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***Effects of agricultural management and landscape on farmland biodiversity
and associated ecosystem services***

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Padova, 28 January 2016

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Riassunto

I prossimi decenni saranno testimoni di un rapido aumento della domanda di prodotti agricoli. Entro il 2050 l'attuale domanda alimentare sarà raddoppiata a seguito della crescita della popolazione mondiale e della crescita socio-economica (FAO 2015). Lo sviluppo urbano sarà sempre più in concorrenza con l'agricoltura per l'uso del suolo. Si rende quindi necessario soddisfare la maggior parte della crescente domanda di prodotti agricoli attraverso l'intensificazione colturale (ossia produrre di più sulla stessa superficie agricola investita) poiché non vi è sufficiente spazio per incrementare la superficie agricola senza causare danni irreparabili agli ecosistemi naturali vitali. Il costante aumento della produttività agricola per unità di superficie, a cui si è assistito nell'ultima parte del 20° secolo si è ormai stabilizzata, con scarse possibilità di ulteriori aumenti di efficienza attraverso metodi convenzionali. La dipendenza dell'agricoltura convenzionale e dell'approvvigionamento alimentare sulle risorse non rinnovabili (ad esempio i combustibili fossili, fosfato) la rende insostenibile nel lungo periodo.

L'intensificazione eco-funzionale è stata proposta come una soluzione promettente (Niggli et al. 2008). Per Intensificazione Eco-funzionale si intende l'ottimizzazione dell'approvvigionamento, della regolazione e del mantenimento dei servizi ecosistemici nel processo di produzione agricola (Niggli et al. 2008). Come tale, promuove il mantenimento o la crescita della produzione agricola sostenendo la biodiversità e i connessi servizi ecosistemici. Tuttavia, l'implementazione della intensificazione ecologica nei sistemi di produzione delle colture agrarie specifiche conoscenze oltre che una comprensione globale delle relazioni tra i diversi servizi ecosistemici.

Lo scopo di questa tesi è stato quello di studiare come la struttura del paesaggio (habitat seminaturali) e l'uso del suolo (gestione in azienda) interagiscono nell'approvvigionamento di alcuni servizi ecosistemici, quali sono il servizio di impollinazione e il controllo biologico. Abbiamo indagato gli effetti del tipo di margine del campo coltivato, a scala locale, sul servizio di impollinazione e sulla comunità degli impollinatori, valutando le caratteristiche delle siepi presenti lungo i campi coltivati, ad esempio, la densità degli alberi, l'eterogeneità in termini di specie e di struttura. Inoltre, abbiamo indagato la relazione tra le pratiche gestionali agricole, gli habitat semi-naturali

e il controllo biologico delle colture nonché l'effetto combinato dei tipi di lavorazione del suolo e del grado di urbanizzazione presente sulle comunità dei coleotteri Carabidi. (Coleoptera: Carabidae).

Nel capitolo 2 sono state valutate le potenziali interazioni tra impollinazione e la qualità degli elementi del paesaggio semi-naturale. Abbiamo testato se l'abbondanza e la ricchezza di specie vegetali, a scala locale, possono incentivare la ricchezza e l'abbondanza di impollinatori.

Nel capitolo 3 abbiamo esplorato come le pratiche locali di gestione agricola delle colture, (fertilizzanti e di insetticidi, pratiche colturali che favoriscono una maggiore SOC) in combinazione con il paesaggio influiscono sulla resa delle colture, sul controllo biologico dei parassiti e sull'abbondanza dei predatori. Questo modello sperimentale ha permesso di testare le generali conseguenze interattive, dovute ad una diversa gestione colturale, tra i servizi ecosistemici e il rendimento degli input esterni sui raccolti.

Nel capitolo 4 abbiamo esplorato le possibili interazioni tra intensità di gestione delle colture agrarie, la complessità del paesaggio e la comunità dei predatori. Attraverso un esperimento in campo abbiamo testato l'effetto combinato della gestione dell'aratura (lavorazione convenzionale rispetto a lavorazione conservativa) e della urbanizzazione sulle comunità di coleotteri Carabidi (Coleoptera: Carabidae).

Summary

The next few decades will witness a rapidly increasing demand for agricultural products.

By 2050 current food demand will be doubled by world population and socio-economic growth (FAO 2015). The expanding bio-based economy will increase the demand for agricultural products. Urban development will increasingly compete with agriculture for land use. The growing demand for agricultural products needs to be met largely through intensification (produce more from the same land surface) because there is little scope for an increase in agricultural area without doing irreparable damage to vital natural ecosystems. The steady increases in agricultural productivity per unit area seen through the latter part of the 20th century have now plateaued with little opportunity for further increases in efficiency through conventional methods. The dependency of conventional agriculture and food supply on non-renewable resources (e.g. fossil fuels, phosphate) makes it unsustainable in the long run.

Eco-functional intensification has been proposed as a promising solution (Niggli et al. 2008). Eco-functional intensification is the optimization of all provisioning, regulating and supporting ecosystem services in the agricultural production process (modified after Niggli et al. 2008). As such it advocates to maintain or enhance agricultural production through the promotion of biodiversity and associated ecosystem services. However, the implementation of ecological intensification into crop production systems is knowledge-intensive and it requires a comprehensive understanding of the relationships among multiple ecosystem services.

The aim of this thesis was to investigate how landscape structure (semi-natural habitats) and land-use (on-farm management) interact in the provisioning of some ecosystem services, that is pollination service and biological control. We explored the effects of field boundary quality, at local scale, on pollination service and pollinators community, assessing the quality of field boundaries, e.g. tree diversity, tree structural heterogeneity. Furthermore we assessed the relationship between farm management, semi-natural habitats and biological control then the combined effect of tillage management and urbanization on ground beetle communities (Coleoptera: Carabidae).

In chapter 2 we evaluated the potential interactions between pollination services and quality of semi-natural landscape elements. We tested whether abundance and richness of plant species at local scale can enhance richness and abundance of pollinators.

In chapter 3 we explored how local management practices, fertiliser and insecticide inputs and management for enhanced SOC, combined with land use at the landscape (1 km diameter circle) determined the crop yield, biological pest control, and abundance of predators. This experimental design allowed us to test for general interactive effects of management for ecosystem services and of efficiency of external inputs to crop yields.

In chapter 4 we explored the potential interactions between management intensity, landscape complexity and predators community. In a field experiment we tested the combined effect of tillage management (conventional vs. conservation tillage) and urbanization on ground beetle communities (Coleoptera: Carabidae).

Our study stresses the importance of considering both local management and landscape composition when planning strategies to support farmland biodiversity. These principles are taken up by the EU agricultural policy that through the mediation of the member states and their regions promotes the introduction of environmentally friendly farming systems, such as minimum tillage or no tillage (MT, NT) in order to increase the organic carbon into the soil and the related environmental benefits, such as conservation of nutrient cycling, species diversity and productivity.

Chapter I

Introduction

Biodiversity and ecosystem services

By 2050 current food demand will be doubled by world population and socio-economic growth (FAO 2015). The expanding bio-based economy will increase the demand for agricultural products. Urban development will increasingly compete with agriculture for land use. The growing demand for agricultural products needs to be met largely through intensification (produce more from the same land surface) because there is little scope for an increase in agricultural area without causing irreparable damage to vital natural ecosystems (Bongaarts 1996). Moreover, urban development will increasingly compete with agriculture for land use (Rounsevell et al. 2005). The steady increase in agricultural productivity per unit area observed during the latter part of the 20th century has now reached a plateau, with little opportunity for further increase in efficiency through conventional methods. Moreover, further intensification of farming practices such as increased agrochemical and energy inputs and simplification of agricultural landscapes through removal of semi-natural habitats have already been shown to dramatically impact farmland biodiversity (Tilman et al. 2001, Green et al. 2005, Norris 2008). The dependency of conventional agriculture and food supply on non-renewable resources (e.g. fossil fuels, phosphate) makes the production system unsustainable in the long run. In many parts of Europe, agricultural productivity is amongst the highest in the World, but it depends on unsustainable high levels of external inputs. Agriculture faces the dual challenge of feeding a 9-12 billion global population by 2050 and reducing its footprint on the environment.

Ecosystem services are the direct and indirect contributions of ecosystems to human well-being. They support directly or indirectly our survival and quality of life. Ecosystem services can be categorized in four main types: provisioning services, regulating services, habitat services, cultural services (figure 1). Provisioning services are the products obtained from ecosystems such as food, fresh water, wood, fiber, genetic resources and medicines. Regulating services are defined as the benefits obtained from the regulation of ecosystem processes such as climate regulation, natural hazard regulation, water purification and waste management, pollination or pest control. Habitat services highlight the importance of ecosystems to provide habitat for migratory species and to maintain the viability of gene-pools. Cultural services include non-material

benefits that people obtain from ecosystems such as spiritual enrichment, intellectual development, recreation and aesthetic values.

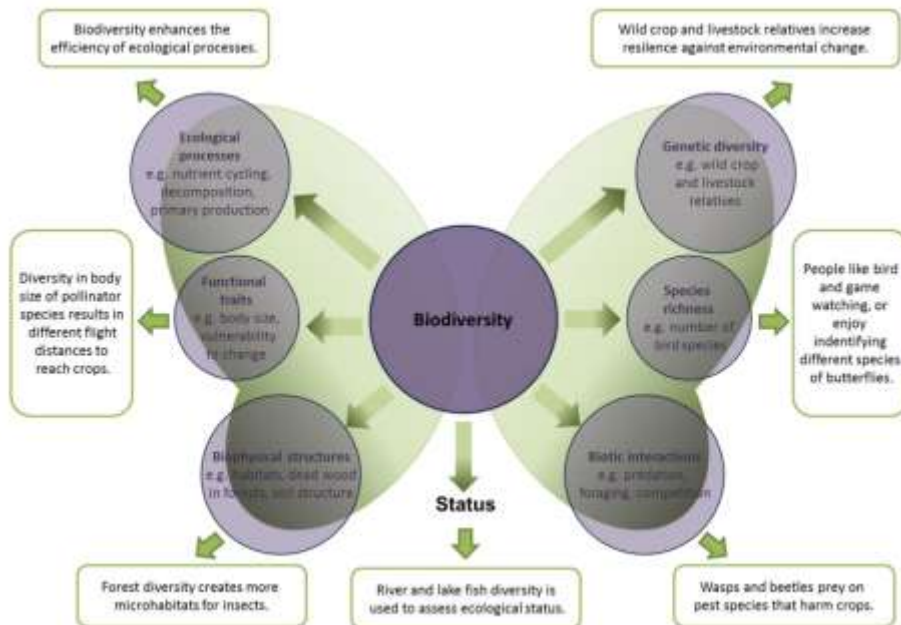


Figure 1 The multi-faceted role of biodiversity to support the delivery of ecosystem services and to assess the status of ecosystems (<http://biodiversity.europa.eu/maes>)

With growing global pressure on food and environmental systems, we must absurdly expect more from agriculture. While the impact of agriculture intensification on the environment is widely recognized, the management of ecosystem services involved in crop production presents an alternative to support and improve agriculture minimizing negative effects on ecosystems. Moreover, it will contribute to enhance the capacity of agricultural landscapes to provide multiple functions (DeClerck et al. 2015). It is fundamental to consider biodiversity if we are to increase agricultural productivity or to enhance the capacity of these ecosystems to provide multiple services. Biodiversity in essence serves as the global operating system: similarly to the operating systems that run computers allowing users to complete both simple and complex functions, biodiversity serves the same role for ecosystem services. The abundance and combination of species in space and time determine in fact which services are provided, when, where, and to what degree (Naeem et al. 2012).

