known to improve soil fertility and to increase availability of both macro- and micronutrients essential to crop growth (Edmeades 2003, Morari et al. 2008). For example Rasool et al. (2013) found that farmyard manure application (20 t ha^{-1}) increases both number of fruits per head and yield in sunflower crop under field condition. Contrary to our results, Rasool et al. also found an increase in seed weight when farmyard manure was applied. This could be explained by the fact that whereas the number of florets per head depends on the nutrient availability before the flowering onset, higher seed weight resulted from a higher nutrient uptake during the phase of floret growth (Steer et al. 1984) that could has been limited at that time by the limited amount of soil in the pots.

We found an interaction between pollination and soil input, where insect pollination enhanced seed set and yield only under high soil fertility condition. Exclusion of insect pollinators led to high values of fruit set which indicates high levels of self-compatibility, similar to those observed for other sunflower hybrids (Degrandi-Hoffman and Chamber 2006). Under high soil fertility condition, insect pollination increased seed set and yield by 18.2 and 19.6 % respectively, confirming that insect pollination is an important resource in sunflower crop (Greenleaf and Kremen 2006). Under low soil fertility condition insect pollination did not lead to any difference in both fruit set and yield respect to pollinator exclusion. Variation in fruit set and seed production has been largely investigated during the last decades on both wild and crop species. Nutrient and pollen availability are considered important factors affecting reproduction of plants. Pollinator scarcity or inefficiency are well known to affect fruit set and yield owing to an inadequate supply of pollen to female reproductive organs (Klein et al. 2007). Moreover, the proportion of fruits and seeds that mature may depends on the ability of maternal parent to provide the resources necessary to growth and develop and, when these are limited, competition among fruits leads to fruit abortion (Stephenson 1981). Resource availability can also indirectly impact on plant fertility, affecting floral traits and the consequent attractiveness to pollinators (Burkle and Irwin 2009). Our results suggest that

sunflowers might be principally limited by nutrient availability and secondarily by insect pollination. The lack of difference in fruit set between plants exposed and excluded to insect pollination under poor soil fertility indicated a potential limit in the carrying capacity of the plants. Under higher nutrient availability instead, pollinated florets developed manifesting the pollination benefit to yield formation.

Only recently few studies explored relationships between pollination and nutrient availability and their effects on yield formation for important crop species showing, to our knowledge, no evidence for interactive mechanisms. Probably this is due by the fact that the manipulation of nutrient availability mainly consisted in mineral fertilization application: if the initial soil fertility is high enough, constraining effects on pollination caused by limits in the plant carrying capacity might be not visible. For example Klein et al. (2014) found no effects of fertilization on almond fruit set or yield, arguing that almond tree may had already accumulated sufficient nutrients for flowering and fruit maturation. Groeneveld et al. (2010) found that background high soil fertility caused the lack of direct effects of nutrient addition, or in interaction with pollination, on reproductive success in cacao crop. Bommarco et al. (2013) hypothesized that in agricultural ecosystems, limitations to yield production might be set by the lowest regulating or supporting service, even if other services could sustain higher levels of yield.

The management of ecosystem services needs a deep understanding of the mechanisms shaping yield formation, including tradeoffs and synergies between multiple ecosystem services. Our study provides important implications for estimating direct and interactive contribution to crop production of two important ecosystem services. In particular we highlighted the crucial role of soil fertility in supporting not only production of goods but also other services, such as pollination. We showed that poor soil input management that does not include strategies to maintain and enhance soil fertility might nullify or reduce the positive effects of an efficient pollination service. Several studies have already shown that agricultural intensification and land-use change have dramatically reduced soil fertility in agricultural systems and threaten associated soil services (Tilman et al. 2002, Barrios et al. 2007, Montgomery 2007). The negative effects of soil fertility loss on co-acting ecosystem services are difficult to estimate because it is a slow process, often hidden by the application of inorganic fertilizer that rapidly enhance crop production. Therefore there is an urgent

need for studies investigating consequences of soil management on soil services and its repercussion on other ecological processes. Interactions between above- and belowground services and processes need to be better understood to implement sustainable farming practices in agricultural ecosystems.

Acknowledgments

Supporting Information

Input management history in the long-term trial

Every 12 years the type of intensification has been modified to keep the experiment up-to-date (Table S1): in the first cycle (1964–1975), the intensification factor was irrigation vs. rainfed; in the second (1977–1988) intensive inter-annual successions and farmyard manure application vs. main crop only and residues incorporation; in the third and fourth (1990–2012) residues incorporation and liquid manure vs. residues incorporation without any organic fertilization. Farmyard manure was applied at average doses of 20 t ha^{-1} per year whereas liquid manure at a rate of 40 t ha^{-1} per year. All the soils selected received the same amount of inorganic fertilizers (70, 70, 90 kg ha^{-1} per year of N, P_2O_5, K_2O).

Table S1. Input management history from 1964 to 2012.

Cycle	Input intensity level	
	Low Input (LI)	High Input (HI)
(1964-1975)		
Crop	MC-IS	MC-IS
Residue incorporation	No	No
Irrigation	No	Yes
Farmyard manure (t ha^{-1}) [a]	20	20
Liquid manure (t ha^{-1}) [b]	0	0
(1977-1988)		
Crop	MC	MC-IS
Residue incorporation	Yes	No
Irrigation	No	No
Farmyard manure (t ha^{-1}) [a]	0	20
Liquid manure (t ha^{-1}) [b]	0	0
(1990-2012)		
Crop	MC	MC
Residue incorporation	Yes	Yes
Irrigation	No	No
Farmyard manure (t ha^{-1}) [a]	0	0
Liquid manure (t ha^{-1}) [b]	0	40

MC = Main crop; IS = inter-annual succession with forage crop.

[a] Average composition 20% dry matter, 0.5% N, 0.11% P; 0.58% K.

[b] Average composition 10% dry matter, 0.4% N, 0.13% P; 0.33% K.

Soil properties

The soil is a fluvi-calcaric cambisol (FAO-UNESCO, 1990), silty or sandy loam, with a pH of 7.8. Morari et al. (2008) studied the evolution of the soil chemical parameters in the long-term trial and developed a soil quality index (SQI) based on a small number of carefully chosen indicators (Andrews et al. 2002): available water content (AWC), water filled porosity (WFP, ratio between water content at -33 kPa and total porosity), pH, soil organic carbon (SOC), cation exchange capacity (CEC), available phosphorus (P_{av}) and exchangeable potassium (K_{ex}).

Soil collection

The experimental layout of the long-term trial consists in a split plot with three 3 blocks of 78 plots (7.8 x 6 m each, 234 plots in total), with the intensification level as the split factor. The soils used for the experiment were collected in February 2013 from 12 different plots (2 plots per block for each soil input level).

Chapter 4

Soil organic matter modifies nitrogen and drought effect on aphid performance in spring wheat

Tamburini G, van Gils S, Kos M, van der Putten W, Marini L
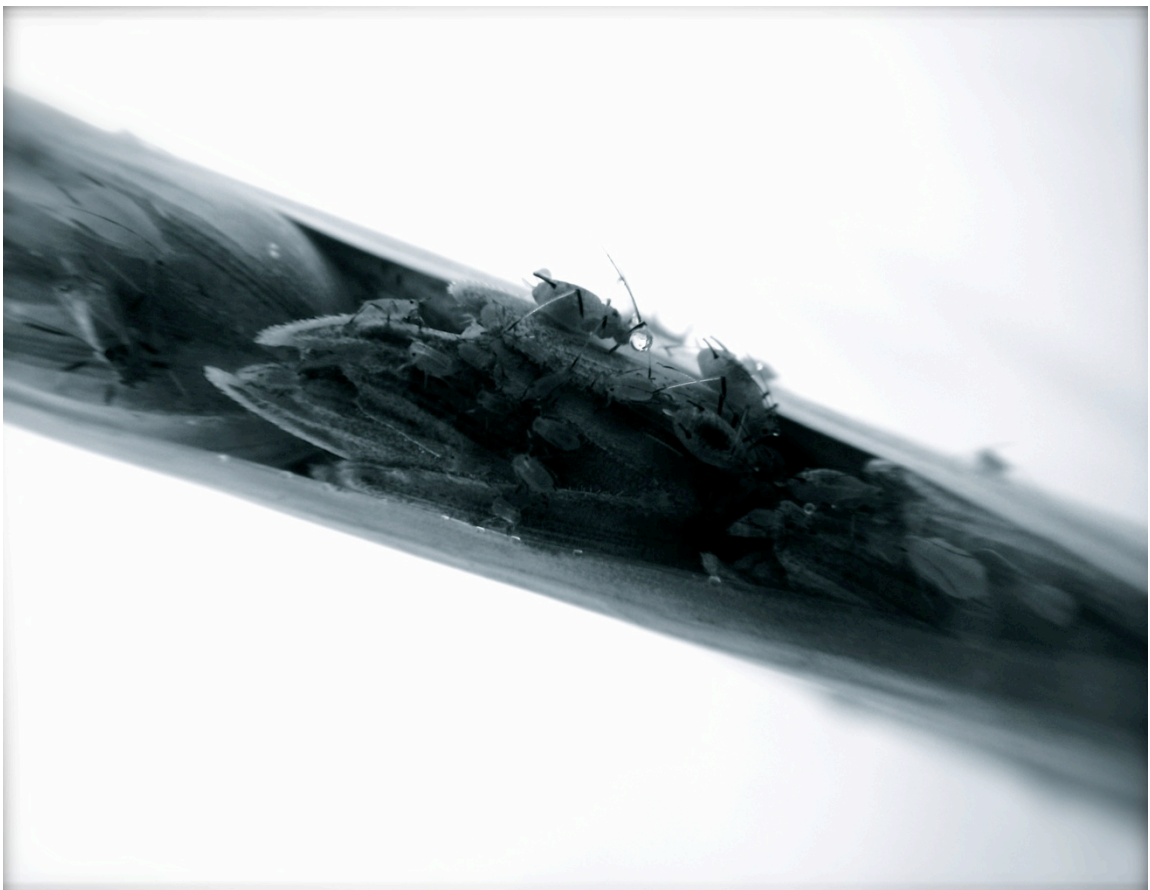
Abstract

Agricultural ecosystems receive a vast array of services and disservice that interplay together with farming practices and abiotic conditions in shaping yield. Long-term soil management influences nutrient and water retention capacity by altering soil properties (e.g. soil organic matter content). Drought and nitrogen inputs can interplay with the effect of long-term soil management in modifying plant-herbivore interactions. To explore the effects of soil fertility, input management and drought on aphid performance and crop production, we conducted a pot experiment in greenhouse adopting the English grain aphid, *Sitobion avenae*, on spring wheat as study system. We exposed infested wheat plants to contrasting levels of soil organic matter (SOM), mineral nitrogen fertilizer and water availability. We found that high fertilization levels under normal water condition led to the higher aphid biomass and crop growth, irrespective of SOM levels. High levels of SOM had minor positive effects on aphid performance than high nitrogen applications, maintaining similar crop growth. Drought condition was found to strongly decrease both aphid growth and crop growth, limiting the positive effects of higher nitrogen applications. Our study shows that different strategies to enhance soil fertility (SOM vs. inorganic fertilizer) might differently affect pest performance and the resulting yield production and that climate change can alter herbivore-plant interactions. These findings provide a new insight into how above- and belowground processes, farming practices and abiotic conditions interact in cropping systems.

Introduction

Agricultural ecosystems rely upon a suite of supporting and regulating soil services such as soil fertility and water regulation (MEA 2005) that are crucial for yield formation. At the same time agriculture is also affected by ecosystem disservices (e.g. pest damage) that constrain productivity or increase production costs (Zhang et al. 2007). Recently, a growing body of evidence has shown that ecosystem services and disservices interplay together with farming practices and abiotic conditions shaping yield (Classen et al. 2014, Wielgoss et al. 2014). Moreover, climate change has been forecasted to alter the provision of services by ecosystems (Schröter et al. 2005). The

mechanisms driving these complex systems are far to be completely understood (Seppelt et al. 2011), and comprehensive studies for many important crop species are still lacking. The implementation of sustainable farming practices able to maximize yield production and minimize detrimental effects on environment requires specific knowledge about interactions occurring between above- and belowground ecosystem processes, level of external inputs and abiotic environmental properties in order to successfully integrate the management of ecosystem services and disservices into crop production systems (Bennet et al. 2009, Bommarco et al. 2013).

Soil organic matter (SOM) is a recognized key indicator of soil quality and sustainability, as it greatly influences the provision of belowground ecosystem services, affecting soil structure and porosity, water infiltration rate, moisture holding capacity and plant nutrient availability (Magdoff and Weil 2004, Bot and Benites 2005). Agricultural intensification has been shown to decrease SOM through repetitive harvesting of crop biomass together with inadequate organic replacement (Paul et al. 1996) consequently constraining soil-based ecosystem services (Pan et al. 2009). Inorganic fertilizers are widely used to rapidly replace nutrient availability in the soil, although they have multiple negative side-effects on the environment (e.g. eutrophication, accelerated SOM decomposition rate). On the other hand, farming practices able to restore and maintain high level of SOM such as addition of manure and residue incorporation, need several years to be effective (Birkhofer et al. 2008). The interactions between long- and short-term strategies to enhance crop productivity (SOM vs. inorganic fertilizer) and their effects on aboveground processes are still poorly explored (Bommarco et al. 2013).

Water availability is one of the most important environmental factors driving ecological processes in agricultural ecosystems. It influences both plant growth and organisms at different trophic levels. Climate change models predict an increase in the frequency, duration and/or severity of summer droughts in different geographical areas of the planet (Solomon 2007, Dai 2011). Changes in precipitation patterns are likely to strongly influence agricultural ecosystems and the services they provide (Schröter et al. 2005). For example insect pests that feed on crops may diversely affect yield production under water stress condition. Drought can in fact alter herbivorous insect performance via changes in plant physiology (e.g. Hale et al. 2003,

Tariq et al. 2012). Water availability is therefore an important factor to take into account when considering interactions between ecosystem services and disservices.

The English grain aphid (*Sitobion avenae*) is a threat to cereal crops worldwide having significative economic impact on yield (e.g. Larsson 2005). Sap-feeding insects are known to strongly respond to host plant quality (Huberty and Denno 2004). Nitrogen level in the diet of herbivorous invertebrates is in fact the most important factor limiting their performance (Awmack and Leather 2002). Many authors found positive correlation between aphid fecundity and nitrogen levels in their host plant (e.g. Schütz et al. 2008, Nowak and Komor 2010). Increased nitrogen fertilizer applications to wheat have been shown to increase aphid performance such as development time and fecundity (Aqueel and Leather 2011). Furthermore many studies focused on the effects of drought on aphid growth. Prolonged water stress condition in plants is known to affect aphids negatively (Huberty and Denno 2004). Although drought increases concentration of nitrogen in the plant tissues (Johnson et al. 2011), phloem properties in stressed plants (e.g. increased sugar concentration, sap viscosity) and decreased turgor compromise aphid feeding activity and consequently their performance (Hale et al. 2003, Pescod et al. 2007).

Aphid performance and its effect on plant growth are therefore expected to respond to soil quality, fertilizer applications and water availability. Nevertheless, these factors have never been tested together. In a greenhouse experiment we exposed wheat plants infested with *S. avenae* to contrasting levels of SOM, nitrogen fertilizer and water availability. Our study examines for the first time potential interactions and tradeoffs between soil services (soil fertility and water holding capacity), input management and drought on an important pest of cereal crops and the related effects on plant growth. The study was specifically designed to test for interactions between services and not to unveil the biological mechanisms behind the response of aphids to plant quality. Our results will provide a first insight on the consequences of adopting different farming strategies to enhance yield production on aboveground processes in a world exposed to climatic changes.

Materials and methods

Soil organic matter

The soil was collected in May 2013 at the experimental field of Wageningen UR located in Vredepeel (Limburg, The Netherlands) where a long-term experiment on soil health has been underway since 2006 (Korthals et al. 2014). The soil texture is 1.1% clay, 3.7% silt and 94.9% fine sand. In order to investigate the effect of SOM on aphid performance keeping unchanged all the other physical and chemical properties, the soil was collected from the same plot at two different depths: 0-20 cm (c. 3.4% SOM, from Korthals et al. 2014) and from C horizon (0% SOM) and then stored in plastic bags under the same environmental condition. In December 2013 upper soil was mixed with 10% soil from deeper layers (high SOM) and with 50% soil from deeper layers (low SOM). We therefore achieved two levels of SOM, 3.1 and 1.7%, reflecting realistic soil conditions in crop systems. After being sieved (1.0 cm mesh) the soils were set in 112 pots (5 L, 56 pots per SOM treatment).

Experimental set-up

Six pairs of spring wheat seeds (*Triticum aestivum* var. Tybalt) were sown into the prepared pots and randomly placed in a climate controlled greenhouse at 60% RH, 16 L : 8 D, and 20 ± 1 °C at day and 14 ± 1 °C at night. Overhead lighting (sodium lamps, SON-T Philips, Eindhoven, The Netherlands) was supplied to ensure a minimum light intensity of 200 W/m^2 during the light period. After emergence, the best performing plant per pair was selected and the other clipped and removed in order to have six plants per pot. Plants were watered three times per week, with quantities as required, before applying drought treatment. *Sitobion avenae* (Hemiptera: Aphidae) adults were obtained from the Laboratory of Entomology, Wageningen University, and reared in a growth chamber maintained at 20 – 22°C in a 16 h/8 h day/night regime. To support aphid population, new plants were added roughly every two weeks, and old plants were removed after the aphids had settled on the new plants.

   Drought. To assess the effect of drought on aphid performance, two weeks old wheat plants were assigned to two different irrigation regimes: 300 ml water/week (normal water condition), and 150 ml/week (drought stress). The mentioned amounts

of water were selected at the beginning of the drought treatment in order to maintain leaf turgor (normal water condition) and to allow the soil to dry until 70% of the plants showed wilting signs (drought stress). Individual pots were placed on 3 cm deep plastic saucer to retain any excess water.

Fertilization. Nitrogen fertilizer was added in the form of calcium nitrate $Ca(NO_3)_2$ at two different levels: 0.3 g/pot (high fertilization) and 0.03 g/pot (low fertilization) reflecting an amount of nitrogen of 130 and 13 kg/ha respectively. Following normal farming practices, nitrogen application was split in two occasions, at plant sprouting (60%) and 4 weeks later (40%). All the treatments were randomly distributed over the greenhouse. Pots were randomly rearranged once before the aphid inoculum and not moved again to not interfere with aphid growth.

A single adult apterous aphid of standard age and size was carefully placed with a fine brush on the first leaf of each plant (6 aphids per pot) three weeks after sprouting. Each pot was placed individually in gauze nets. In order to measure aphid fecundity and development time (number of days between birth and reproduction), clip cages were placed on one adult aphid per pot. Once reproduced, only one neonate nymph was left inside the clip cage. The adult and the other nymphs (if present) were gently placed on another leaf. Clip cages were monitored daily and the nymph development time was scored 11 days after the clip cage placement, the day by which most of individuals had reproduced. Five days later, fecundity was measured (number of third-generation-offspring) and the clip cages removed. Four weeks after inoculum all the aphids from each pot were removed with a soft brush, collected and stored at 4°C for weighting (aphid fresh biomass). One week later (57 days after sprouting) the experiment was destructively sampled. Harvested plant tissue was separated into roots, shoots and ears. The roots were washed to remove adhering soil residues. Subsequently, the plant material was dried at 60°C for 24 h and weighted. The plant biomass (root, shoot and ear) refers to the total biomass per pot (6 plants).

Statistical analysis

Data were analyzed with general linear models using the R statistical language, version 3.1.1 (R Development Core Team 2013). We investigated the effect of SOM (high vs. low content) fertilization (high vs. low fertilization) and drought (standard water regime vs. drought stress) and their interactions on aphid performance and plant

growth (root, shoot and ear biomass, three different models). Each treatment combination had 14 replicates. The aphid biomass was log transformed before the analyses to achieve normal distribution of model residuals. Since there were missing values, the analyses regarding aphid development time and fecundity were based on data from 94 pots, and those regarding aphid biomass on 109 pots. We first built full models and then we simplified them by removing not significant interactions. Tukey multiple comparison test was applied to determine significant differences among treatments.

Results

Aphid performance

Fertilization treatment was found to affect development time: nymphs developed faster when higher nitrogen input was deliver to the plants (Tukey test; development time under low fertilization 11.1 ± 0.3 days, under high fertilization 10.1 ± 0.3; Table 1). Fecundity was significantly higher under high SOM condition (7.4 ± 0.5 and 4.9 ± 0.5) and positively affected by high fertilization input only on unstressed plants (interaction fertilization x drought). Under drought stress condition, fertilization treatment did not affect fecundity. The effects of the three treatments and their interactions on aphid performance were stronger and more evident on the aphid biomass measured at the end of the experiment. High SOM and high fertilization generally increased aphid biomass by 30 and 110% compared to low levels of the same treatments, whereas drought significantly reduced it (21.5%) respect to standard water regime condition. However, fertilization interacted with both SOM and drought: the increment in aphid biomass owing to higher SOM content was observable only under low fertilization condition (SOM x fertilization; Fig. 1*a*) whereas the effect of higher nitrogen input was higher under standard water regime (fertilization x drought; Fig. 1*b*).
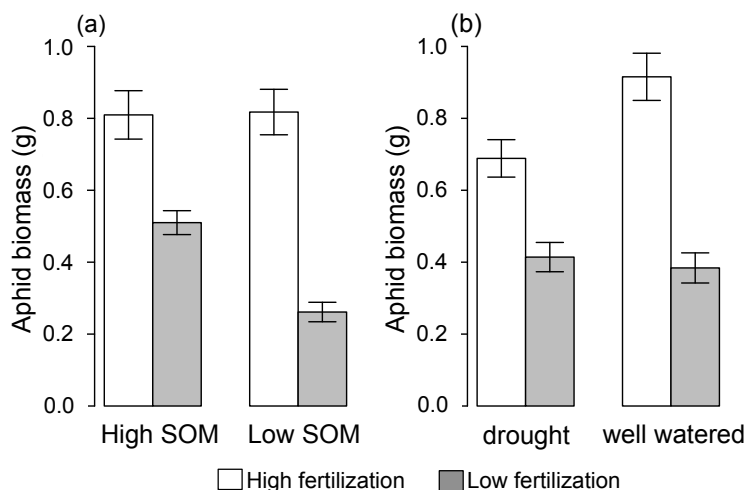
Fig. 1. Effects (mean ± SEM) of SOM, fertilization and drought on aphid biomass; high (+) and low (-) levels of SOM (S), fertilization (F); bars with the same letter are not significantly different ($P > 0.05$). Interactions between (a) SOM and fertilization and (b) drought and fertilization; high (white bars) and low (grey bars) levels of fertilization.

Plant performance

All the treatments significantly affected plant growth (Fig.2). At the end of the experiment biomass of both roots and shoots was increased by high SOM and fertilization and reduced by drought. However, drought neutralized the positive effects of both high levels of fertilization for root biomass (fertilization x drought) and high levels of SOM for shoot biomass (SOM x drought). Fertilization treatment influenced shoot biomass only in unstressed plants under low levels of SOM (SOM x fertilization x drought). Furthermore drought generally reduced ear biomass and neutralized the positive effect of high fertilization levels (fertilization x drought). High levels of SOM compensated the reduction in ear biomass owing to low fertilization in well watered plants (SOM x fertilization x drought).

Table 1. Effects of soil organic matter (SOM), fertilization (Fert), drought (Drought) and their interactions on performance parameters of *Sitobion avenae* and on plant growth.

|	SOM		Fert		Drought		SOM Fert		xSOM Drought		xFert Drought		xSOM x Fert x Drought	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Aphid performance														
Development time	0.78	0.387	5.66	0.027	0.34	0.562	-	-	-	-	-	-	-	-
Fecundity	8.42	0.004	4.87	0.029	0.80	0.371	-	-	-	-	4.51	0.036	-	-
Final biomass	23.53	<0.001	93.55	<0.001	5.11	0.025	15.36	<0.001	-	-	11.66	<0.001	-	-
Plant performance														
Root	8.53	0.004	5.03	0.027	10.85	0.001	-	-	-	-	4.84	0.029	-	-
Shoot	18.32	<0.001	19.66	<0.001	480.6	<0.001	4.36	0.039	4.86	0.029	1.03	0.311	4.59	0.034
Ear	1.13	0.289	1.03	0.311	261.1	<0.001	3.82	0.053	2.87	0.093	41.91	<0.001	4.63	0.033
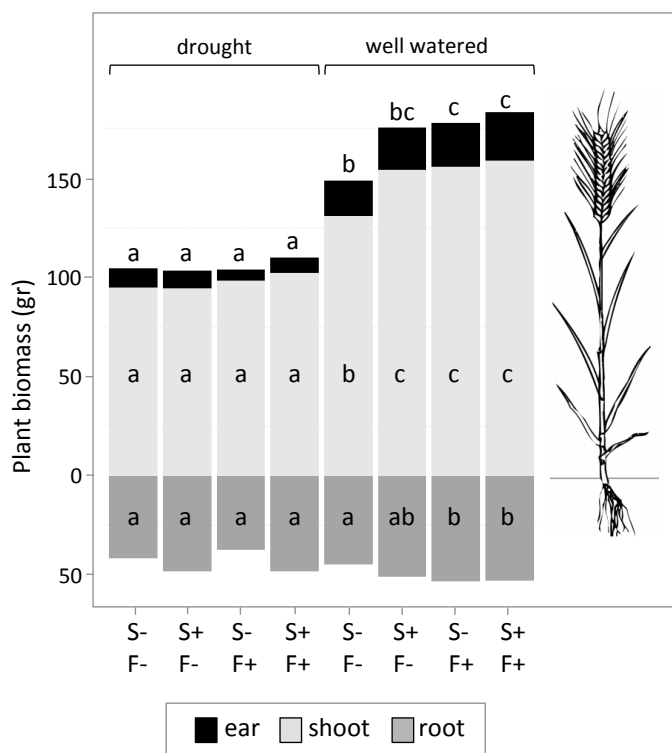
Fig. 2. Effects of treatments on the biomass of wheat plants (roots, shoots, ears) at the end of the experiment; high (+) and low (-) levels of SOM (S), fertilization (F). Bars with the same letter are not significantly different (*P* > 0.05).