Ecological intensification

Eco-functional intensification of food production is the optimization of all provisioning, regulating and supporting ecosystem services involved in the agricultural production process (modified after Niggli et al. 2008). Ecological intensification may be formally defined as a knowledge-intensive process that requires optimal management of nature's ecological functions and biodiversity to improve agricultural system performance, efficiency and farmers' livelihoods. Ecological intensification should improve soil quality (nutrient stocks and soil organic matter), rely on integrated pest management, and provide a net positive energy balance for food, feed, fiber, and biofuel.

Eco-functional intensification is needed to significantly increase yield on currently cultivated lands, to both protect soil and water resources in arable lands, and to avoid potential expansion to new areas, especially on marginal land not suited for continuous crop production or natural ecosystems that provide habitats for wildlife.

Successful eco-functional intensification requires a basic insight on how biodiversity contributes to various ecosystem services and subsequently how ecosystem services contribute to yield and farm income.

On-field practices to sustain ecosystem services

Agroecosystems are both providers and consumers of ecosystem services. Human management can greatly influence the provision of services to agriculture, including pollination, pest control, genetic diversity for future agricultural use, soil retention, regulation of soil fertility and nutrient cycling. Management practices can also influence the provision of 'disservices' from agriculture, including loss of habitat for conserving biodiversity, nutrient runoff, sedimentation of waterways, and pesticide poisoning of humans and non-target species (Zhang et al. 2007). However, appropriate management can ameliorate many of the negative impacts of agriculture, while largely maintaining provisioning services.

On-field management practices can significantly enhance the provision of ecosystem services to agriculture. Farmers routinely manage for greater provisioning services by using inputs and practices to increase yields, but management practices can also enhance

other ecosystem services, such as pollination, biological pest control, soil fertility and structure, water regulation, and support for biodiversity. For example, evidence suggests that management systems that emphasize crop diversity through the use of polycultures, cover crops, crop rotations and agroforestry can often reduce the abundance of insect pests that specialize on a particular crop, while providing refuge and alternative prey for natural enemies (Andow 1991). Similar practices including minimal use of pesticides, reduce use of fertilizers, no-till systems and crop rotations with mass-flowering crops may also benefit wild pollinators therefore sustaining pollination service. Managing for increased soil organic carbon (SOC), e.g. through diversified crop rotations or addition of manure and organic residues, correlates positively with the flow of soil services (Magdoff & Weil 2004). On the other hand, low content of soil organic carbon (SOC) resulting by intensive cultivation and poor soil management, affects mineral nutrient use efficiency (Brady et al. 2015) and it is a contributing cause for observed yield declines in major agricultural regions globally, jeopardizing food security (Ray et al. 2012). Farming practices that increase SOC have potential also to enhance arthropod natural enemies and, therewith, biological pest control above and below ground. Adding organic residues can bolster the soil fauna, which, in turn, represents additional food resource for generalist predators (Halaj & Wise 2002; Bell et al. 2008; von Berg et al. 2009, 2010), potentially increasing pest control (Scheu 2001). However, a comprehensive understanding of how the interplay between SOC and other farming practices such as nitrogen fertilization affect crop yield is still to be achieved. This kind of knowledge could reveal precious options for ecological intensification. Crop rotation and the use of spatial or temporal crop diversity can reduce the dependency to agro-chemical inputs such as pesticides and chemical fertilizers, sustaining natural enemies population and therefore biological pest control (Altieri 1992).

Off-field practices to sustain ecosystem services

Agricultural intensification has dramatically transformed agricultural landscapes into simplified monocultures with low cover of semi-natural habitats (Tschardt et al. 2005). Landscape simplification (Meehan et al. 2011), has led to significant biodiversity loss and to the reduction in the provision of key ecosystem services to agriculture

(Tilman et al. 2001). In fact, communities of natural enemies and pollinators are often found to be more abundant in complex landscapes where semi-natural habitats are more abundant (Chaplin-Kramer et al. 2011, Garibaldi et al. 2011). These habitats provide higher and more stable availability of hosts, nectar and pollen resources for natural enemies and pollinators, as well as refuge sites during overwintering (Bianchi et al. 2006, Winfree et al. 2011). However, amongst off-field interventions, field-margin diversification through the conservation and restoration of hedgerows is becoming a prominent intervention for promoting biodiversity and ecosystem services in intensive agricultural landscapes. Recent studies have shown that hedgerows can help to mitigate the negative effects of agricultural intensification on biodiversity (Batáry et al. 2010, Dainese et al. 2015). In particular, hedgerows can promote pollinator populations and export wild bees to adjacent fields (Morandin & Kremen 2013, Hannon and Sisk 2009) as well as support natural enemies (Morandin & Kremen 2014, Bianchi et al. 2006). The introduction of hedgerows seems to locally compensate the lack of semi-natural habitats mainly in simplified landscapes, providing therefore greater benefits than in landscapes with already a complex hedgerow network (Tschardt et al. 2005). Increased environmental heterogeneity can be also an effective way of decreasing the abundance of problematic weeds by shifting weed species abundance distribution (Dornelas et al. 2009).

Research objectives and thesis structure

The general objective of this thesis is to investigate how landscape structure (semi-natural habitats) and land-use (on-farm management) affect the provisioning of some important ecosystem services to agriculture, i.e. pollination service and biological control. The thesis is divided into two parts: in Part I we explored the effects of field margin quality (e.g. tree diversity, tree structural heterogeneity) on local pollinator community and pollination service. In Part II we explored the effects of farm management and landscape composition on biological control and then on ground beetle biodiversity (Coleoptera: Carabidae).

Part I: Pollination

Chapter 2 evaluated the effect of the quality of different semi-natural elements in the landscape on pollination service. We selected eight study areas of 10 x 10 km in Veneto region, in order to cover all levels of landscape composition from structurally simple to structurally complex landscapes.

Part II: Farm management and biological control

Chapter 3 investigated how local management practices, i.e. fertilizer and insecticide inputs, SOC, and landscape composition (1 km scale) affect crop yield, natural enemy abundance and pest control. Paired wheat fields with different levels of SOC were distributed along a gradient in landscape complexity. Within each field, a full factorial plot experiment was established to test the effects of mineral nitrogen and insecticide use.

Chapter 4 explored the potential interactions between soil management, landscape composition on beetle communities inhabiting cereal fields. In a field experiment we tested the combined effect of tillage management (conventional vs. conservation tillage) and urbanization on ground beetle communities (Coleoptera: Carabidae).

Chapter 5 provides a summarizing discussion on the fundamental and applied research findings

Chapter 2

Influence of hedgerows on bee communities and pollination in contrasting landscapes

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Abstract

Pollinators are a key component of global biodiversity, providing vital ecosystem services to crops and wild plants. An aspect that needs to be investigated more is the potential effects of both local habitat quality and landscape composition on pollinator diversity and pollination service. This study has the scope to assess the effects of field margin quality on pollination service and pollinator communities in contrasting landscapes. In this study we showed that in Veneto region there was an efficient pollination service irrespective of landscape composition and margin quality. Moreover our study indicates that the conservation of pollinator habitat can enhance overall biodiversity but not always the pollination service.

Introduction

The next few decades will witness a rapidly increasing demand for agricultural products owing to the world population and socio-economic growth (FAO 2014). This demand needs to be met largely through intensification (produce more from the same land surface) because there is little scope for an increase in agricultural area without causing irreparable damage to vital natural ecosystems (Bongaarts 1996), also considering that urban development will increasingly compete with agriculture for land use (Rounsevell et al. 2005). The steady increase in agricultural productivity per unit area observed during the latter part of the 20th century has now reached a plateau, with little opportunity for further increase in efficiency through conventional methods. Moreover, further intensification of farming practices such as increased external inputs and simplification of agricultural landscapes through removal of semi-natural habitats have already been shown to dramatically impact farmland biodiversity (Tilman et al. 2001, Green et al. 2005, Norris 2008). The dependency of conventional agriculture and food supply on non-renewable resources (e.g. fossil fuels, phosphate) makes the production system unsustainable in the long run. Eco-functional intensification of food production is the optimization of all provisioning, regulating and supporting ecosystem services involved in the agricultural production process (modified after Niggli et al. 2008). Successful eco-functional intensification requires a basic insight in how biodiversity contributes to various ecosystem services and subsequently how ecosystem services contribute to yield and farm income.

The inadequate supply of one single service can dramatically reduce production even if all the other services that support yield production are optimized; no or little additional output will be attained until this service short-fall is addressed (Bommarco et al. 2013). Even if a lot of studies have been conducted in the last years about ecosystem services, there are still a lot of questions concerning how landscape structure (semi-natural habitats) and land-use (on-farm management) affect the provision of ecosystem services (e.g. Kleijn et al. 2011), how farmland biodiversity is related to multiple ecosystem services (e.g. Van Der Putten et al. 2001; Isbell et al. 2011), whether there are trade-offs between different ecosystem services (Foley et al. 2005; Tylianakis et al. 2008; Power

2010), how ecosystem services are influence farm income (Zhang et al. 2007) and how ecosystem services can be managed through policy measures at the local, national or EU scale.

Pollinators are a key component of global biodiversity, providing vital ecosystem services to crops and wild plants. Though most of the world's staple foods, including wheat, corn, and rice reproduce without insect pollination, almost 35% of food production depends on pollinating animals (Kleijn et al. 2007). Pollination services depend on both domesticated and wild pollinator populations, that can both be affected by a range of environmental changes, such as habitat loss and climate change, with unknown consequences for pollination service delivery (Potts S. et al. 2010). Several studies on pollinator communities showed that pollinator loss is caused indirectly by agricultural intensification and habitat fragmentation at the landscape scale (Kremen et al. 2002, Winfree et al. 2009) and, at the local scale, by intensive crop production and habitat loss (Ricketts et al. 2008, Kremen et al. 2002). Furthermore, other research highlighted that pollinator persistence depends on both the maintenance of high-quality habitats around farms and on local management practices that may offset impacts of intensive farming (Lonsdorf et al. 2013).

The conservation of farmland biodiversity needs therefore a multiple-scale perspective (Gonthier et al. 2014). An aspect that needs to be investigated more is the potential effects of both local habitat quality and landscape composition on pollinator diversity and pollination service. Major pollinator groups nest or overwinter in semi-natural habitats and exploit agricultural fields mainly for foraging (Kremen et al. 2004, Holzschuh et al. 2008). Therefore pollinator richness and the pollination services they provide, decline exponentially with increasing distance from natural or semi-natural habitats such as field margins, species-rich grasslands or forests (Albrecht et al. 2007, Kohler et al. 2007). At the local scale, field margin quality (herbaceous, shrubs and trees) is known to influence pollination service affecting the abundance and diversity of wild bee populations. On the other hand, landscape variability, heterogeneity and diversity seem to not significantly influence pollinator diversity (Jeanneret et al. 2003). Some studies showed that changes in agricultural management, such as the conversion of species-rich hay meadows to silage production and the degradation of perennial

vegetation in field margins and hedgerows, are likely to have instead a detrimental effects on all *Bombus* spp. (Osborne & Corbet 1994). This study has the scope to assess the effects of field margin quality, at local scale, on pollination service and pollinators community in contrasting landscapes.

Materials and Methods

Study area and sampling design

Forty-eight field margins were selected in the Veneto Plain (north-eastern Italy). The plain is characterized by a continental climate, with relatively rigid winters and warm summers. The average temperatures in this area are comprised between 13 ° C and 15 ° C. Rainfall is distributed fairly evenly throughout the year and the total annual average of between 600 and 1100 mm (Table 1).

Table 1 Type of landscape at different scales, ID hedge, location, and latitude/longitude coordinates (accuracy of 10 m) of margin types included on the study

Land 1x1km	Buffer 500 m	Hedgerow type	ID hedge	Location	Latitude (N)	Longitude (E)
High	High	Complex	HC_ASOLO	Fonte (TV)	45,7993	11,8819
High	High	Grass margin	GM_2_ASOLO	Fonte (TV)	45,7997	11,8827
High	High	Simple	HM_ASOLO	Fonte (TV)	45,7963	11,8817
High	Low	Complex	SC_ASOLO	Asolo (TV)	45,7823	11,8920
High	Low	Simple	SM_ASOLO	Asolo (TV)	45,7834	11,8995
High	Low	Grass margin	GM_1_ASOLO	Asolo (TV)	45,7833	11,9000
Interm	Low	Simple	SM_CITTADELLA	Cittadella (PD)	45,6293	11,7715
Interm	Low	Complex	SC_CITTADELLA	Cittadella (PD)	45,6303	11,7815
Interm	Low	Grass margin	GM_1_CITTADELLA	Cittadella (PD)	45,6290	11,7819
Interm	High	Simple	HM_CITTADELLA	Fontaniva (PD)	45,6200	11,75163
Interm	High	Complex	HC_CITTADELLA	Fontaniva (PD)	45,6151	11,75759
Interm	High	Grass margin	GM_2_CITTADELLA	Fontaniva (PD)	45,6139	11,75761
High	Low	Simple	SM_COLLI	Abano Terme (PD)	45,3696	11,7737
High	Low	Complex	SC_COLLI	Abano Terme (PD)	45,3686	11,7749
High	Low	Grass margin	GM_1_COLLI	Abano Terme (PD)	45,3700	11,7729
High	High	Simple	HM_COLLI	Teolo (PD)	45,3609	11,7435
High	High	Grass margin	GM_2_COLLI	Teolo (PD)	45,3612	11,7419
High	High	Complex	HC_COLLI	Teolo (PD)	45,3594	11,7448
Low	Low	Complex	SC_MARTELLAGO	Martellagao (VE)	45,5534	12,1296
Low	Low	Simple	SM_MARTELLAGO	Martellagao (VE)	45,5588	12,1318
Low	Low	Grass margin	GM_1_MARTELLAGO	Martellagao (VE)	45,5546	12,1274
Low	High	Complex	HC_MARTELLAGO	Salzano (VE)	45,5383	12,1344
Low	High	Simple	HM_MARTELLAGO	Salzano (VE)	45,5293	12,1261
Low	High	Grass margin	GM_2_MARTELLAGO	Salzano (VE)	45,5302	12,1252
Low	High	Simple	HM_PIOVE	Piove di Sacco (PD)	45,2898	12,0438
Low	High	Grass margin	GM_2_PIOVE	Piove di Sacco (PD)	45,2883	12,0450
Low	High	Complex	HC_PIOVE	Piove di Sacco (PD)	45,2870	12,0477
Low	Low	Complex	SC_PIOVE	Arzzergrande (PD)	45,2761	12,0580
Low	Low	Grass margin	GM_1_PIOVE	Arzzergrande (PD)	45,2756	12,0580
Low	Low	Simple	SM_PIOVE	Santangelo di Piove di Sacco (PD)	45,2673	12,0563
Low	Low	Simple	SM RONCADE	Roncade ((TV)	45,6354	12,3841
Low	Low	Complex	SC RONCADE	Roncade ((TV)	45,6393	12,3886
Low	Low	Grass margin	GM_1 RONCADE	Roncade ((TV)	45,6402	12,3894
Low	High	Complex	HC RONCADE	Roncade ((TV)	45,6377	12,4018
Low	High	Grass margin	GM_2 RONCADE	Roncade ((TV)	45,6395	12,4010
Low	High	Simple	HM RONCADE	Roncade ((TV)	45,6361	12,4010
Interm	Low	Simple	SM_THIENE	Sarcedo(VI)	45,6982	11,5175
Interm	Low	Complex	SC_THIENE	Sarcedo (VI)	45,6982	12,0580
Interm	Low	Grass margin	GM_1_THIENE	Sarcedo(VI)	45,2756	12,0580
Interm	High	Simple	HM_THIENE	Sarcedo(VI)	45,6991	11,5176
Interm	High	Complex	HC_THIENE	Sarcedo(VI)	45,7040	11,5221
Interm	High	Grass margin	GM_2_THIENE	Sarcedo(VI)	45,7032	11,5221
Low	Low	Complex	SC_VIGODARZERE	Vigodarzere (PD)	45,4864	0,8848
Low	Low	Simple	SM_VIGODARZERE	Vigodarzere (PD)	45,4823	0,8857
Low	Low	Grass margin	GM_1_VIGODARZERE	Vigodarzere (PD)	45,4815	0,8853
Low	High	Simple	HM_VIGODARZERE	Vigodarzere (PD)	45,4766	0,8764
Low	High	Complex	HC_VIGODARZERE	Vigodarzere (PD)	45,4793	0,8791
Low	High	Grass margin	GM_2_VIGODARZERE	Vigodarzere (PD)	45,4795	0,8800

Three types of field boundary with increasing structural complexity were chosen within a landscape at 0.5 km: (i) grass margin, a perennial grass buffer strip without any nearby tree; (ii) simple hedgerow, a grass buffer strip adjacent to a single storied hedge (a so-called ‘treeline – full grown trees’, formed by a few dominant nectar species, either plan tree *Acer campestre* L., *Gleditsia triacanthos* L., *Robinia pseudoacacia* L., *Salix alba* L.); (iii) complex hedgerow, a grass buffer strip with a multi-storied hedgerow on the edge. We only selected hedgerows having a grass margin associated with them. For each field boundary, we obtained from interviews the number of cuts executed yearly in each grass buffer strip and we used it as measure of management intensity. Each field margin was characterized by sampling plant species composition. In total, 16 field boundary triplets were sampled. (The average number \pm SD of vascular plant species was 71.50 ± 95.11 in grass margins, 57.45 ± 3.32 in simple hedgerows, 44.13 ± 12.30 in complex hedgerows). We focused the effort on selecting field boundaries adjacent to the three main arable crops cultivated in the study region, i.e. maize and wheat. The occurrence of the adjacent arable crops within the different scale was done to obtain an interspersed arrangement. In fact, we found that the occurrence of the adjacent arable crops was independent of landscape at 0.5 km and field boundary type. In fact, we found that the occurrence of the adjacent arable crops was independent of landscape at 0.5 km and field boundary type. Overall, 48 field boundaries were sampled across the study region with the following procedure.

First, a large number of field margins were selected to cover a gradient of cover of arable land in a 0.5 km buffer (from 38 to 100%). Information on arable land cover was derived from a detailed vector-based land-cover map (Geoportal of Regione Veneto: <http://idt.regione.veneto.it>). Arable land cover was defined as the proportion of arable land within each buffer and quantified by GIS analysis (Quantum GIS 1.7, Open Source Geospatial Foundation Project, <http://qgis.osgeo.org>). Second, around each of the margins selected hedgerow cover was defined as the proportion of hedgerow patches in a 0.5 km buffer. In each site three field boundaries were sampled. Around each field boundary, landscape composition was assessed using a buffer with a 500 m radius. In each buffer hedgerow, forest and grassland patches were manually digitised from a visual inspection of high-resolution satellite images (Google Earth). In GIS (Quantum

GIS 1.7, Open Source Geospatial Foundation Project, <http://qgis.osgeo.org>), we quantified the area of each patch and then we calculated the proportion of the different land-use classes within the buffers. Was also measured cover of arable land in the 0.5 km buffer.

Pollination service

To assess pollination we used radish plants (*Raphanus sativus* L. variety Cherry belle) as phytometer. Six pots, each one containing an individual plant of radish, were placed adjacent to each margin. Three of these pots served as control, i.e. plants were protected with a tulle barrier, to prevent insect pollination. Radish was sown in little pots of 0.35 L with standardized soil, at 8°-10° C, in February and placed in a green house. After 15 days, in March, the plants were transferred into large 7L (22 x 22 cm in diameter) pots. The phytometers were placed outside between 4th and 12th of May, to allow visitation of native pollinators. All the experimental plants were irrigated when necessary. The flowering period lasted from April 29th to July 15th. When the phytometers were placed they were all of similar size and growth stage. Plants of *R. sativus* were removed between 13 to 17 June and relocated to a greenhouse. Once ripen, we estimate plant reproductive success and pollination service a number of measurements were done on flowers, pods and seeds. Seeds were weighed after drying for a week at 55°C. The following four parameters were considered:

- the proportion of flowers producing fruits, i.e. “fruit set” (numbers of pods/numbers of flowers) the numbers of seeds per pod
- the weight of 1000 seeds
- the total weight of seeds per plant

Damaged and predated pods were not used in the analyses of the numbers of seeds.

To test the effect of pollinators on plant reproductive success in relation to local and landscape factors, we calculated, for each cluster in the field margin, the difference in fruit set between the open and bagged plant. Such measure was then used as response variables in the statistical analyses.

Vascular plants and bees

Vascular plant species richness was estimated once before the first cut of the grass buffer strip in June 2013. Sampling was conducted in the grass buffer strip along a transect parallel to the each field boundary. We established three plots of $1 \times 2 \text{ m}^2$: one plot was placed in the middle part of the transect and the other plots at the two margins at least 10 m apart along transect. Within each sampling plot, vascular plants to species level (presence/absence data) were recorded.