Discussion

Although aphid performance is known to respond to fertilizer application and to host water stress, the impact of soil organic matter on pest growth and its interaction with input management and water availability was never tested before. We explored the combined effects of SOM, nitrogen fertilizer and drought on the performance of the grain aphid and the consequent effects on wheat growth. We found that high fertilization levels under normal water condition led to the higher aphid biomass, irrespective of SOM levels. Nevertheless high levels of SOM also increased aphid performance, but this effect was visible only under low levels of fertilization. Drought conditions negatively affected *S. avenae* and limited the positive effect of high fertilizer levels on the pest performance. Ear biomass was primarily affected by drought and secondly by nutrient availability. Under normal water conditions, although high fertilization levels scored the highest yield, the combination of high SOM and low fertilization did not show differences in ear biomass. Even if understanding the relationships between ecosystem services and disservices is considered important for a more sustainable management of cropping systems (Bennet et al. 2009), the interactions between above and belowground processes are still poorly understood (Wardle et al. 2004). Our study gives a first insight on possible interactions occurring between soil services, an aboveground herbivore performance, input management and drought and the consequent effects on crop growth.

Increased fertilizer inputs to wheat plants led to the higher aphid performance, decreasing development time, and increasing fecundity and final biomass. As mentioned before, the positive effect of nitrogen fertilizer on aphid growth parameters via enhanced plant quality has been already shown in many studies (Moon et al. 1995, Duffield et al. 1997, Khan and Port 2008). For example Aqueel and Leather (2011) found that N-fertilizer application had a positive effect on fecundity, longevity and adult weight of *S. avenae*. High levels of SOM increased fecundity by 30% and final biomass by 35% but the latter effect was visible only under low fertilization levels. These results indicate that SOM acted as a source of crop nutrition, altering plant quality and therefore affecting aphid performance. Even if smaller in size (e.g. no effect on development time), the effects of SOM on aphid growth were similar to those of fertilization. SOM is in fact known to provide nitrogen for crop growth and

to increase the availability to plants of many essential micronutrients (Matus and Rodríguez 1994, Loveland and Webb 2003). Nevertheless, high levels of fertilizer application canceled the gain in aphid biomass owing to high SOM observed under low levels of fertilization. The interaction between SOM and fertilization has been explored mainly in the contest of crop production. For example Zhukov et al. (1993) found similar patterns in winter wheat: under unfertilized condition, crop production depended of SOM content, whereas fertilizer application nullified the effect of SOM. In our experiment aphid populations probably reached a biological limit in growth rate caused by high fertilization application, hiding the effect of higher levels of SOM.

Drought strongly affected both aphid fecundity and final biomass. These results are in line with previous findings showing decreased aphid performance in response to water stress in the host plant (e.g. Sumner et al. 1986). Moreover, drought limited the positive effect of high fertilizer levels on aphid growth respect to standard water regime. Although it is know that nitrogen uptake by wheat plants is constrained by limited water availability (Barraclough et al. 1989), the lack of higher fertilization effect on aphid performance indicated that the prolonged drought condition might have primarily affected the ability of the aphids to access to plant resources via reduced turgor and increased sap viscosity (Hale et al. 2003). Surpriseling higher levels of SOM did not reduce the negative effect of drought on aphid growth. SOM is in fact expected to increase soil water holding capacity, increasing water availability to plants under drought conditions (Bot and Benites 2005). However, soil texture is also known to strongly influence water holding capacity and its interaction with SOM has been explored in previous studies, with contrasting results (e.g. Bauer and Black 1992; Hudson 1994).

As expected, the biomass of roots and shoots increased under high levels of fertilizer and SOM and decreased under drought condition. Interaction between fertilization and SOM in shoot biomass and fertilization and drought in root biomass were similar to those observed for aphid growth. Moreover high level of SOM increased shoot biomass only under normal water conditions whereas it increased ear biomass only under low fertilization in well watered plants. All together these results suggest again that high level of fertilization generally canceled the positive effect of high level of SOM and that water availability had a strong effect on nutrient uptake by

plants (e.g. Barraclough et al. 1989). In our experiment we were not able to disentangle the direct effects of treatments on plants from the indirect effects via aphid performance changes and pest damage. However, we found that under normal water conditions the combination of high SOM and low fertilization led to the same average ear biomass than the high fertilization treatments. The increase in yield expected under higher fertilizer applications might has been limited by the higher aphid growth caused by the same treatment. These results suggest that long-term strategies to enhance soil fertility (high SOM, low mineral fertilization) may lead to higher nitrogen use efficiency by infested plants, limiting pest development and maintaining high the production. Modern intensive crop management makes wide use of inorganic fertilizers to ensure high yields, often ignoring negative side effects such as nitrogen leakage to ground water or increased herbivore performance. Furthermore nitrogen is a major production cost in agriculture. Finding options to reduce mineral fertilizer applications in agroecosystems is of primary importance in order to promote ecologically and economically sustainable crop management.

Tradeoffs among ecosystem services and disservices can lead to unwanted outcomes when management considers only one service at time (Bennet et al. 2009). Our study shows that different strategies to enhance soil fertility differently affect pest performance and the resulting yield production. Under normal water conditions, long-term strategies to enhance soil fertility have minor positive effects on aphid performance than high nitrogen applications, maintaining similar yield production. Moreover drastic changes in precipitation patterns resulting in prolonged droughts might disrupt the capacity of crops to access nutrients both applied by farmers and naturally present in the soil, altering herbivore-plant interactions. Our findings could be used to forecast the consequences of adopting long- or short-term strategies to enhance crop productivity (SOM vs. inorganic fertilizer) on aboveground pest performance and can be included in decision-making tools for a more sustainable management of cropping systems.

Acknowledgments

Chapter 5

Conservation tillage enhances natural pest control in winter cereal crops

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Chapter 5

Abstract

Biological control (BC) of pests by natural enemies is a major ecosystem service delivered to agriculture and it depends on multiple factors acting from the field to the landscape scale. Complex landscapes support more diverse and abundant communities of natural enemies and low-intensive farming practices such as diverse crop rotation can locally enhance control of pests. Much less is known about the effect of soil management on BC and about its potential interaction with the landscape. In a field exclusion experiment, we examined the relative effect of conservation vs. conventional tillage on the BC of aphids along a landscape complexity gradient. We evaluated the relative contribution of three different guilds of natural enemies in winter cereal crops: birds, flying insects/vegetation-dwelling predators and ground-dwelling predators. On the one hand, conservation tillage management supported more abundant arthropod communities and higher BC, in particular that provided by ground-dwelling predators. On the other hand, high proportion of semi-natural habitats in the landscape enhanced the BC provided by vegetation-dwelling predators. Moreover, complex landscapes showed higher parasitism rate but only in fields managed with conventional tillage. Our results emphasize the importance of considering both the local habitat and the landscape quality when planning strategies for maximize the BC service in agroecosystems, and of adopting a functional guild approach to reveal hidden processes behind the provision of ecosystem services.

Introduction

Insect natural enemies have been estimated to be responsible for the 50-90% of the pest control occurring in crop fields (Pimentel 2005) saving $4.5 billion per year in agricultural crops in the United States alone (Losey & Vaughan 2006, Power 2010). A large body of evidence suggests that agricultural intensification is threatening the BC service (Winqvist et al. 2001, Kleijn et al. 2009, Geiger et al. 2010, Bommarco et al. 2013). Moreover, despite the steadily increasing use of chemical pesticides worldwide during the last 50 years, estimated crop losses to animal pests have also significantly increased (Oerke 2006). In a future where agriculture will face severe environmental,

economic, and social challenges (Foley et al. 2005, MEA 2005), improving the BC service provided by natural enemies arises as an ecologically and economically promising solution.

BC depends on multiple factors acting from the local to the landscape scale (Tscharntke et al. 2007, Rusch et al. 2010,). Various farming practices have been found to affect natural enemy communities and the associated BC. For instance, organic farming has been often shown to locally support higher BC compared to more intensely managed systems (Crowder et al. 2010, Wingvist et al. 2011). However, the effect on BC of other key agricultural practices has not been tested yet (Rusch et al. 2010). In particular, much less is known about the mechanisms linking soil management (e.g., crop rotation, soil tillage) to aboveground ecosystem services such as BC (Rusch et al. 2013). Conservation tillage is a farming practice that includes all the techniques characterized by non-inversion of soil often combined with a permanent vegetation cover of the soil. Globally it is applied on 45 million ha, mainly in North and South America but its adoption is increasing worldwide (Holland 2004). It has been pointed as a promising soil management able to minimize negative impacts of farming operations with several beneficial consequences on soil structure, hydrology and biodiversity (Kladivko 2001, Holland et al. 2004, Collette et al. 2011, Soane et al. 2012). Which are the actual effects of conservation tillage on BC service and whether it interacts with landscape composition is, by now, only speculative.

Landscape composition is a further key factor shaping natural enemy communities. Complex landscapes with large proportions of semi-natural habitats provide a more stable environment than landscapes dominated by annual crops. Semi-natural habitats can maintain populations of alternative hosts and preys for parasitoids and predators, protecting natural enemies against crop disturbance, offering additional nectar resources during the vegetative season and shelter during overwintering (Denys and Tscharntke 2002, Bianchi et al. 2006). A growing number of studies have shown how complex landscapes support more diverse and abundant communities of natural enemies (Bianchi et al. 2006, Chaplin- Kramer et al. 2011, Chaplin- Kramer & Kremen 2012, Martin et al. 2013, Rusch et al. 2013; Winqist et al. 2011). However, soil management has been rarely considered in relation to landscape complexity.

In a field exclusion experiment, we examined the relative importance of tillage management and landscape complexity on the BC of aphids provided by three different guilds of natural enemies in winter cereal crops: birds, flying insects/vegetation-dwelling predators and ground-dwelling predators. In winter cereal crops the food web interactions contributing to the BC of aphid population include specialized aphid-suppressors such as parasitoids and more generalist predators such as carabid beetles or cursorial spiders (Brewer & Elliott, 2004). The role of birds in controlling pest population or in constraining BC by intraguild predation has been studied for several systems (Martin et al. 2013; Railsback and Johnson, 2014) but never in winter cereal crops. Quantifying the level of BC provided by different natural enemy guilds might be considerably important if we are to plan sustainable management strategies in agricultural landscapes (Loreau et al. 2003).

This study is among the first experimental ones trying to link soil management, landscape complexity, natural enemy communities and BC service. Using a design where landscape complexity and tillage management (conservation vs. conventional tillage) were statistical orthogonal factors, we hypothesized that: (1) conservation tillage management will enhance BC service and in particular the BC provided by ground-dwelling predators. Populations of generalist predators such as carabid beetles, spiders and rove beetles, are expected to be enhanced under conservation tillage conditions because of reduced soil disturbance, increased surface residues and greater weed diversity, that all together provide a more suitable environment at multiple life stages (Ball et al. 1998, Kendall 2003, Thorbek & Bilde, 2004, Soane et al. 2012); (2) all natural enemy guilds should influence the control of aphid population in the field; (3) the level of BC services will increase with landscape complexity which may act additively or synergistically with local tillage management.