We sampled pollinators using a passive sampling with pan-traps (750 ml plastic bowl). Within each field boundary, six pan-traps were placed in three clusters with two traps for each cluster. Each cluster was composed of one pan-trap painted with UV-bright yellow and one painted with standard yellow. The three clusters were placed along the grass buffer strip in the plant sampling plots. The traps were placed directly on the ground among open or low-growing vegetation and were filled with water and a drop of detergent (2% dilution). Pan traps were exposed for 72 hours, Apoidea and Hoverflies were collected. The first collection of insects was carried out between 26-29 May, while the second occurred between of 29 June to 2 July. In laboratory collected insects washed with distilled H_2O and transferred into vials (falcon) containing 70% ethanol, where they were stored for later identification. All specimens' bees were pinned, within a year of their collection. Abundance was quantified as the number of individual bees collected from pan trapping while richness was to species-level.

Bee identification

The species identification was determined taking 200 photos for each morph group. In order to properly take photos both wings were removed from each individual. The photos were then elaborated by reference database created by Naturalis. This database contains most of the species of Apoidea that are present in the Netherlands, as well as other European countries, including Italy. The comparison was made using software (“tpsUtil” and “tpsRelw”, files utility program by F. James Rohlf) that allows to digitize wing nerves of each photo. Then we compared the wing veins of all the pictures performing a PCA using R. Different “geomorph 2.1.3” packages were used: (Adams & Otárola-Castillo, 2013), package morpho 2.0.2 (Stefan Schlager, author and maintainer) and geometry package (Barber [cph], Kai Habel [cph, aut], Raoul Grasman [cph, aut], Robert B. Gramacy [cph, aut], Andreas Stahel [cph, aut], David C. Sterratt [cph, aut, cre]). 352 *Lasioglossum* specimens were not identified and therefore excluded from the analyses.

Table 2 List of the bees species

FAM	SPECIES
Andrenidae	<i>Andrena (Chlorandrena) boyerella</i>
Andrenidae	<i>Andrena (Euandrena) bicolor</i>
Andrenidae	<i>Andrena (Melandrena) cf. nitida cf. thoracica</i>
Andrenidae	<i>Andrena (Micrandrena) minutula</i>
Andrenidae	<i>Andrena (Micrandrena) minutuloides</i>
Andrenidae	<i>Andrena (Micrandrena) strohmella</i>
Andrenidae	<i>Andrena (Micrandrena) subopaca</i>
Andrenidae	<i>Andrena (Notandrena) nitidiuscula</i>
Andrenidae	<i>Andrena (Plastandrena) cf. tibialis</i>
Andrenidae	<i>Andrena (Simandrena) dorsata</i>
Andrenidae	<i>Andrena (Taeniandrena) ovatula</i>
Andrenidae	<i>Andrena (Zonandrena) flavipes</i>
Andrenidae	<i>Andrena (Zonandrena) gravaida</i>
Apidae	<i>Apis mellifera</i>
Apidae	<i>Bombus pascuorum</i>
Apidae	<i>Bombus terrestris</i>
Apidae	<i>Ceratina cf. cucurbitina</i>
Apidae	<i>Ceratina cf. dallatorreana</i>
Megachilidae	<i>Chalicodoma ericetorum</i>
Halictidae	<i>Halictus fulvipes</i>
Halictidae	<i>Halictus leucaheneus</i>
Halictidae	<i>Halictus ligatus</i>
Halictidae	<i>Halictus quadricinctus</i>
Halictidae	<i>Halictus scabiosae</i>
Megachilidae	<i>Hoplitis claviventris</i>
Megachilidae	<i>Hoplitis leucomelana</i>
Halictidae	<i>Lasioglossum (D.) brevicorne</i>
Halictidae	<i>Lasioglossum (D.) intermedium</i>
Halictidae	<i>Lasioglossum (D.) leucopus</i>
Halictidae	<i>Lasioglossum (D.) lucidulum</i>
Halictidae	<i>Lasioglossum (D.) minutissimum</i>
Halictidae	<i>Lasioglossum (D.) morio</i>
Halictidae	<i>Lasioglossum (D.) parvulum</i>
Halictidae	<i>Lasioglossum (D.) punctatissimum</i>
Halictidae	<i>Lasioglossum (D.) pygmaeum</i>
Halictidae	<i>Lasioglossum (D.) quadrinotatum</i>
Halictidae	<i>Lasioglossum (D.) sabulosum</i>
Halictidae	<i>Lasioglossum (D.) semilucens</i>
Halictidae	<i>Lasioglossum (D.) tarsatum</i>
Halictidae	<i>Lasioglossum (D.) villosulum</i>
Halictidae	<i>Lasioglossum (E.) albipes</i>

Halictidae	<i>Lasioglossum (E.) calceatum</i>
Halictidae	<i>Lasioglossum (E.) fratellum</i>
Halictidae	<i>Lasioglossum (E.) fulvicorne</i>
Halictidae	<i>Lasioglossum (E.) laticeps</i>
Halictidae	<i>Lasioglossum (E.) lineare</i>
Halictidae	<i>Lasioglossum (E.) malachurum</i>
Halictidae	<i>Lasioglossum (E.) pauxilus</i>
Halictidae	<i>Lasioglossum (L.) costulatum</i>
Halictidae	<i>Lasioglossum (L.) leucozonium</i>
Halictidae	<i>Lasioglossum (L.) majus</i>
Halictidae	<i>Lasioglossum (L.) pallens</i>
Halictidae	<i>Lasioglossum (L.) zonulum</i>
Megachilidae	<i>Megachile alpicola</i>
Megachilidae	<i>Megachile cf. alpicola cf. versicolor</i>
Megachilidae	<i>Megachile willughbiella</i>
Megachilidae	<i>Osmia caerulescens</i>
Halictidae	<i>Sphecodes dichrous</i>
Halictidae	<i>Sphecodes monilicornis</i>

Statistical analysis

Effects of boundary field and landscape composition (at local scale, around each field) on pollination service were analyzed using linear mixed effects model (landscape composition analyzed was land10 = percentage cover of semi-natural habitat at large scale, land500, = percentage cover of semi-natural habitat at local scale). In the model we used as response variable “fruitset” while the explanatory variables were margin (type of edge) and % landscape use. Land ID and Cluster ID were included in all the models as random factors. The response variables were ln-transformed to achieve normal distribution of model residuals. All the analyses were performed using the “nlme” and “lme4” packages (Pinheiro et al., 2014) implemented in R Statistical Software 3.1.1 (R Development Core Team, 2013).

Results

Pollination

We found that pollination was not dependent on margin quality or landscape composition. However, several plant parameters were affected by the presence of insect pollinators.

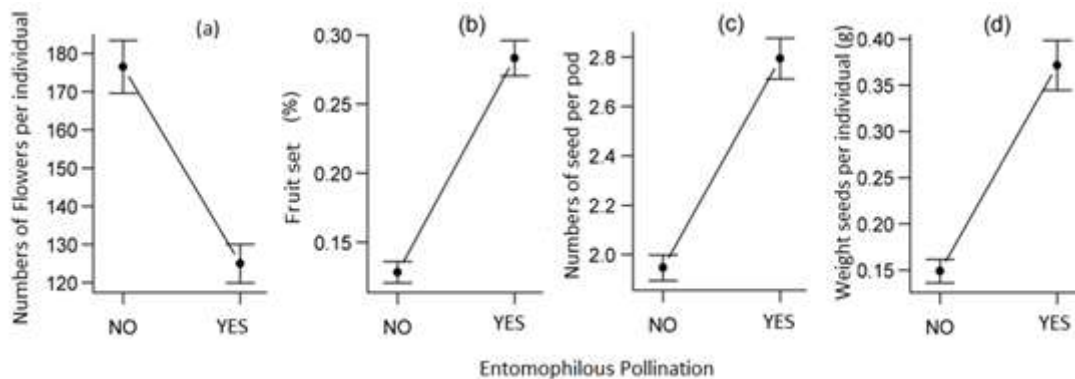


Figure 2 Yield parameters in plants exposed to insect pollination (YES) compared to control plants (NO).

All measured parameters were related to pollination (Table 3). There was a tendency for reduced number of flowers in exposed plants (Fig 2a), with increasing pollination, plants protected by tulle produced an average of 50 flowers more than the exposed ones. As

result of pollination, exposed plants produced a higher number both pods and seeds (Figs 2b and c).

Table 3 Results of the mixed model testing the insect pollination and landscape on the yield parameters

(a) Weight 1000 seeds	df	F	P
Landscape (10km)	1, 6	0.3798	0.5604
Landscape (0,5km)	1, 36	4.3393	0.0444
Pollination	1, 186	1.2782	0.2597
Hedgerow	2, 36	0.9947	0.3798
Landscape (10km) x Landscape (0,5km)	1, 36	0.6885	0.4121
Landscape (10km) x Pollination	1, 186	0.9906	0.3209
Landscape (0,5km) x Pollination	1, 186	1.42	0.2349
Landscape (10km) x Landscape (0,5km) x Pollination	1, 186	1.1419	0.2866
(b) Fruitset(%)			
Landscape (10 km)	1, 6	15.5396	0.0076
Landscape (0,5km)	1, 35	1.0532	0.3118
Pollination	1, 203	139.3311	< 0.0001
Hedgerow	2, 35	0.6789	0.5137
Landscape (10km) x Landscape(0,5km)	1, 35	0.4778	0.494
Landscape (10km) x Pollination	1, 203	7.7813	0.0058
Landscape (0,5km) xPollination	1, 203	3.2452	0.0731
Landscape (10km) x Landscape (0,5km) x Pollination	1, 203	1.6771	0.1968
(c) Seeds per pods			
Landscape (10km)	1, 6	4.4836	0.0786
Landscape (0,5km)	1, 35	0.2037	0.6545
Pollination	1, 203	99.5348	< 0.0001
Hedgerow	2, 35	0.9163	0.4094
Landscape (10km) x Landscape (0,5km)	1, 35	1.4049	0.2439
Landscape (10km) x Pollination	1, 203	0.0015	0.9695
Landscape (0,5km) x Pollination	1, 203	1.4731	0.2263
Landscape (10km) x Landscape (0,5km) x Pollination	1, 203	0.0061	0.9377
(d) Weight of seeds per individual			
Landscape (10km)	1, 6	2.96185	0.136
Landscape (0,5km)	1, 35	0.66289	0.421
Pollination	1, 198	82.73084	< 0.0001
Hedgerow	2, 35	1.39196	0.262
Landscape (10km) x Landscape (0,5km)	1, 35	0.00256	0.96
Landscape (10km) x Pollination	1, 198	0.9747	0.3247
Landscape (0,5km) x Pollination	1, 198	1.42176	0.2345
Landscape (10km) x Landscape (0,5km) x Pollination	1, 198	1.89858	0.1698

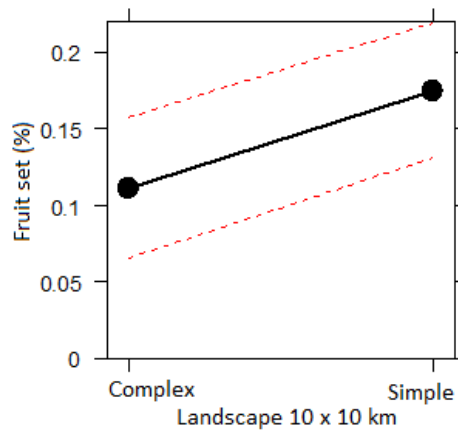


Figure 3 Correlation between fruit set and landscape structure.