Material and methods

Study area and sampling design

The study was conducted between April and June 2014 in 30 fields located in the agricultural landscape of the Udine province, NE Italy (longitude 46°4′0″N, latitude 13°14′0″E). This region is an extensive lowland area (c. 615 km^2) characterized by

temperate climate with a mean annual precipitation of c.1300 mm and a mean annual temperature of 13°C. Our sampling consisted of 15 pairs of neighboring winter cereal fields. Within each pair, one field was managed with conservation tillage and the other with conventional tillage (distance range: 0-400 m). Field pairs were separated by at least 1 km except for two that were distant by 300 m. Of the 15 pairs, seven were planted with winter wheat and 8 with barley in autumn 2013. Crop species was consistent within the pairs and environmental characteristics comparable because of the short distance between fields. Conservation tillage included all techniques characterized by non-inversion of soil for at least 5 years (10 years on average, ranging 5 to 20 years), whereas under conventional tillage the seedbed was prepared by moldboard plowing (30 cm depth). Conservation tillage management also included the adoption of cover crops through the year. The most used cover crop was grass (*Lolium multiflorum* L.) sown after the summer crops such as Maize.

In each field, we identified one 60x20 m strip located on one side of each field. Within each pair, the strips bordered with an edge habitat of similar structure and composition (either a grass margin or a hedgerow). Each strip was divided into 6 10x20 m plots, of which the outer ones were considered as buffer zones. Among the four left, two non-adjacent subplots were randomly selected for the exclusion experiment and the natural enemies sampling. Nitrogen fertilizer was applied to the selected plots following farming recommendations (80 kg ha^{-1} in two applications). No chemical pesticides and herbicides were applied on the experimental plots.

Landscape analysis

Eight nested spatial scales were considered to measure the landscape composition around each plot within windows of varying radii (95, 135, 190, 265, 375, 530, 750 m and 1060 m). Each increment in scale doubled the surrounding area from 0.028 (95 m) to 3,5 km^2 (1060 m) (Fig. 1b). Field pairs were selected along a gradient in landscape complexity ranging from 1.2 to 22.4 % of semi-natural habitats (forests, shrubby areas, grasslands, hedgerows, and field margins) in a 1060-m radius around each field. The increase in % semi-natural habitats was consistent with the increase in landscape complexity (correlation index = 0.62). ArcGIS 9.3 was used for landscape analyses of regional land use maps, verified and ameliorated with aerial photographs to increase class discrimination accuracy.

Exclusion experiment

Exclusion treatments consisted of cylindrical cages (height: 1.5 m, diameter: 0.3 cm) designed to exclude combinations of three different guilds of natural enemies: flying insects and vegetation-dwelling predators F (parasitoids, flying beetles, larvae of ladybirds, hoverflies,lacewings, and web spiders); ground-dwelling predators G (carabid beetles, cursorial spiders and rove beetles); birds and other vertebrates B. Flying insects and vegetation-dwelling predators were excluded using polyester fine mesh (mesh size: 1 mm), birds and other vertebrates with an anti-bird net (mesh size: 1.5 cm). In treatments excluding ground-dwelling predators a plastic ring 0.3 m in diameter and 0.25 m in height was dug 10 cm into the soil and an 8-cm-wide band of insect glue was applied along the perimeter. One live pitfall trap was placed inside each barrier, checked and eventually emptied for the duration of the experiment. In the other exclusion treatments, access of ground-dwelling predators was guaranteed fixing nets to the support poles 5 cm above the ground. An opening at the side of the cages sealed with blinder clips was used to examine plant material during the experiment. A total of six exclusion treatments were installed and randomly located within one plot in each field. Natural colonization of crop plants by aphids can be very irregular both in time and in space. Preliminary surveys showed comparable low density of naturally occurring aphids among the fields that was always below the economic threshold, *i.e.* no outbreak was present in our experimental fields. To avoid bias due to differences in the initial aphid abundance, we inoculated field plants with aphids reared in lab condition. Ten days before the inoculation, seven tillers were selected for each treatment cage to standardize the plant density. The selected plants and the ground were cleared from natural enemies and then covered by a nonwoven fabric supported by sticks to exclude recolonization. Aphid material (*Sitobion avenae*) was provided by Katz Biotech AG® and directly placed on the plants (c. 150 aphids per treatment including both adults and nymphs). Inoculation was done at the heading stage of the cereals (BBCH50-55) during good weather conditions (absence of precipitation and strong wind, minimum air temperature 18°C). After 5 days established aphids were counted and plants were re-inoculated if needed (i.e. cages with less than 15 aphids). After 10 days from the first inoculation aphids were counted (time 0) and exclusion treatments started. Aphids were counted visually in each treatment at two occasions, 5 and 10 days after the onset of the exclusion

experiment. In each plot, for all the 5 exclusion combinations (-G, -B, -F-B, -G-B and an open treatment O) for each 5 days period, suppression of aphids due to natural enemies was quantified as the proportion of aphids predated in the exclusion treatment compared with the aphid population growth in the close treatment (-F-G-B), calculated following the methodology of Gardiner et al. (2009):

$$Biological\ Control\ index = \frac{N_{treatment\ 5}}{R_{close} \times N_{treatment\ 0}}$$

where $N_{treatment\ 5}$ is the number of aphids counted in each exclusion treatment after 5 days, R_{close} is the aphid population growth in the close treatment and $N_{treatment\ 0}$ is the number of aphids in each exclusion treatment at the beginning of the 5 days period. This BC index ranges from 0 to 1 (0: no net loss of aphids; 1: 100% of aphids predated). Where the index was found to be negative (more aphids in the exclusion treatment than in the close one; 10 cases out of 240) a value of zero was assigned to these cages as this indicates no effective BC (Gardiner et al. 2009, Rush et al. 2013). In all the treatments where flying insects were not excluded (-G, -B, -G-B, O) parasitized aphids were visually counted after 10 days from inoculation. Parasitism was calculated as the ratio of mummies to total aphids (mummies + non-parasitized aphids). Just before the onset of the exclusion experiment a local storm event damaged the cages in 3 field pairs (6 fields) compromising the aphid establishment. The analyses regarding the exclusion experiment were thus based on data from 12 field pairs (24 fields).

Natural enemy sampling

In each field, vegetation-dwelling predators were visually monitored once, three days after the onset of the experiment. The sampling was conducted along two 20-m transect inspecting 50 randomly chosen tillers each. Abundance of flying beetles, web spiders, larvae of hoverflies, ladybirds and lacewings was observed as the total number of individuals per 100 tillers. Ground-dwelling predators were caught with three plastic pitfall traps per plot (9.5 cm in diameter and 13 cm deep) placed along a linear transect spaced at 3 m intervals. The pitfall traps were filled with 150 ml of 50% ethylene glycol. Plastic roofs fixed with nails to the soil prevented flooding by rain. The first sampling period coincided with the exclusion experiment (10 days) and the second lasted for the following 10 days. Invertebrates were preserved in 70%

ethanol. Abundance of carabid beetles, rove beetles and cursorial spiders was measured as the total number of individuals per field (the total catch of the three pitfall traps for each sampling period). The pitfall traps in one field were disrupted during the second sampling period. The analyses regarding the pitfall catches were thus based on data from 30 fields for the first sampling period and from 29 fields for the second.

Data analysis

The BC index, parasitism, predator abundance and the relation between BC index and predator abundance were analyzed using general linear mixed-effects models (lme, 7 models) and generalized mixed linear model (glmer, 1 model) (Table 1). We first built full models and then we simplified them by removing one-by-one the non-significant interaction terms. We used traditional analyses based on p-values due to the very low collinearity between our factors.

BC index (model 1) was calculated for each 5-day-period of the exclusion experiment (from time 0 to the 5th day and from the 6th to the 10th day) for each exclusion treatment. Two data points, highly influential outliers, were excluded from the analyses. Tukey multiple comparison test was applied to determine significant differences among exclusion treatments. Parasitism and abundance of ground-dwelling predators were log-transformed to achieve normal distribution of model residuals. Abundance of carabid beetles, rove beetles and cursorial spiders were analyzed, separately (model 3,4 and 5, respectively). Because of the large amount of zeros in the data, abundance of vegetation-dwelling predators was analyzed as one group (sum of ladybirds, hoverflies and web spiders) with generalized

mixed linear model with a poisson distribution (model 6).

To test the relation between abundance of natural enemies and the BC recorded during the exclusion experiment, we calculated the total BC index (from time 0 to the 10th day) and the predator: prey ratio (Thies et al., 2011). For vegetation-dwelling predators (model 7) the ratio was calculated as the number of predators recorded divided by the number of all the aphids (both alive and parasitized) counted in each treatment at the end of the exclusion experiment (10 days from the inoculation). BC index data came from both -G and -G-B exclusion treatments since

we found no significant difference in aphid predation between the two (see the results). For ground-dwelling predators (model 8) the ratio was calculated as the number of predators caught in the pitfall traps after 10 days divided by the number of aphids counted in each treatment (-F-B). For both vegetation- and ground-dwelling predators the predator: prey ratio was log-transformed (Brose et al. 2006). Where the landscape variable was included in the models, analyses were performed at all spatial scales between 95 and 1060 m around the fields. Only scales that gave significant main effects and interactions were presented in the results. The analyses were performed using the "nlme" and "lm4" packages (Pinheiro et al. 2013) implemented in R Statistical Software 3.1.1 (R Development Core Team 2013).

Table 1. Attributes of models used in the analyses (treatment, exclusion treatment; tillage, tillage management; counting, counting period; sampling, sampling period; pair, field pair).

n.	transformation	model distribution	response variables	explanatory variables	random effects
1	-	normal	BC index	treatment + tillage + % semi-natural + interactions	counting/crop/pair/plot
2	log(y+0.01)	normal	Parasitism rate	tillage + % semi-natural + aphid density + interactions	crop/treatment/pair/plot
3,4,5	log(y+1)	normal	Ground-dwelling predators' abundance (carabid beetles, cursorial spiders, rove beetles)	tillage + % semi-natural + interactions	sampling/crop/pair
6	-	poisson	Vegetation-dwelling predators' abundance	tillage + % semi-natural + interactions	crop/pair
7	-	normal	BC index (Vegetation-dwelling predators)	predator: prey ratio.	crop/treatment
8	-	normal	BC index (Ground-dwelling predators)	predator: prey ratio.	crop

Results

An average of 57±49 aphids successfully colonized the plants in each treatment. There was no significant difference in initial number of aphids between exclusion treatments and between tillage managements at the beginning of the experiment. Overall predation was 11% higher in fields managed with conservation tillage respect to those managed with conventional tillage. The effect of natural enemy guild on BC was, however, influenced by tillage practice (Table 2).The exclusion of birds and other vertebrates did not lead to significant differences in BC compared to the same treatments in which they were not excluded (-G, O). In the fields managed with conventional tillage, BC index was 1.3%, 11.5%, 8.2% and 20.5 % lower in the treatments -B, -G, -B-G and -F-B respectively, compared to open treatments O (Figure 1). In the fields managed with conservation tillage BC index was 4.2%, 16.9%, 24.6% and 43.0 % lower in the treatments -B, -G, -B-G and -F-B respectively, compared to O. Only the BC provided by ground-dwelling predators (-F-B) significantly differed between tillage practices, being 22.6% higher in fields managed with conservation tillage. Vegetation-dwelling predators showed only an indication of higher predation in conventional tillage fields (p=0.105, from Tukey multiple comparison test). The effect of natural enemy guild on BC also interacted with landscape composition (Table 2). BC index best responded to landscape complexity at 190 m scale. From simple to complex landscapes, BC index increased only in treatments excluding ground-dwelling predators (interaction treatment x % semi-natural, Figure 2). This result indicates that the strength of BC was higher in complex than in simple landscapes mainly for vegetation-dwelling predators, for which BC index increased from ~ 0.4 in simple landscapes to ~ 0.7 in complex landscapes. On the contrary for ground-dwelling predators BC index did not show significant variation along the landscape complexity gradient. We found no significant interaction between tillage management and landscape complexity related to BC index.

Table 2. Results of (generalized) linear mixed effects models relating the BC index, parasitism, predator abundance, the relation between BC index and predator abundance to explanatory variables.