Only fruit set had a correlation with the landscape (Fig. 3).

Relationship between pollination service and abundance of pollinators

There was no significant relationship between the number of pollinators caught in pan traps and the yield parameters analyzed. Overall 2231 bees were caught in the 48 field boundaries and were identified 61 species and 11 genus. The average species richness per field boundary was 9.71 (\pm 5.59 SD), range between 1 and 26, while abundance average was 46.48 (\pm 35.98 SD), range between 7 and 179. In GLMMs we found no effect of field boundaries type or landscape variables on abundance of pollinators, while grassy margins have significant effect on pollinator species richness (Fig. 4).

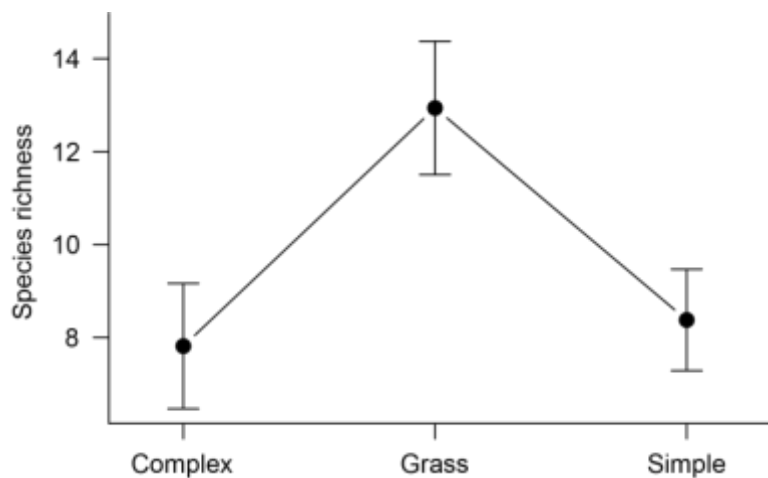


Figure 4: Relation between species richness and margins type

Opposite effect was found for evenness that was strongly correlated with complex hedgerows within landscape at 0.5 km (Fig. 5).

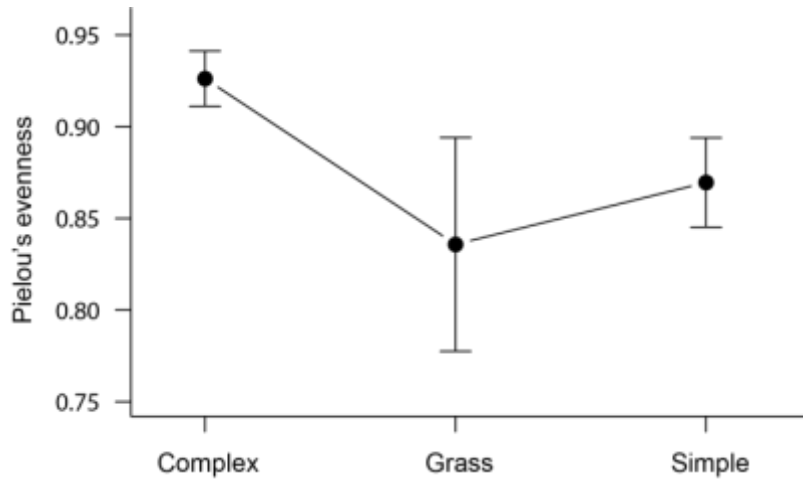


Figure 5 Relation between evenness and margins type

Discussion

Our results showed that in Veneto region there is a considerably efficient pollination service irrespective of landscape composition. The heterogeneous landscapes are able to support species that use different habitats for various ecological requirements, such as the need to differentiate ecological niche in the various stages of age (Rundolf et al. 2006). Simple hedgerows and complex hedgerows did not have a significant effect on the abundance of pollinators and we found even higher pollinator abundance in the grass margins. These findings can be explained by the presence of higher diversity of herbaceous species in the grassy margin compared to hedgerows.

Excluding fruit set, all other productive parameters considered were not significantly different between simple and complex landscapes. Wild bees can utilize plant communities across multiple habitats in agricultural landscapes including non-crop areas (Hannon and Sisk 2009, Mandelik et al. 2012), so these habitats should be included in floral resource assessments. Some studies suggest that the observed increase of pollinator diversity in the grassy margins are due to increases in abundance and diversity of herbaceous flora compared to hedgerows (Thomas et al. 1999, Andersson et al. 2012, Ricou et al. 2014). Landscape had no significant effect on pollinator abundance and species richness at both scales.

The success of Agri-environment schemes (AESs) can vary (Kleijn and Sutherland, 2003; Kleijn et al. 2006) and may depend on acceptance and popularity to farmers and the public. Furthermore, it is difficult to successfully link the enhancement of pollinator habitat adjacent to crop field with increased yield, a factor that may affect widespread adoption of such practices by farmers. This in turn can create difficulties in grower acceptance due to a perceived potential conflict between crop productivity and biodiversity conservation (Power, 2010). With regard to AES efforts it is important to note that studies have also shown that such strategies also provide secondary benefits to the farm and the surrounding landscape. In particular, our study indicates that the conservation of pollinator habitat can enhance overall biodiversity but not always the pollination service. Incorporating these secondary benefits into decision-making processes is likely to help stakeholders to assess the trade-offs implicit in supplying

ecosystem services (Wratten Stephen et al. 2012). One key finding of our study is that biodiversity and pollination delivery responded differently to both local management and landscape composition.

Aknowledgments

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Chapter 3

Reducing the yield gap between low and high chemical inputs: the benefit of soil organic carbon and natural pest control

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Abstract

Agricultural intensification has led to a loss of semi-natural habitats in landscapes and regions, while chemical inputs have increased and soil organic carbon (SOC) stocks decreased. This has raised concerns about degraded biological pest control, pest outbreaks and yield loss, but such combined effects of aspects of agricultural intensification have been difficult to disentangle. We analyzed the interactive effects of SOC, mineral fertilizer and insecticide inputs, as well as landscape and regional simplification on pest control and crop yield in 114 European wheat fields. We found yield loss due to the reduction of fertilizer inputs to decrease with SOC when no insecticide was applied, indicating indirect effects of pest pressure. This was paralleled by a higher suppression of aphid numbers and increase of ground-dwelling predators and pest control with SOC, especially in non-fertilized plots. Furthermore, we show that although landscape complexity enhance some natural enemies to pests, this benefit can be small in intensified regions.

Introduction

In response to emerging environmental and societal challenges and increased demand for agricultural products, future agriculture will need to better balance production with minimizing negative impacts on the environment and biodiversity (Foley et al. 2005). There is little scope to further increase the area of cultivated land globally (Pretty 2008), and agricultural intensification has had pervasive negative impacts not only on the environment, but possibly also on the crop production itself. One means to sustainably reduce current yield gaps is to replace crop production that largely depends on external inputs of agrochemicals, with ecologically intensified cropping that to greater extent rely upon yield-supporting ecosystem services generated by biodiversity within the agroecosystem (Bommarco et al. 2013). For this to be effective, ecosystem services above and below ground need to be understood, harnessed and managed for high and resilient yields with fewer inputs.

Above ground, rove beetles, spiders, parasitoid wasps and a plethora of other predatory arthropods can prevent or dampen outbreaks of herbivorous pest insects and secure harvests that would otherwise largely be lost (e.g. Settle et al. 1996; Losey & Vaughan 2006; Thies et al. 2011). Below ground, soil organisms release nutrients to the crop, control below-ground pests, form soil, affect carbon sequestration, water retention, and several other soil services that support crop yields (Barrios 2007). Well acknowledged yield-supporting ecosystem services such as these are generally examined individually, and are often implicitly considered as additive in their contribution to crop yield (Seppelt et al. 2011). Interplay among ecosystem services in their contribution to final crop yield has only recently been revealed (Lundin et al. 2013; Bartomeus et al. 2015). Likewise, there is an emerging interest, and huge remaining knowledge gaps, in how agricultural management and external inputs combine with ecosystem services in determining yield (Boreux et al. 2013; Klein et al. 2015; Marini et al. 2015; Tamburini et al. 2015). Although above- and below-ground food webs and ecological processes are clearly interlinked (de Deyn & van der Putten 2005; Kostenko et al. 2012), large scale patterns in the combined contribution to yield from services generated above and below ground and their interplay with agricultural management, remain poorly explored.

Managing for increased soil organic carbon (SOC), e.g., through diversified crop rotations, or addition of manure and organic residues, correlates positively with the flow of soil services (Magdoff & Weil 2004). Decreased contents of SOC as a result of intensive cultivation and poor soil management is jeopardising food security, mineral nutrient use efficiency (Brady et al. 2015), and is probably a contributing cause for observed yield declines in major agricultural regions globally (Ray et al. 2012). SOC feeds the diverse community of soil organisms that help increase soil fertility through breakdown of soil organic matter, and can thereby reduce the need for mineral fertilisers. Farming practices that increase SOC have potential also to enhance arthropod natural enemies to pests and, therewith, biological pest control above ground. Adding organic residues can bolster the soil fauna, which, in turn, becomes additional prey to generalist predators (Halaj & Wise 2002; Bell et al. 2008; von Berg et al. 2009, 2010), thereby possibly contributing to more efficient pest control (Scheu 2001), but evidence of such effects on agricultural soils are scarce. Better understanding of how the interplay between SOC and nitrogen fertilisation directly (through nutrient availability) and indirectly (through biological pest control) affect crop yield can reveal options for ecological intensification.

Natural enemies to insect pests are generally highly mobile, and a landscape perspective is essential to correctly predict predator community composition and pest control levels above ground (Tscharntke et al. 2015). Pest control is generally lower in arable fields embedded in intensively cultivated landscapes that extend approximately 1km around the field, because such landscapes lack refuge habitat and alternative prey to support viable predator populations throughout the year (Chaplin-Kramer et al. 2011; Schellhorn et al. 2015). However, agriculture is commonly intensified beyond the spatial scale of one or few kilometres. Entire regions have become devoid of natural and semi-natural habitat, and are cultivated with comparably few crops with high input levels. But how intensive agriculture over such large areas has affected communities of natural enemies and the services they provide is not well understood. Two contrasting hypothesis have been suggested: local management interventions benefit more biodiversity in more simplified (Tscharntke et al. 2005), or in more complex landscapes (Klein et al. 2009), but the evidence supporting either of these hypothesis when ecosystem services are considered is still sparse. It is even less

clear which of these hypotheses is true when agricultural intensification at larger, landscape and regional scales is considered.