Explanatory variables	nDF	dDF	Test	P	
BC index (model 1)			F		
treatment	4	174	18.98	<0.001	***
tillage	1	20	10.14	0.004	**
% semi-natural (190 m)	1	20	1.95	0.177	
treatment x tillage	4	174	2.61	0.036	*
treatment x % semi-natural (190 m)	4	174	2.56	0.039	*
Parasitism (model 2)			F		
n. of aphids	1	33	2.86	0.099	
tillage	1	33	7.75	0.008	**
% semi-natural (750 m)	1	33	0.54	0.466	
tillage x % semi-natural (750 m)	1	33	4.72	0.037	*
Ground-dwelling predators abundance			F		
carabid beetles (model 3)					
tillage	1	26	5.98	0.021	*
cursorial spiders (model 4)					
tillage	1	26	10.28	0.003	**
rove beetles (model 5)					
tillage	1	26	0.13	0.713	
Vegetation-dwelling predators abundance (model 6)			Deviance		
tillage	1	-	-2.81	0.004	**
BC index (vegetation-dwelling predators) (model 7)			F		
predator : prey ratio	1	39	6.30	0.001	**
BC index (ground-dwelling predators) (model 8)			F		
predator : prey ratio	1	10	8.21	0.003	*

Explanatory variables are exclusion treatment (five levels of natural enemy exclusion), tillage management (conservation or conventional) landscape complexity (% semi-natural habitats in a 190 or 750-m radius around plots) and their interaction. dDF, denominator degrees of freedom; nDF, numerator degrees of freedom. Significance codes: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.
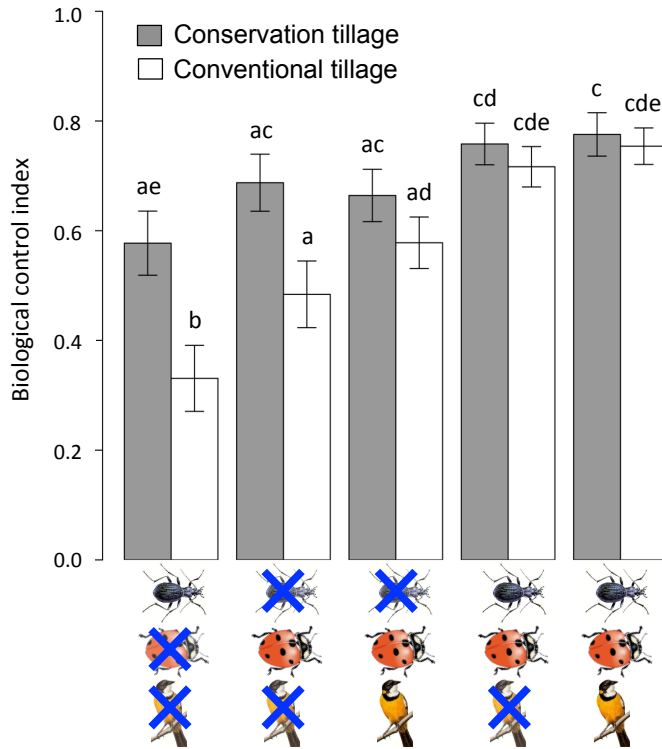
Fig. 1. Effects of natural enemy exclusion on means (± SEM) BC index per exclusion treatment and tillage management (grey bars, conservation tillage; white bars, conventional tillage). Different letters indicate significant differences among guilds (adjusted P values < 0.05). Crossed-out symbols signify exclusion of corresponding natural enemy functional guilds. Guilds of natural enemies are as follows: flying insects and vegetation-dwelling predators (ladybird symbol); ground-dwelling predators (beetle symbol); and birds and other vertebrates larger than 1.5 cm (bird symbol).
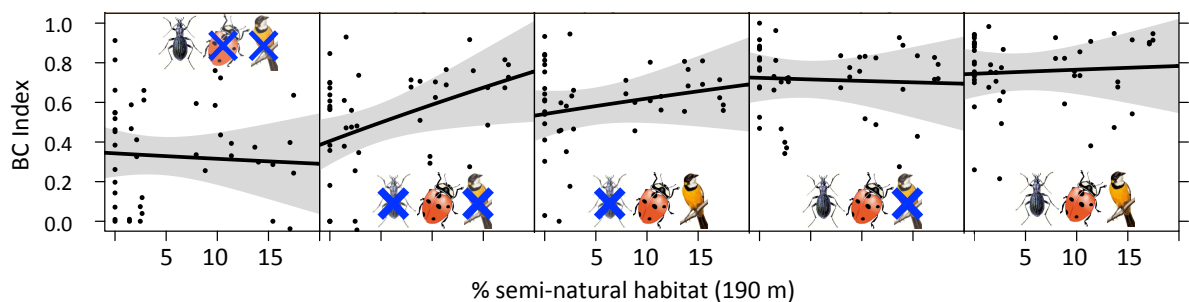

Fig. 2. Effects of exclusion of natural enemy guilds across a gradient in landscape complexity (% of semi-natural habitats in a 190 m radius) on BC index. Interaction of treatment and % semi-natural habitats is significant (Table 2).

Parasitism rate was found to be higher in fields managed with conservation tillage and to be positively affected by landscape complexity (750 m radius) only in the fields managed with conventional tillage (interaction tillage x % semi-natural). In the fields managed with conservation tillage, parasitism rate remained stable along the landscape complexity gradient (Figure 3).
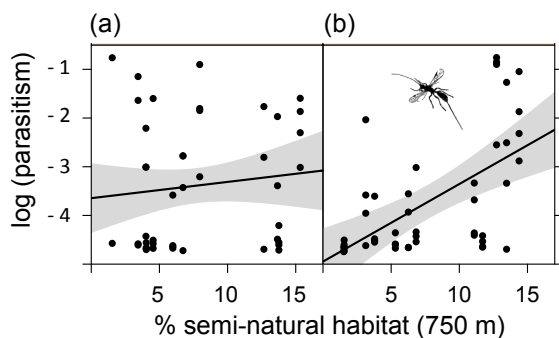

Fig. 3. Effect of landscape complexity (% of semi-natural habitats in a 750 m radius) on log-transformed parasitism rate in fields managed with (a) conservation and (b) conventional tillage.

During the monitoring of vegetation-dwelling predators a total of 5 ladybirds, 3 larvae of hoverflies and 50 web spiders were recorded. The total abundance of vegetation-dwelling predators was significantly higher in the fields managed with conservation tillage (an average of 2.6 individuals per 100 tillers, SE = 0.8) respect to those managed with conventional tillage (1.2, SE = 0.2) (Figure 4), but it did not respond to landscape variables at any scale.
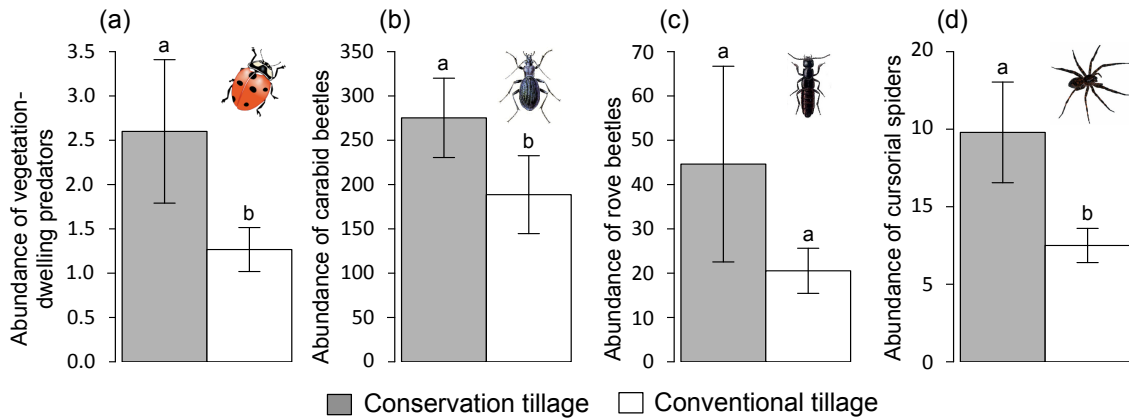
Fig. 4. Abundance of (a) vegetation-dwelling predators, (b) carabid beetles, (c) rove beetles and (d) cursorial spiders in response to tillage management (grey bars, conservation tillage; white bars, conventional tillage). Abundance of vegetation-dwelling predators was calculated as the total number of individuals per 100 tillers (flying beetles, web spiders, larvae of hoverflies and ladybirds). Abundance of ground-dwelling predators referred to the two periods of pitfall trap sampling (10 days each). Different letters indicate significant differences among tillage management (P values < 0.05).

With pitfall traps a total of 13,641 carabid beetles, 1,910 rove beetles, 654 cursorial spiders were caught during the two sampling periods. Abundance of carabid beetles and cursorial spiders was significantly affected by tillage practice, i.e. abundance of carabid beetles was on average 275.2 (SE = 44.7) in fields managed with conservation tillage and 188.9 (SE = 45.5) in fields managed with conventional tillage, and abundance of cursorial spiders was 14.8 (SE = 3.2) and 7.5 (SE = 1.1), respectively. Rove beetle abundance showed no differences in response to tillage management. BC index correlated positively with both vegetation- and ground-dwelling predator: prey ratio. Positive relation was steeper for ground-dwelling predators (Fig.5).
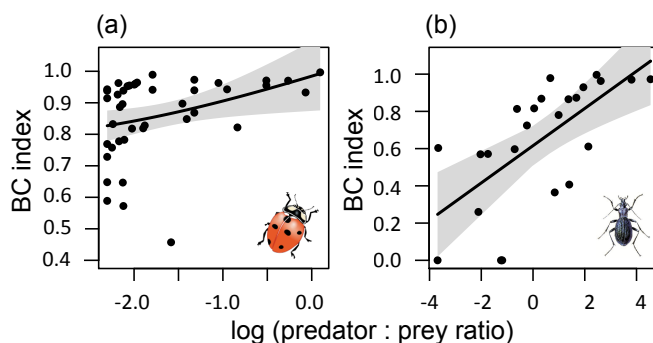

Fig. 5. Relation between BC index and log-transformed predator : prey ratio for (a) vegetation-dwelling predators and (b) ground-dwelling predators.

Discussion

Our study explored the combined effect of soil management and landscape complexity on the BC of cereal aphids provided by different guilds of natural enemies. The combination of an exclusion experiment and the predator sampling enabled us to directly link the abundance of natural enemies in the field and the provided BC and to reveal how conservation tillage management and the proportion of semi-natural habitats in the landscape improve BC service through the enhancement of specific predator guilds. Moreover, we found complex landscapes to enhance the parasitism in the fields managed with conventional tillage. Our study showed for the first time an interaction between a specific soil management and the landscape content for a pivotal component of the BC service.

Conservation tillage management was found to support higher overall BC (11% higher than in the fields managed with conventional tillage) and more abundant arthropod communities. In particular, both the abundance and the predation of ground-dwelling predators were significantly higher under conservation tillage condition (35 and 24% respectively). We found no differences in BC index between tillage management in the open treatments indicating that small populations of aphids were equally well controlled under both conservation and conventional tillage management when aphids are accessible by all the guilds of natural enemies. These results generally support the first hypothesis showing that a soil management that limits the detrimental effects of farming practices on soil enhances predator abundance and consequently the BC service provided.

Our findings confirm previous studies showing a response of ground-dwelling arthropods to within-field habitat quality and, specifically, to conservation tillage. For instance more abundant ground beetle communities were found in conservation tilled fields compared with conventional ones (Cárcamo 1995, Kromp et al. 1999, Kladivko 2001). Conventional tillage was shown to affect carabid populations directly by mechanically injuring or killing individuals (Holland and Luff 2000) and indirectly degrading habitat quality and alternative prey availability (Hance 2002, Holland 2004). Moreover, residue cover is important in maintaining soil moisture and temperature conditions suitable for the survival and development of the numerous carabid species that spend their larval stage in the soil (Cochran et al. 1994). Holland

and Reynolds (2003) showed that spiders are affected by tillage management as well: the more stable soil environment and the higher weed density promoted by conservation tillage create a deeper layer of litter and a more structurally complex vegetation, ideal for spider colonization and establishment (Rypstra et al. 1999, Holland 2004). This could also explain why we found vegetation-dwelling predators to be more abundant in fields managed with conservation tillage: the 86% of the specimens were web spiders that have been demonstrated to respond negatively to tillage intensity and positively to vegetation complexity (Diehl et al. 2013). Abundance of rove beetles did not respond to tillage management. Kroos and Schaefer (1998) reported the same pattern for the total group abundance: differences among tillage systems were found only for different species.

In accordance with previous studies (Schmidt et al. 2003, Thies et al. 2011), we found vegetation-dwelling predators to be more effective than ground-dwelling predators while the combination of both additively contributed to the suppression of aphid population. Contrary to our expectation (second hypothesis), not all the natural enemy guilds contributed to aphid population control: the exclusion of birds and other vertebrates did not lead to any significant differences in BC index. Although it is known that birds can play an important role in the provision of BC service in tropical agro-ecosystems (Martin et al. 2013; Karp et al. 2013, Railsback and Johnson, 2014) and that some species feed on polyphagus predators and consume aphids as alternative preys in European environments (Cowie and Hinsley, 1988, Snow and Perrins 1998, Naef-Daenzer et al. 2000, Eeva et al. 2009), our study suggests that they may not be important in cereal fields. Probably the structure of these crops did not allow birds to easily locate and hunt their preys.