Here, we explored how local management practices, fertiliser and insecticide inputs and management for enhanced SOC, combined with land use at the landscape (1 km diameter circle) and regional scales (~100 x100 km) determined the crop yield, biological pest control, and abundance of predators. Replicated experiments were set up in seven regions across Europe differing widely in agricultural intensity. Paired wheat fields with different levels of SOC were distributed across landscape complexity gradient within each region and factorial plot experiment adding, or not, mineral nitrogen and insecticides was established within each field. This experimental design allowed us to test for general interactive effects of management for ecosystem services and of efficiency of external inputs to crop yields. We found that yield loss due to cessation of N fertilisation depends not only on the amount fertilisers used, but also on SOC content and presence of pests and their natural enemies. We show that pests and predators can modify the effect of SOC content and amount N fertilisation on yield loss and demonstrate decreased yield gap between intensively fertilised and non-fertilised plots when fields are not sprayed. This is because in non-sprayed and non-fertilised plots, SOC benefits predators and increases biological pest control and simultaneously decreases pest pressure.

Material and methods

Experimental design

We performed replicated experiment in seven countries across Europe (Italy, Hungary, Sweden, Poland, Germany, Netherlands, and UK) in year 2014. We selected conventional winter wheat fields within an approximately 100 x100 km region within each country. The regions lie in a gradient of agricultural intensity with percentage crop (“Rcrop”) in the region ranging from 40 to 77% and average grain yield from 3.8 to 9.5 t/ha. In each country, we selected between 7 and 9 field pairs resulting in total of 114 fields overall. Paired fields were selected to have contrasting levels (high and low) of soil organic carbon (SOC) due to different history of contrasting management and not due to confounding environmental

factors (e.g., more or less peaty soils, drier or wetter parts of farms). To control for management effect, fields had different management for increasing SOC within each region (e.g. management for low SOC - through mono-culture rotation, or short rotation, and no organic matter added; for high SOC longer rotation, rotation with legumes and perennial grasses or organic matter added). The maximum distance between the two fields within each pair was 5 km.

Field pairs within each country were chosen across a gradient in landscape complexity surrounding the fields calculated as proportion arable land (“Lcrop”) in 1km radius circle around focal field. Landscape gradient was as extended as possible representative for each region. Landscapes with low complexity had at least 50% annually tilled land. Proportion arable land at the landscape (Lcrop) and regional level (Rcrop) were not correlated (Pearson's product-moment correlation = 0.08, $p > 0.05$). Experimental treatments were established near the edge of each field. Edge characteristics were matched within pairs (e.g., grassy open) and edge types were balanced among landscapes (e.g., we avoided having only forested edges in the complex landscapes). The treatments consisted of all combinations of pesticide application (0, 1) and mineral (inorganic) fertilizer application (0, 1) randomly assigned to each plot of minimum 10 x 12 m size. Applied insecticides were products with pyrethroids, applied using backpack sprayer after the first visual counts. Fertilizers were NPK, or ammonium nitrate and the amount of insecticides and fertilizers was applied according to the regional recommended rate (see Appendix Table 5). Farmers were allowed to use herbicides and fungicides in the treatment area. Percentage crop in the region was highly negatively correlated to the amount of fertilizers (Spearman's rank correlation rho: -0.813, $p < 0.0001$). The treatments were situated along the long side of a field and at least 10m from field edge. Experimental area was 18-24m wide and 40 m long.

We sampled soils to validate that SOC did not co-vary with other soil characteristics (pH, percentage clay). We took five 15 x 3 cm soil cores from each experimental area before fertilization. The samples were homogenized into one and stored at 5°C before analyses of soil properties (Mason 1983). SOC levels significantly differed between field pairs (Wilcoxon test: mean of the differences = 0.5, $p < 0.0001$). SOC did not correlate with soil pH (Pearson's product-moment correlation = 0.077, $p > 0.05$), but soil pH differed between field pairs (Wilcoxon test: $p < 0.0001$). Soil clay did not differ between field pairs.

Biological control of aphids

We measured biological control of aphids using cage experiment (Rusch *et al.* 2013) in plots without insecticide application. Visits to each field and establishment of the experiment were aligned with crop growth stage. We inoculated ~ 50-100 aphids (*Sitobion avenae*) per plot at BBCH 40-50. We placed one pitfall trap per inoculation place to remove aphid predators from the cage. The pitfall trap was removed from control treatment (no cage) at the time of the establishment of cage experiment. In each treatment without insecticide we established one cage and one open control. Cages consisted of plastic net with 5 x 5mm mesh size (30cm diameter and 100 cm high) inserted in a metal or plastic barrier (25 cm high, 32 cm diameter). We dug barriers 10cm into the soil to prevent ground-dwelling predators from entering the cages. To prevent flying predators to enter the cages we sprayed them with sticky glue. We removed aphid natural enemies prior to the cage establishment. We counted aphids non-destructively in each plot on 10 randomly selected tillers after 10 and 15 days when the experiment ended and we removed the cages. Pitfall traps were emptied and removed. Inoculation of aphids was not successful in Hungary.

Abundance of pests and flying predators and activity-density of ground-dwelling predators

We visually counted aphids, predators (Coccinellids (adults and larvae); Syrphids (larvae); Chrysopids (larvae), and parasitized aphids (mummies) on 50-100 randomly selected shoots per plot three times at BBCH 35+, 40-50 and 5 days later. To assess activity-density of ground dwelling predators we use pitfall traps placed 10 m from the edge covered with roofs (10 cm above each trap) to prevent flooding by rain. The pitfall traps were polypropylene beaker height 154.5mm and diameter 95mm filled with 200mL propylene glycol (antifreeze) solution 1/3 glycol, 2/3 water and a drop of detergent to reduce surface tension. The pitfall traps were emptied after 10 days and activity density of major functional groups (carabid beetles, staphylinid beetles and wolf spiders) was determined. We installed one pitfall trap in each treatment plot in each field.

All treatment plots were hand-harvested at 1m² per plot. We cut the tillers at the ground surface, let the plants dry and thresh them with a small thresher or by hand. We measured yield as dry weight (kg/ha). We interviewed farmers to obtain background information on the yield, fertilization and crop protection inputs in the experimental field.

Statistical analyses

We used Linear Mixed-Effects models to test the effects of agricultural intensification on grain yield, counts of pests and flying predators, aphid parasitism rates, activity-density of ground-dwelling predators and biological pest control. Explanatory variables for the models of grain yield (“GY”) were insecticide application (“I”, 2-level factor: 0, 1), N fertilization (“N”, 2-level factor: 0, 1), soil organic carbon (“SOC” - continuous). Since insect abundances and aphid biological pest control can depend on the landscape and regional agricultural intensification, percentage crop in the landscape (Lcrop) and region (Rcrop) was additionally included in the models for counts of aphids, flying predators, parasitism rates, activity-density of ground-dwelling predators (carabids, wolf spiders, rove beetles) and aphid biological pest control. In these models we tested 2-way interactions between agricultural intensification at different scales: regional and landscape (Rcrop x Lcrop), landscape and field (Lcrop x SOC), field and within field (SOC x N, SOC x I).

To examine the effects of SOC through nutrient retention and cycling, i.e. the buffering capacity of SOC when N fertilization ceases, we analyzed the change in grain yield (“GYnet”) in plots with minus plots without N fertilization in relation to SOC (continuous variable), amount of N applied (“Ntotal” – continuous variable ranging from 80 to 190 kg/ha), Insecticide (“I” factor: 0, 1), and their 3-way interaction. Calculating GYnet emphasizes the benefit of our fertilizer treatment to yield, while accounting for the underlying variation in baseline (non-fertilized) yield due to for example previous year crop or fertilization (see Appendix Fig 10). Biological pest control index (“BSI”) was calculated as difference in aphid growth with and without cages. Aphid grow was calculated as $\log_{10}(AT2+1) - \log_{10}(AT1+1)$ where AT is number of aphids per tiller at the beginning (AT1) and at the end of the experiment (AT2). In the analyses of BSI we excluded data where aphid growth within cages was negative as it indicates unsuccessful treatment of

natural enemy exclusion. To estimate the local management effects on aphid growth in the absence of aphid natural enemies, we analyzed aphid growth rate within the cages as a function of SOC, mineral fertilizers and their interaction.

Continuous explanatory variables were scaled to reduce collinearity and improve interpretability of the parameters (Zuur *et al.* 2009; Schielzeth, 2010). Collinearity was low in all models as indicated by Variance inflation factor (VIF) that was always lower than 3. The models residuals were visually checked for normality, homoscedasticity and spatial auto-correlation. When necessary, we used variance functions (constant – “varIdent” or exponential – “varExp”) to model heteroscedasticity and log or square root transformation of response variable to model normality of residuals. Count data were averaged across the 2 sampling dates after all treatments were applied. The random structure included field identity within the pair (1:2), nested within field pair identity (1:9), nested within country (1:7). Two fields had SOC content much higher compared to all other fields (SOC > 4) and to obtain more reliable and conservative estimates they were not included in the analyses. However, we did conduct all analyses with the full dataset and the results did not substantially change. All analyses were performed in R (R Development Core Team 2015) using packages “nlme” (Pinheiro *et al.* 2015) and “MuMIn” (Barton 2015) and “ncf” (Bjornstad 2013).

Results

Crop grain yield

As expected, grain yield (GY, t/ha) increased in plots with nitrogen added (N: 0.37 ± 0.03 , $p < 0.0001$), but less so in fields with high SOC (SOC x N interaction: -0.07 ± 0.03 , $p = 0.025$). SOC and insecticide application did not have any main effects on grain yield (SOC: 0.06 ± 0.04 , $p > 0.1$; I: -0.01 ± 0.03 , $p > 0.1$).

The net yield decrease when N fertilizer application was ceased (GY_{net}) was higher in the regions with high amounts of fertilizer applied (N_{tot}: 1.32 ± 0.39 , $p = 0.02$), tended to diminish with increasing levels of SOC (SOC: -0.35 ± 0.18 , $p = 0.058$) and did not change

with insecticides (I: 0.07 ± 0.14 , $p > 0.1$). However, insecticides changed the effect of SOC on GYnet from negative to positive (SOC x I interaction: 0.27 ± 0.14 , $p = 0.061$). GYnet tended to decrease with high SOC when mineral fertilization was high (SOC x Ntot interaction: -0.25 ± 0.14 , $p = 0.071$), but not when insecticides were applied (SOC x Ntot x I interaction: 0.24 ± 0.09 , $p = 0.012$, Figure 6). Including topsoil type as a covariate did not change the results. Percentage land covered with cropped fields in the region was strongly negatively correlated to the average regional yield (Pearson's correlation: -0.71 , $p < 0.0001$).

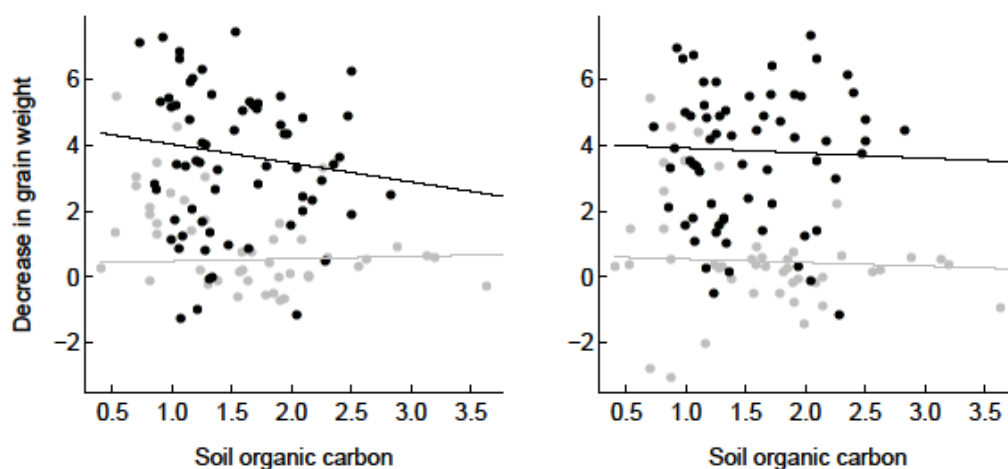


Figure 6 Difference in grain yield (GYnet, t/ha) due to added N fertilizers in relationship to high N added (black dots Ntotal >150, black line N.tot=190 (3rd Qu.)) and low N added (gray dots Ntotal<150, gray line N=90 (1st Qu.)). Left is a graph without insecticides and right with insecticides.

Counts of aphids and their flying natural enemies

Aphid number per tiller decreased with insecticide application (I: -0.61 ± 0.08 , $p < 0.0001$) and increased in plots with N (N: 0.17 ± 0.07 , $p = 0.016$). There was no main effect of SOC (SOC: -0.05 ± 0.09 , $p > 0.1$), or landscape simplification (Lcrop: -0.06 ± 0.07 , $p > 0.1$) on aphid counts, but aphids decreased with SOC only in non-fertilized plots (SOC x N interaction: 0.17 ± 0.05 , $p = 0.001$, Figure 7c) and increased with SOC in simplified landscapes (SOC x Lcrop interaction: 0.17 ± 0.07 , $p = 0.017$). Aphid growth in the absence of predators did not respond to SOC or mineral fertilization.

Number of vegetation-dwelling predators per tiller decreased in plots with insecticides (I: -0.40 ± 0.15 , $p = 0.01$) and with landscape simplification (Lcrop: -0.16 ± 0.08 , $p = 0.044$), but did

not change with SOC (SOC: -0.15 ± 0.11 , $p > 0.1$) or nitrogen fertilization (N: -0.18 ± 0.15 , $p > 0.1$). There were interactions between SOC and fertilization, and between SOC and landscape simplification such that predators increased with SOC in fertilized plots (SOC x N interaction: 0.22 ± 0.11 , $p = 0.04$), and had a tendency to increase with SOC in simplified landscapes (SOC x Lcrop interaction: 0.14 ± 0.07 , $p = 0.08$). Parasitism rates increased in fertilized plots (N: 0.42 ± 0.16 , $p = 0.008$) and decreased with increasing SOC (SOC: -0.30 ± 0.14 , $p = 0.039$).

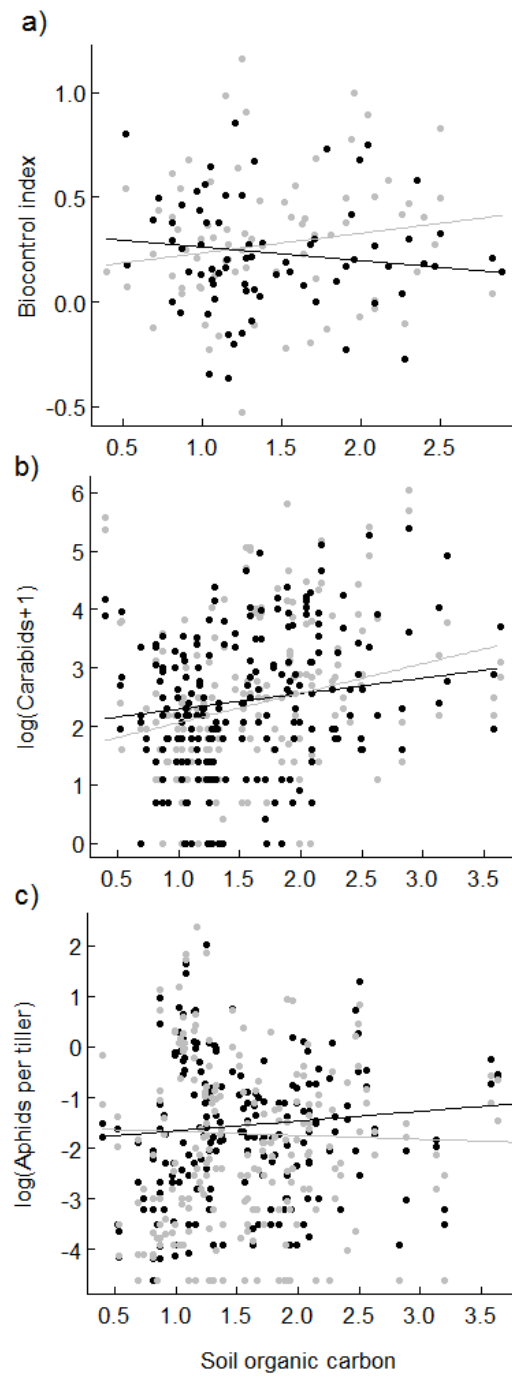


Figure 7 Biological control index (a), carabids (b) and aphids (c) in relationship to SOC in plots with N (black dots and line) and without N (gray dots and line)

Activity-density of ground dwelling predators

Ground-dwelling predator activity-density increased with SOC (SOC: 0.19 ± 0.08 , $p= 0.025$), but taxonomic groups responded differently to our treatments. While increase in SOC was negatively related to aphid pests, it increased carabid and spider activity-density and biological control, especially so in the absence of N fertilization. Carabid activity-density increased with SOC (SOC: 0.32 ± 0.10 , $p= 0.0025$), did not change with the fertilization treatment (N: 0.09 ± 0.09 , $p>0.1$), but increased slower with SOC in plots with N (SOC x N interaction: -0.15 ± 0.07 , $p= 0.04$, Figure 7b). Rove beetle activity-density was higher in fertilized plots (N: 0.51 ± 0.09 , $p< 0001$). Wolf spider activity-density increased with SOC (SOC: 0.37 ± 0.16 , $p= 0.02$) and was lower in plots with N (N: -0.46 ± 0.18 , $p= 0.012$) and in landscapes with high percentage crop fields (Lcrop: -0.34 ± 0.12 , $p= 0.007$). They did not change with regional intensification (Rcrop: -0.40 ± 0.39 , $p>0.1$), but had lower activity-density in landscapes with high percentage crop in simplified regions (Lcrop x Rcrop interaction: 0.26 ± 0.12 , $p= 0.04$, Figure 8).

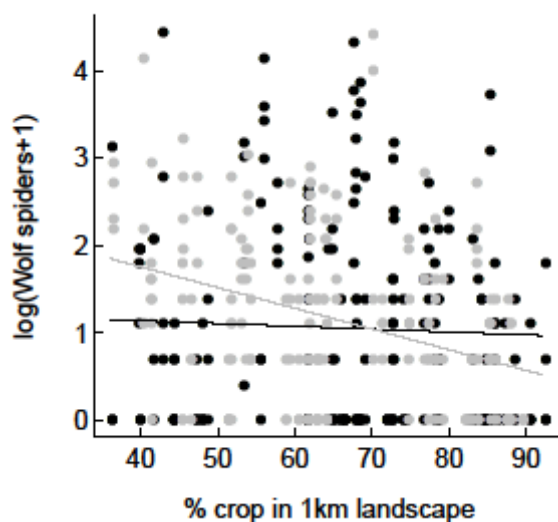


Figure 8 Wolf spider activity-density in relationship to percentage crop in 1km landscape in regions with high AI (Rcrop > 50, black dots, black line at Rcrop = 67 (3rd Qu.)) and low AI (Rcrop < 50, gray dots, gray line at Rcrop = 41 (1st Qu.))

Biological control of aphids

Biological control of aphids (BSI), i.e. the difference in aphid growth inside and outside the cages, increased with SOC (SOC: 0.05 ± 0.02 , $p=0.037$), did not change with N fertilization (N: -0.04 ± 0.03 , $p>0.1$), but decreased with SOC when N was added (SOC x N interaction: -0.09 ± 0.03 , $p= 0.003$, Figure 7a). The biological control index was correlated to ground-dwelling predator activity-density (Spearman correlation = 0.20, $p= 0.014$), in particular to carabid (Spearman correlation = 0.18, $p= 0.027$) and wolf spider (Spearman correlation = 0.18, $p= 0.02$), but not to rove beetle activity density.

Discussion

Our results demonstrate consistent interactive effects between mineral nitrogen fertilization and SOC on crop yield, aphid pests, their predators and biological control. The yield gap between plots with and without mineral fertilization was higher in regions with high amounts of fertilizer applied, but this difference in yield tended to become smaller with increasing levels of SOC indicating that SOC mitigated nutrient poverty (Brady *et al.* 2015). Interestingly, this mitigating effect by SOC on the yield-decline in non-fertilized plots became even more pronounced when insecticides were not applied. A likely cause for this was that SOC and natural pest control (in contrast to chemical pest control) together minimized the yield reduction at cessation of high mineral nutrient inputs. This contention is supported by the observation that aphids decreased, while natural enemy abundance and biological pest control were enhanced by SOC, especially so in non-fertilized plots. Important to note is that ceasing to apply mineral nitrogen consistently, as expected reduced yields, but that SOC and biological control to surprisingly great extent mitigated this reduction. If future agriculture is to maintain sufficient production levels while minimizing negative impacts on the environment and climate by reducing agro-chemical inputs, our study points to the dual, and probably ecologically coupled, benefit of supporting SOC and biological control services to this end.