The positive correlation between BC index and both vegetation- and ground-dwelling predator: prey ratio strengthened the link between the predator sampling and the exclusion experiment, showing higher BC in those fields where predators were more abundant. Vegetation-dwelling predators provided high BC index also in fields where a few predator individuals were sampled. Indeed many vegetation dwelling predators are commonly recognized as aphid specialists (e.g. ladybird and hoverfly larvae) able to control aphid populations even at low densities. On the contrary the diet of ground-dwelling predators (e.g. carabid and rove beetles) comprises a much wider number of species leading to a less efficient BC despite the larger populations

(Brewer and Elliot, 2004). Furthermore ground-dwelling predator efficiency is also constrained by their limited mobility (Winder et al. 2005). Similarly, in winter wheat fields web spiders have been demonstrated to have a narrower diet breadth compared to cursorial spider one (Nyffeler et al. 1999) and to greatly rely on aphids for their sustenance (Harwood et al. 2004).

Landscape composition positively affected the BC provided by vegetation-dwelling predators at 190 m scale. This result supports the hypothesis that high proportion of semi-natural habitats in the landscape benefits the BC provided highly mobile insects (Gardiner et al. 2009, Chaplin-Kramer 2011, Holland et al. 2012, Rush et al. 2013). Surprisingly we did not find any effect of landscape composition on the abundance of vegetation dwelling predators, although ladybirds have been demonstrated to strongly respond to landscape composition (Bianchi et al. 2004, Gardiner et al. 2009). Neither the abundance nor the BC provided by ground-dwelling predators were influenced by landscape composition at any scale. Although several studies highlighted the importance of landscape in building the population of natural enemies (Chaplin-Kramer et al. 2011), some authors showed that it is not always possible to find a general positive effect of landscape complexity on overall abundance because different functional groups or species may respond differently to the landscape (Purtauf et al. 2005, Schmidt and Tscharntke 2005, Shackelford et al. 2013). The effect of the landscape may have been mediated by other factors known to have a strong impact on ground-dwelling community, such as seasonality or prey abundance. For instance Östman, et al. (2001) showed how both BC provided by ground-dwelling predators and aphid establishment depended on landscape complexity only early in the season.

Parasitism rate was enhanced by high proportion of semi-natural habitats in the landscape only in the fields managed with conventional tillage, whereas under conservation tillage parasitism did not depend on landscape complexity. Parasitoids are known to profit from semi-natural habitats in the landscape due to higher availability of overwintering sites, refuges from disturbances and more diverse and abundant food sources (Tscharntke et al. 2007, Thies et al. 2005, Thies et al. 2011, Rand et al. 2012). Moreover, floral nectar has been shown to be an important component of the diet of adult parasitoids and that its availability in the landscape may influence parasitism (Lavandero et al. 2005, Rusch et al. 2010, Araj et al. 2011).

This can explain why fields managed with conservation tillage showed higher parasitism rate. Several studies in fact reported non-conventional tillage management (conservation, reduced or no-tillage) to increase weed abundance and diversity (Holland 2004, Soane et al. 2012) that might act as an important within field food resource. This, together with limited disturbance by farming practices, might have contributed to sustain and enhance parasitoid populations both in complex and simple landscapes.

Our study provides evidence for the effect of both soil management and landscape complexity on the biological control of aphids in cereal crops and these effects change for different guilds of natural enemies. Our results emphasize the importance of considering both the local habitat and the landscape quality when planning strategies for maximize the BC service in agro-ecosystems, and of adopting a functional guild approach to reveal hidden processes behind the provision of ecosystem services. More research is needed to better understand consequences and opportunities of endorsing sustainable soil management.

Aknoledgment

Chapter 6

Effects of conservation tillage on multiple ecosystem services supporting cereal production

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Chapter 6

Abstract

Soil management is expected to affect belowground properties linked to multiple ecosystem services. We analyzed the effects of conservation vs. conventional tillage on the provision of multiple ecosystems, i.e. grain production, control of weeds and pest (aphids) and soil fertility. In addition, we examined whether landscape complexity affected the delivery of pest and weed control. The experiment was performed in 15 pairs of fields (conventional tillage vs. conservation tillage) of winter cereals along a gradient of landscape complexity located in the agricultural landscape of Friuli Venezia Giulia Region (North-East Italy). Grain production showed no differences between conservation and conventional tillage. Conservation tillage decreased weed control, but it enhanced weed diversity. Moreover, conservation tillage management was found to increase the pest control provided by ground-dwelling predators. Parasitism rate was not affected by tillage management, but it increased with landscape complexity. Finally conservation tillage positively affected soil fertility enhancing soil organic matter. Conservation tillage is a potential win-win practice in our study area, able to maintain levels of productivity similar to conventional tillage and simultaneously to enhance multiple ecosystem services.

Introduction

Agricultural ecosystems cover nearly 40 per cent of the terrestrial surface of the Earth (FAO 2009) and they are principally managed to maximize provisioning services (e.g. food or fiber). However, they also receive a large array of ecosystem services and disservices (Zhang et al. 2007) that are greatly influenced by farming practices (Power 2010). Agricultural intensification has been broadly shown to negatively affect biodiversity and the associated services with potential negative feedbacks to crop productivity (e.g. Daily 1997, MA 2005a, Schröter et al. 2005). Considering the growing demand of agricultural goods worldwide (Tilman et al. 2011) there is a need to identify farming practices able to maintain high levels of productivity while minimizing detrimental effects on the environment and sustaining ecosystem services (Tilman et al. 2002). Nevertheless, comprehensive studies exploring the effects of different farming practices on multiple ecosystem services are still scarce.

Soil management has been shown to affect belowground properties linked to multiple ecosystem services (Paul et al. 1996, Smukler et al. 2012). Conservation tillage (CT) is a soil management characterized by non-inversion of soil often combined with other farming practices such as cover crops, incorporation of crop residues and diverse crop rotation. CT has been shown to minimize the negative effects of farming operations improving general soil quality (e.g. enhanced soil fertility, reduced surface run-off, mitigated leaching of nutrients, reduced disruption of soil structure and erosion), and soil biodiversity (Holland 2004; Trewavas 2004; Aina 2011). Although several studies have investigated the effects of CT management on soil environment, its repercussions on multiple ecosystem services are still unknown.

Along with local management, landscape composition plays a key role in shaping the provision of several ecosystem services such as biological control or pollination (e.g. Rusch et al. 2010). Non-crop habitats in fact provide alternative food, hosts and winter refuges for a wide range of predators and parasitoids (Tscharntke et al. 2007). Landscape simplification with the consequent loss of semi-natural habitats, has been shown to negatively affect the abundance and the diversity of natural enemy communities (Bianchi et al. 2006; Maron et al. 2012) potentially compromising the biological control of pests (Tscharntke et al. 2005-2007, Flynn et al. 2009, Gardiner et al. 2009). The interactions between local management and landscape composition have been principally investigated in relation to one specific service (e.g. Rusch et al. 2013) and rarely considering local soil management.

Although several studies investigated specific ecosystem services and specific agro-ecosystem management (see Kremen and Miles 2012), the effects of both soil management and landscape composition on multiple ecosystem services remain largely unknown. The aim of this study was to analyze the effects of tillage (conservation vs. conventional tillage), and landscape complexity on the provision of multiple ecosystems services in cereal crops. The ecosystem services considered in the experiment were yield production, weed control, aphid pest control and soil quality. We further included two levels of nitrogen fertilizer application, to test potential interactions between tillage, landscape and local management intensification.

Materials and methods

Study area and sampling design

The study area was located in the agricultural landscape of the lowlands of Friuli Venezia Giulia Region in North-East Italy (46°10′08″N, 12°57′44″E to 45°46′18″N, 13°30′56″E). Lithology is characterized by Holocene alluvial and Pleistocene fluvioglacial sediments (Martinis, 1993; Carulli, 2006). The climate is temperate with average annual temperatures of 13° C, while the average annual rainfall ranging from 1100 mm in low plain areas to 1600 mm in high plains areas (Osmer - Regional Meteorological Observatory, http://www.osmer.fvg.it/).

The experiment was carried out in 15 pairs of fields (i.e. 30 fields) of winter cereal crops (i.e. 7 pairs of barley and 8 pairs of wheat). In each pair, one field was managed with conventional tillage (CoT) and the other with conservation tillage (CT). Conservation tillage includes all techniques characterized by non-inversion of soil for at least 5 years (range=5-20 years, mean=10 years). Field pairs were separated by at least 1 km. Typical rotation of the fields included as cash crops maize, wheat, and soybean. In the CT field this rotation was always coupled with cover crops between harvests, generally with graminaceous species (*Lolium multiflorum* L.). In the fields managed with conventional tillage practice the seedbed was prepared by deep primary tillage with inversion of the surface layer of soil (30 cm), followed by one or two tills for seedbed preparation.

Field pairs were selected along a gradient of landscape complexity ranging from 1.2 to 22.4 % of semi-natural habitats (forests, shrubby areas, grasslands, hedgerows, and field margins) in a 1 km radius around each field (see SI). The proportion of agricultural and urban areas was also measured. Preliminary analyses showed no differences in landscape composition between tillage management for all the three land use classes considered, (Linear Mixed Models for % agricultural areas, *p*-value = 0.48; % semi-natural areas, p-value = 0.70; % urban areas; p-value= 0.76). ArcGIS 9.3 was used for landscape analyses of regional land use maps, verified and ameliorated with aerial photographs to increase class discrimination accuracy.

The experiment was conducted during spring-time (April to June) in 2014. In each field, we selected a 20x60 m strip placed on one side of each field. No pesticides

and herbicides were applied on the strip during the experiment. Within each pair, the strips bordered with an edge habitat of similar structure and composition. The strips were then split into six (10 x 20 m) plots, of which the outer ones were considered as buffer zones. Among the four left, only two were fertilized (80 kg ha^{-1} of ammonium nitrate). Data collection was performed in the different plots as described in the following section (Fig.1).
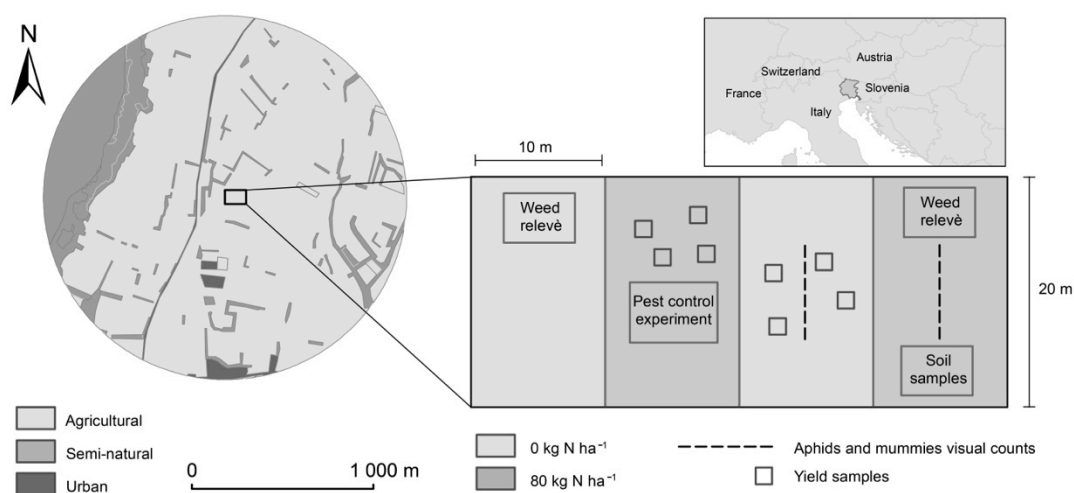

Fig.1 Study area and experimental plots.

Data Collection

Production service. Yield production was recorded at crop maturity harvesting 4 samples of 0.25 m^2, randomly selected in two plots per field (fertilized and not-fertilized plots). Subsequently, the plant material was dried at 60°C for 24 h. Production was measured as the dry weight of grains per square meter (kg/m^2).

Weed control service. Weed cover and species richness were measured in two plots for each field (fertilized and not-fertilized plots) during the 3rd decade of May, at the maximum weed development stage. Plant surveys were conducted in a rectangular area of 10 m^2 (2 X 5 m) placed along the midline of each plot. We recorded the presence and the estimated cover value of all vascular plants. The cover values of each species were then summed together to quantify the overall weed cover in each plot.

Aphid control service. We examined the biological control of aphids provided by both ground-dwelling predators and parasitoids. Aphid pest control by ground-dwelling predators was measured with an exclusion experiment with two treatments: a close treatment, where all natural enemies were excluded, and an open treatment, where only ground-dwelling predators had access. In one fertilized plot per field we placed two cages. For the treatment excluding all the natural enemies a plastic ring (0.3 m in diameter, 0.25 m in height) was dug 10 cm into the soil and an 8-cm-wide band of insect glue was applied along the perimeter. Moreover a polyester fine mesh (mesh size: 1 mm) supported by poles was sealed to the cylinder. For the open treatment, the access of ground-dwelling predators was guaranteed by fixing the net to the support poles 5 cm above the ground (no plastic ring). One pitfall trap was placed inside each cage, checked and emptied for the duration of the experiment. To avoid bias due to differences in the initial aphid abundance, we inoculated field plants with aphids reared in lab condition. Ten days before the inoculation, to standardize the plant density only seven tillers were left for each treatment cage. The selected plants and the ground were cleared from natural enemies and then covered by a nonwoven fabric supported by sticks to exclude recolonization. Aphid material (*Sitobion avenae*) was provided by Katz Biotech AG® and directly placed on the plants (c. 150 aphids per treatment including both adults and nymphs). Inoculation was done at the heading stage of the cereals during good weather conditions (absence of precipitation and strong wind, minimum air temperature 18°C). After 5 days established aphids were counted and plants were re-inoculated if needed (i.e. cages with less than 15 aphids). After 10 days from the first inoculation aphids were counted (time 0) and exclusion treatments started. Aphids were counted visually in each treatment at two occasions, 5 and 10 days after the onset of the exclusion experiment. For each 5 days period, predation rate was calculated as the proportion of aphids predated in the open cages compared with the aphid population growth in the close cage, as following:

$$Predation\ rate = \frac{N_{treatment\ 5}}{R_{close} \times N_{treatment\ 0}}$$

where $N_{treatment\ 5}$ is the number of aphids in the open cage after 5 days, R_{close} is the aphid population growth in the close cage, $N_{treatment\ 0}$ is the number of aphids in the open cage at the beginning of the experiment. Predation rate values ranged from 0 to 1, where 0 indicates no net loss of aphids in the open cage and 1 indicates that 100% of aphids was predated (Gardiner et al. 2009). The exclusion experiment was performed only in the fertilized plots because we did not expect any short-term effect of N fertilization on predation rate.

We also measured the parasitism rate through visually inspection of 50 tillers per field. The sampling was repeated twice, the first time during stem elongation stage and the second during fruit development stage. The parasitism rate in each field was calculated as the ratio between the number of parasitized aphids (mummies) and the total number of aphids in the field. Preliminary analyses on the natural denisty of aphids per field showed no differences between treatments.

Soil fertility service. To analyze soil fertility, 5 soil samples (15 cm in depth and 3 cm in diameter) were randomly collected in each fertilized plot with an auger. Samples were then mixed together and SOM content was measured (Walkley-Black procedure, Soltner 1988). We did not sample non-fertilized plots because we did not expect any short-term effect of N fertilization on SOM.

Disease incidence. A preliminary survey on the incidence of diseases was conducted to avoid bias due to different health conditions of the fields. Disease incidence was assessed visually inspecting 50 randomly selected leaves per plot, and it was calculated as the number of leaves affected by fungal disease per plot (i.e. Rust, Leaf Spot, Mildew and Fusarium). Generalized linear mixed model (family = Poisson) was used to analyze disease incidence. Tillage management and fertilization treatment were included as fixed factors and number of leaves affected as independent variable. The type of crop (barley or wheat), pair id and field id were included as random factors. GLMM showed no effect of any of the variables considered on disease incidence (p-value> 0.05).

Data Analysis

Linear mixed models were used to explore the combined effects of tillage management, fertilization and landscape composition on the provision of the ecosystem services considered ("nlme" package in R environment; Pinheiro et al. 2009, R Core Team 2013). Linear mixed model assumptions were verified using diagnostic plots of model residuals.

A total of six models were run. For the analyses of yield production (production service), weed cover and weed species richness (weed control), tillage management, fertilization and landscape composition were included as fixed factors and crop type, pair id and field id as random factors. For the analyses of aphid predation (aphid control service) tillage management and landscape composition were included as fixed factors and crop type, pair id and counting round as random factors. The parasitism rate was log-transformed to achieve normal distribution of model residuals. The model included fertilization as covariate, tillage management and landscape composition as fixed factors and crop type, pair id and counting round as random factors. The analyses of SOM content (soil fertility service) included tillage management as fixed factor and crop type and pair id as random factors. Before the onset of the experiment, a local storm event damaged the cages in 3 pairs (6 fields) compromising the aphid establishment. The analyses regarding predation rate were thus based on data from 12 field pairs (24 fields).

Results

The results from LMM showed no effect of tillage management on yield production (Fig. 2a), whereas nitrogen fertilization increased crop yield (Tab.1). A total of 91 plant species were recorded during the weed sampling, 63 species in conventional tilled fields and 76 in conservation tilled ones. LMMs results showed tillage management to affect both weed cover and weed species richness: conservation tilled fields presented higher weed cover and species richness (Fig. 2b and 2c). Landscape composition did not influence the weed communities. Ground-dwelling predators reduced aphid populations, showing stronger biocontrol under conservation tillage management (Fig. 2d). Landscape composition did not affect aphid predation.

Parasitism rate instead responded to landscape composition but not to tillage management (Fig. 2e). Parasitism rate decreased at the increase of proportion of agricultural areas in the landscape. Finally, the results of LMM for soil fertility service indicated that conservation tillage enhanced SOM content (Fig. 2f).

Tab.1 Results of linear mixed effects models relating yield production, weed cover and diversity, predation rate, parasitism rate and SOM content to explanatory variables.

| Ecosystem service	Response variable	Factors	DF	F-value	*p*-value
Production	yield	tillage	12	2.52772	0.1378
		fertlization	28	74.13785	<0.0001
Weed control	weed cover	tillage	13	4.98717	0.0437
		fertilization	28	0.21914	0.6433
		tillage:fertilization	28	0.20097	0.6574
	weed species richness	tillage	13	5.33301	0.038
		fertilization	28	1.88431	0.1807
		tillage:fertilization	28	3.05228	0.0916
Pest control	predation index	tillage	21	8.84051	0.0073
	parasitism rate	tillage	25	0.04859	0.8273
		% agricultural areas	25	6.25005	0.0193
		tillage: % agricultural areas	25	0.29065	0.5946
Soil quality	SOM	tillage	14	4.37271	0.0552
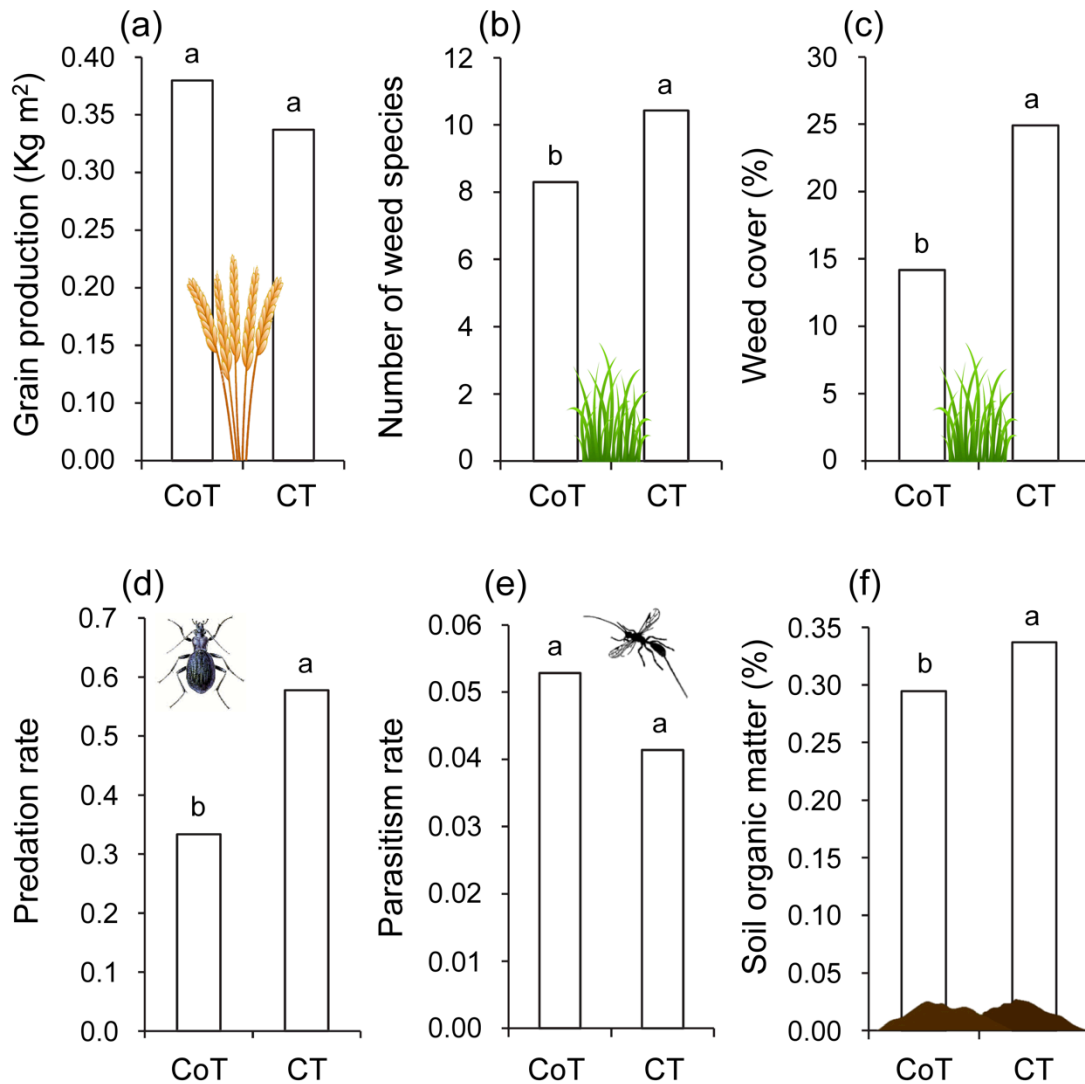
Fig.2 Effects of tillage management (CT, conservation tillage; CoT conventional tillage) on yield production (a; dry weight of grain, kg/m^2), weed species richness (b; number of species), proportion of weed cover (c), aphid predation rate (d; Pr), aphid parasitism rate (e; Pa) and proportion of soil organic matter (f). Bars with the same letter are not significantly different ($P > 0.05$).

Discussion

Conservation tillage enhanced soil fertility, biological control of aphids provided by ground-dwelling predators, but maintaining production levels similar to those achieved under conventional management. Along with the local effect, we found that landscape simplification decreased parasitism rate. Our findings suggest that the adoption of sustainable management strategies both at the local and landscape scale

might be endorsed without causing negative tradeoffs between supporting/regulating and provisioning services.

The two regulating services considered (weed and aphid control) responded differently to both tillage management and landscape composition. Weed control was negatively affected by conservation tillage: both weed cover and species richness increased under conservation tillage management. These results are consistent with previous works supporting the hypothesis that decreased mechanical disturbance to the soil favors weed abundance and diversity (e.g. Murphy et al. 2006, Tolimir et al. 2006, Demjanová et al. 2009, Demjanová et al. 2009). Although the presence of weeds potentially decreases crop productivity (Oerke 2006), recent studies showed that high weed diversity also supports agro-ecosystem functioning (Albrecht, 2003; Franke et al., 2009). For instance, weeds can provide food resources for pollinators (Gabriel and Tscharntke, 2007), habitats for natural enemies (Schellhorn and Sork, 1997), and reduce disease incidence (Ratnadass et al., 2012). Landscape composition did not show any influence on weed cover and diversity. The dispersal of several arable weeds is in fact limited and their occurrence in the field is principally related to the local seed bank (Rew et al. 1996, Bischoff and Mahn, 2000). Although the application of nitrogen fertilizers generally increases weed growth (Dhima and Eleftherohorinos, 2001) we did not find such an effect in our study. However, the effect of fertilization on weeds depends also on management practices and local habitat conditions (Di Tomaso 1995, O'Donovan et al. 2001).

Biological control service is expected to be influenced by both local management and landscape composition (Tscharntke et al., 2007; Rush et al., 2010; Rush et al., 2013). The aphid control provided by ground-dwelling predators and parasitoids presented contrasting responses. We found a strong positive effect of conservation tillage management on pest control provided by ground-dwelling predators on aphids. In fact, the abundance and diversity of ground-dwelling arthropods has been shown to increase with decreasing tillage intensity (Kendall, 2003; Holland, 2004), owing to the reduction of soil disturbance (Altieri, 1999). On the other hand, parasitism rate was affected only by landscape composition, i.e. increasing proportion of agricultural areas decreased parasitism rates. Landscape simplification is in fact known to reduce the availability of natural and semi-natural habitats able to provide key resources for parasitoids such as diverse floral resources

(Olson and Wäckers 2007). In line with previous studies, our results indicate that both local management and landscape composition are important in shaping biological control service (Chaplin-Kramer et al., 2011; Rush et al., 2013).

Finally, we found that conservation tillage in combination with cover crops positively affected soil fertility enhancing SOM content. Reduced tillage intensity is in fact known to enhance SOM content increasing the amount of crop residues incorporated in the soil, favoring the formation of soil aggregates that protect SOM particles from rapid oxidation and modifying edaphic environment limiting SOM degradation (Puget and Lal, 2005; Baker et al., 2007, Soane et al. 2012). Moreover, conservation tillage includes the use of cover crops that are well known to limit soil erosion and to increase soil organic matter (Holland 2004).

Along with the regulating and supporting ecosystem services, we explored the effect of tillage and fertilization on the provisioning service. As expected we found that the application of nitrogen fertilizers increased yield. Fertilizer application is in fact the most common practice to enhance nutrient availability to crops and therefore production (e.g. Campbell et al. 2011, Raun and Johnson, 1999). Interestingly, conservation tillage management did not decrease grain production. The results could be also related to the improved SOM in the CoT fields. Although literature reports contrasting results regarding the effect of conservation tillage on crop production, these discrepancies seem to be caused by local differences in soil and climate properties or concomitant farming practices (Edwards et al. 1988, Nyborg et al. 1995, López and Arrúe 1997, De Vita et al. 2007).

Besides the environmental effects of tillage management, it is also important to consider the cost-effectiveness of promoting conservation tillage practices and the trade-off between environmental and economical benefits. Soil tillage is one of the greatest energy and labor demanding practice in agriculture (Tabatabaeefar et al. 2009). Therefore, a reduction of tillage intensity may lead to a better economic efficiency reducing fuel and energy consumption and decreasing time and energy required for seedbed preparation (Kepner et al. 1978, Bonari et al. 1995, Košutić et al. 2005, Tabatabaeefar et al. 2009). The identification of tradeoffs and synergies between provisioning, regulating, supporting services and farming practices is a crucial step towards sustainable management of agricultural ecosystems (Bommarco

et al. 2013). Our study showed that conservation tillage is a potential win-win practice in our study area, i.e. it performed as well as conventional tillage in providing grain yield and enhanced local soil quality, pest control and weed diversity. Further research is, however, needed to test whether conservation tillage can effectively be applied in other crops and in different agricultural regions.

Supplementary Information

Tab.1 Pair id, tillage management (CT = conservation tillage, CoT = conventional tillage), percent of urban, semi-natural (i.e. woods, hedge-rows, grasslands) and agricultural areas calculated within a radius of 1 km from the field center.

Pair id	Tillage	Years of CT	% Urban	% Semi-natural	% Agricultural
1	CoT	-	10.55	14.54	74.91
	CT	15	9.23	16.61	74.16
2	CoT	-	29.72	10.87	59.41
	CT	20	37.94	10.22	51.83
3	CoT	-	3.78	11.05	85.16
	CT	15	6.86	11.64	81.50
4	CoT	-	18.78	5.82	75.40
	CT	10	19.31	5.89	74.80
5	CoT	-	1.00	4.07	94.93
	CT	7	0.15	3.75	96.11
6	CoT	-	10.79	3.33	85.89
	CT	10	8.30	3.01	88.69
7	CoT	-	1.29	5.58	93.13
	CT	10	6.12	4.48	89.40
8	CoT	-	2.73	44.95	52.32
	CT	5	9.04	22.42	68.54
9	CoT	-	15.02	10.48	74.50
	CT	5	1.57	17.10	81.32
10	CoT	-	2.33	13.70	83.97
	CT	5	2.75	11.92	85.33
11	CoT	-	2.80	20.54	76.66
	CT	5	3.33	20.85	75.82
12	CoT	-	27.78	7.77	64.45
	CT	10	28.23	7.69	64.08
13	CoT	-	11.34	1.54	87.12
	CT	10	1.71	1.25	97.04
14	CoT	-	1.47	2.32	96.21
	CT	10	1.88	3.66	94.46
15	CoT	-	4.44	1.48	94.08
	CT	10	0.74	7.62	91.64

Chapter 7

General conclusions
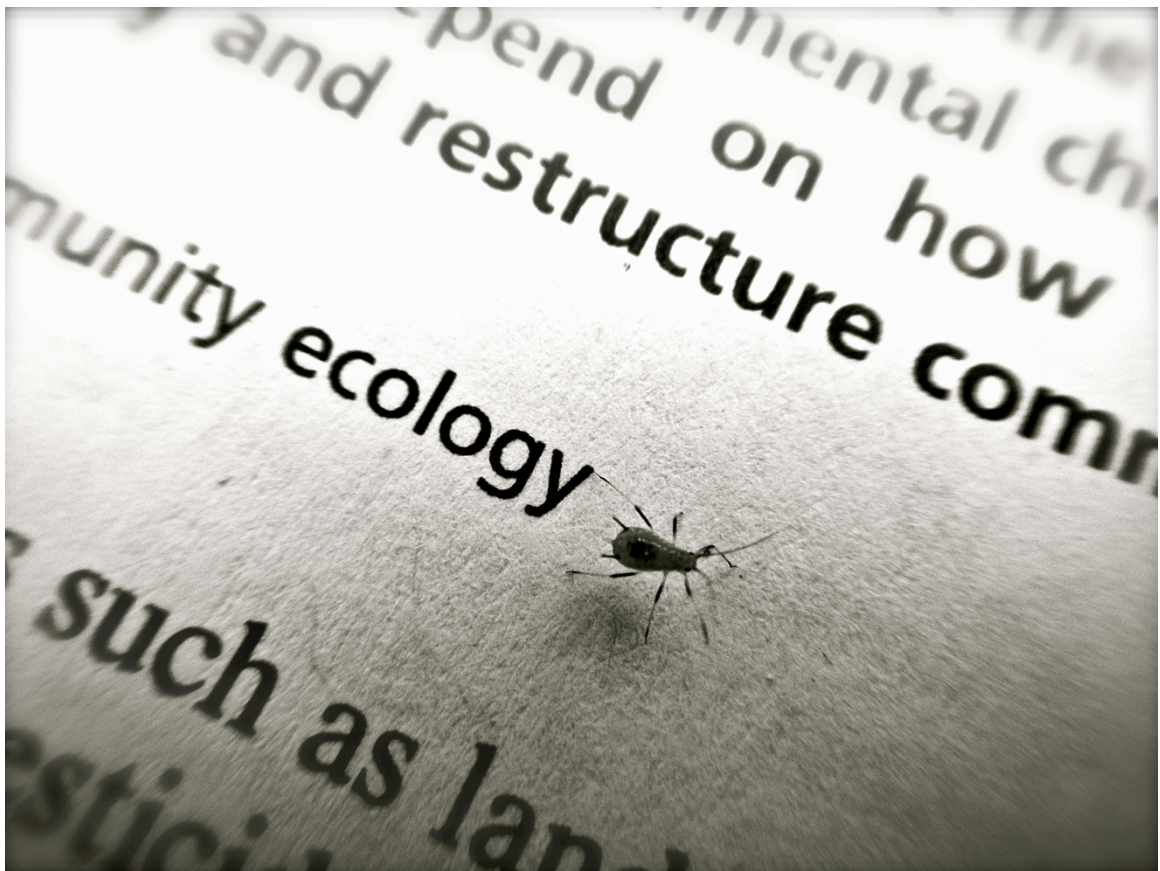
The aim of this study was to explore potential interactions occurring between above- and belowground ecosystem services in agriculture. Despite the growing interest in the topic, a comprehensive understanding about the relationships linking provisioning, supporting and regulating services is still lacking. Our work considered several ecosystem services and disservices crucial to crop production and different management practices expected to affect them. We demonstrated that pollination benefit to yield formation is influenced by nitrogen availability in oilseed rape (Chapter 2) and by soil fertility in sunflower (Chapter 3). Soil organic matter was found to modify nitrogen fertilizer and drought effects on aphid performance in spring wheat (Chapter 4) and general soil quality to enhance multiple ecosystems services in winter wheat. Our study provides evidences for context-dependency of ecosystem services in agriculture and supports ecological intensification as a promising answer to present and future challenges in crop production.

We found important interactions between supporting and regulating services or disservices affecting yield formation. Although it is clear that crop production benefits from healthy ecosystems providing optimal levels of services (e.g. abundant and diverse pollinator and natural enemy communities) our study shows that the final effectiveness of these services is also driven by site-specific factors. Plant and local habitat quality are known to respond to belowground properties and to influence the activity and growth of aboveground organisms. Our results suggest that soil quality indirectly affect aboveground ecosystem services and disservices.

Chapter 2 shows that in oilseed rape, the open pollinated cultivar largely depends on pollination, whereas hybrid cultivars do not. Under normal soil fertility conditions, decreased nitrogen inputs enhance pollination benefits to yield, indicating that nitrogen use efficiency increases with pollination. Hence, the use of hybrid cultivars and the application of fertilizers can compensate degraded pollination service, whereas optimal pollination can replace fertilization. In sunflower (Chapter 3) benefits to crop yield from insect pollination are observable only under good soil fertility conditions whereas they are canceled in highly deteriorated soils, suggesting that limited resources lead to limited yield even where pollination service is optimal. In general, these findings demonstrate that both pollen and nutrient availability are important factors to consider when evaluating pollination effects to crop production. Management practices that aim to augment pollination benefit to yield should include

not only measures to enhance pollinator abundance and diversity but also measures to improve soil fertility together with accurate application of inorganic fertilizers.

Agricultural landscapes also provide resources and habitats for pests that can limit crop productivity. Both long- and short-term farming practices able to increase nutrient availability to plants can positively affect pest performance. The results from Chapter 5 show that in spring wheat increased fertilizer applications strongly enhance aphid performance and that increased soil organic matter has similar but minor effects on pest growth, maintaining similar yield production. The final success of farming practices depends in fact on the tradeoff between services' benefit and disservices' cost to agricultural production. Our findings suggest that high fertilizer applications potentially boost both crop and pest performance leading to yield levels similar to those obtainable with low-input farming systems.

Although it is well known that high proportion of semi-natural habitats in the landscape supports important ecosystem services such as biological control, the effect of local soil management on multiple services and disservices has been poorly explored. In Chapter 6 and 7 the effect of different tillage practices indicates that farming management that reduces soil disturbance in cereal crops enhances soil fertility, arthropod abundance, biological control and maintains levels of crop production similar to those observable under conventional management, despite the increased weed infestation. Moreover, our results show that landscape complexity enhances the biological control provided by highly mobile insects. These findings indicate that both local habitat and landscape quality need attention when managing ecosystem services in agro-ecosystems.

In general our findings indicate that management strategies aiming to maximize crop productivity and to preserve environment through the enhancement of ecosystem services should give attention to both above- and belowground ecosystem health. Aboveground, the conservation and restoration of semi-natural habitats in the landscapes and the limited use of pesticides support the provision of regulating services such as pollination and biological control. Belowground, farming practices that maintain and increase soil fertility and that limit soil disturbance and reduce inorganic fertilizer applications, not only support provisioning services, but also support the benefits of multiple services to crop production. Moreover, this research

provides information useful for a more accurate assessment of ecosystem services in the landscape. The identification of synergies and tradeoffs between different services is in fact crucial for a correct implementation of sustainable management strategies in agricultural ecosystems.

This study provides evidence for complex interactions between above- and belowground services. Supporting services such as soil fertility, are crucial in shaping the effects of other services and disservices on yield formation. Nevertheless, more studies are still needed to comprehensively address the effects of service interactions on agricultural productivity. These findings are also relevant for the mapping of ecosystem services in agricultural landscapes. Currently, evaluation of ecosystem services, essential for land-use decisions, is primarily based on land-cover mapping. For instance, pollination or biological control are directly linked to the presence of semi-natural habitats. Our study suggests that an evaluation solely based on the presencep of pollinator or natural enemies presence could lead to a wrong estimation of the service, especially in landscapes characterized by different soil quality. Expanding the knowledge about service tradeoffs and making it explicit in decision making will improve the efficacy of sustainable management strategies both at the local and regional scale.

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