Ground-dwelling predators appeared to be the main biological control agents as the biological control index was strongly positively correlated to the activity-density of carabids and wolf spiders. Moreover, these ground-dwelling predators and the biological pest control index

increased, while aphids decreased with increased SOC and reduced fertilization. Managing for increased SOC has been suggested to subsidize decomposer communities that provide additional prey to the above ground fauna of generalist arthropod predators higher up in the food web, thereby strengthening top-down control of herbivores, and allowing for increased primary production (Settle *et al.* 1996; Scheu 2001; Halaj & Wise 2002; Bell *et al.* 2008, von Berg *et al.* 2010). Additional gains may also be achieved if soil management not only enhances SOC but also reduces disturbance, such as for low-intensity tilling instead of deep plowing (Tamburini *et al.* 2015). Our results indicate a general positive effect of natural pest control by generalist predators from boosting SOC, and we have found few other cases that demonstrate such ensuing effects of SOC on pest control in agriculture. More generally, these results adds to a view that SOC is linked to a number of yield supporting ecosystem services (Magdoff & Weil 2004) and presents an important natural capital in the soil (Brady *et al.* 2015).

Our experiment also revealed interesting, more local, fertilization treatment effects that had positive effect on pests, but negative on the main predators and biological pest control in high SOC fields. That aphids were less suppressed in unfertilized plots could be because aphids can benefit from mineral fertilizers (Honek 1991; Riedell & Kieckhefer 1993; Hasken & Poehling 1995, Duffield *et al.* 1997), as also shown in our results for aphid counts. Synthetic fertilizers can shift the balance in the plant toward growth and reproduction, while compromising defense capabilities of affected plants and making them more palatable for herbivores (“The mineral balance hypothesis”, Phelan *et al.* 1996). However, the effect of nitrogen fertilization on aphids is likely to be due to the leaf-dwelling aphids in our study (see also Honek 1991; Hasken & Poehling 1995), as the growth of the main cereal aphid pest, *S avenae*, did not change with fertilization within our enclosure cages. Mineral fertilizers can also affect the soil fauna that, in turn, affects generalist predators (Ngosong *et al.* 2009; Cluzeau *et al.* 2012; Birkhofer *et al.* 2008) and changes the plant's capability to resist above-ground herbivores (Bezemer & Van Dam 2005; van Dam *et al.* 2003; Wurst *et al.* 2004). Aphid parasitism rates also increased with fertilizer application, either due to plant nutrition affecting parasitoids indirectly through the host insect (see also Wurst & Jones, 2003; Bukovinszky *et al.* 2008), or as a response to increased aphid abundances. These results are in line with the recent discovery that nitrogen fertilizers

negatively affect aphid predator-prey ratio and positively affect aphid primary parasitism at a field scale (Zhao et al. 2015). Our findings demonstrate that mineral nitrogen input, even at a local, within-field scale, can strongly influence above-ground arthropod food webs presumably due to its effects on plants and soil fauna that has low dispersal ability.

While our results show that local cropping practices directly and indirectly affect pests and their natural enemies, these organisms are mobile and are, in addition, affected by land management at spatial scales well beyond the arable field (Tscharntke et al. 2005). We found that increased crop cover in the surrounding landscape had a negative effect on the densities of flying predators and spiders, a result confirming several other reports of negative impacts on natural enemy communities from intensive land use (Chaplin-Kramer et al. 2011). More interestingly, we revealed that agricultural intensification at the regional scale reduced the benefit of less intensively cropped landscapes for important predator group. Specifically, spider activity-density was higher in complex landscapes in less intensified regions. This means that the “ecological contrast hypothesis” (Tscharntke et al. 2005) that predicts a high effectiveness of changes in field management practices in structurally simple landscapes is not supported in our study when land use at larger scales was considered. Instead, initiatives to support biodiversity and organisms providing ecosystem services in a landscape of few square kilometers can be more effective if implemented in a less intensively cultivated region that supports a richer pool of species and larger populations of beneficial organisms such as natural enemies to crop pests (Kleijn et al. 2009). Reducing area of semi-natural habitat and increasing in the cropped area at large, regional scales might not only weaken the efficiency of actions to support ecosystem services, but also reduce yield, as indicated by the negative relationship between regional intensification and average crop yield that we found.

Our results demonstrate impact of land-use intensity on biological pest control and crop yield at multiple spatial scales: local (management for soil fertility), landscape and regional (change in percentage crop area). In general, our results add to a growing body of recent evidence of non-additive effects among management practices and above- and below-ground ecosystem services (Boreux et al. 2013; Klein et al. 2015; Tamburini et al. 2015; Bartomeus et al. 2015). These results also demonstrate how local management practices for increased soil fertility that enhances SOC, indirectly affect crop yield via changes in pests

and predator abundances and biological control. Our findings pave a way for ecological intensification demonstrating possibilities to reduce inputs of fertilizer and insecticides with enhanced SOC content and bolstering of ecosystem services, thereby minimizing transitional yield losses and avoiding environmental costs. Moreover, bolstering biological control services may require a consideration of previously poorly understood land use at large regional scales, beyond the arable field and landscapes of few square kilometers.

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Chapter 4

Tillage and urbanization affect species richness, abundance and functional richness of carabid beetles inhabiting cereal fields

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Abstract

Urban settlement expansion and agricultural intensification have been widely recognized as major causes of farmland biodiversity decline across multiple taxonomic levels. Nevertheless, comprehensive studies investigating the effects of soil management and landscape urbanization on arthropod biodiversity are still scarce. We explored the combined effect of tillage management (conventional vs. conservation tillage) and urbanization on ground beetle communities (Coleoptera: Carabidae) in 10 pairs of winter cereal fields in the agricultural landscape of Udine province (north-east Italy). Carabids were collected by pitfall trapping during May and June 2014. We found that processes at the local and at the landscape scale interacted: urbanization (750 m scale) strongly decreased species richness, activity-density and functional richness only in fields managed under conventional tillage, while conservation tillage support more diverse (both taxonomically and ecologically) and abundant beetle communities also in highly urbanized landscapes. We also found that different functional groups differently responded to tillage management and landscape composition. The better local habitat quality provided by conservation tillage may thus mitigate the negative effects of urbanization on carabid communities. Our study stresses the importance of considering both local management and landscape composition when planning strategies to support farmland biodiversity.

Introduction

Agricultural ecosystems cover nearly 40 per cent of the terrestrial surface of the Earth (FAO 2015) and they are known to make an important contribution in harboring and sustaining world's biodiversity (Butler et al. 2007). However, agricultural intensification such as loss of landscape complexity, increased farm and field sizes and larger inputs of agrochemicals, has caused a strong decline of farmland biodiversity across multiple taxonomic levels (Benton et al. 2003). Despite decades of agri-environmental policies aimed at supporting and promoting biodiversity in agricultural landscapes, the negative effects of agricultural intensification on wild plant and animal species still persist, threatening the provision of key biodiversity-based ecosystem services such as biocontrol or pollination (Geiger et al. 2010). There is therefore the urgent need to identify strategies both at landscape and local scale to sustain and promote biodiversity in agricultural ecosystems (Power 2010, Bommarco et al. 2013).

At the local scale, many studies explored the impact of different farming practices on biodiversity (e.g. Kromp 1989; Pfiffner and Luka 2003; Bengtsson et al. 2005). Several authors have investigated the effect of soil disturbance on the abundance and diversity of the ground fauna (House and Alzugaray 1989; Holland 2004). In the context of sustainable agriculture, conservation tillage is considered an alternative farming practice aimed at minimizing the negative effects of tillage operation on soil environment, in particular enhancing soil biodiversity because of the reduced physical disturbance, increased soil structure and moisture content that all together provide a more suitable environment for soil fauna (Kromp 1999; El Titi 2002; Holland 2004; Soane et al. 2012). Despite the potential benefit of conservation tillage on soil biodiversity, more research is still needed to understand the potential interactions between landscape processes and local soil management (but see Trichard et al. 2013; Palmu et al. 2014).

Research on landscape-scale effects on farmland biodiversity has often focused on the role of semi-natural habitats since non-crop habitats support a wide range of beneficial organisms providing alternative food, hosts and winter refuges (Tscharrntke et al. 2007). However, although in several regions across the globe agricultural fields are often interspersed with urban settlements, most of the research in agro-ecology has largely

overlooked the potential detrimental effect of urban sprawl in the landscape on local biodiversity. Urbanization is in fact known to cause changes in community structure and diversity of several taxa, because of habitat fragmentation, alteration and loss, noise, local climate warming and increased pollution (e.g. Niemelä et al. 2002; Voogt and Oke 2003; Croci et al. 2008; McKinney 2008). However, which is the effect of urbanization on in-field biodiversity in agricultural ecosystems is still largely unknown.

A large body of research has focused so far on the effects of different farming practices and landscape composition on the communities of beneficial arthropods in arable lands. In particular, carabid beetles (Coleoptera: Carabidae) have been extensively studied (e.g. Kromp 1999) as the majority of species play a potential role in crop pest control being predominantly predatory (e.g. Lang et al. 1999), while many others are of conservation importance in their own (Rainio and Niemelä 2003). Moreover, carabid beetles are considered a reliable monitoring group because of their broad taxonomic and ecological diversity, abundance and their sensitivity to human-caused disturbances both at local and landscape scale (Lövei and Sunderland 1996). For example, they are known to be favored under reduced tillage management (e.g. Holland 2004) and their communities to be shaped by landscape urbanization: highly urbanized areas seem to harbor less diverse carabid assemblages (e.g. Niemelä and Kotze 2009). Whether these local and landscape processes interact, remain unknown.

Here, we want to explore the effects of tillage management (conservation vs. conventional tillage), and landscape urbanization on ground beetles communities inhabiting winter cereal fields. Specifically, we expect that both tillage management and urbanization would affect the abundance, the species richness and the functional richness (i.e. the width of a niche space filled with species) of carabid beetles. We also tested whether urbanization additively or synergistically acted with tillage management in shaping carabid beetle communities.

Material and methods

Study area

Carabid beetles were collected between May and June 2014 in 10 field pairs located in agricultural landscape of the Udine province (Fig. 11), which lowland area (c. 615 km²) is characterized by a temperate climate (mean annual precipitation of c. 1300 mm; mean annual temperature of 13°C). Within each pair, one field was managed under conservation tillage and the other under conventional tillage (distance range: 0-400 m). Field pairs were distant at least 1 km. In autumn 2013, seven pairs were sowed with winter wheat and three with barley. Crop species was consistent within the pairs and environmental characteristics comparable because of the short distance between fields. Under conventional tillage seedbed was prepared by moldboard plowing (30 cm depth) whereas conservation tillage management contemplated non-inversion of soil for at least 5 years (values ranging from 5 to 15, mean=8.7) and used cover crops between main cash crops. The most used cover crop was *Lolium multiflorum* L. that was sown after summer crops. In each field, we identified a plot of 10 x 20 m located on one side of each field. Within each pair, the plots bordered with an edge habitat of similar structure and composition (either a grass margin or a hedgerow). The plot was not sprayed with any pesticides and was fertilized with a dose of 80 N kg ha⁻¹, following local farming recommendation.



Figure 11 Study area and an example of a field pair.

Landscape variables

The ten field pairs were selected along a gradient of urbanization ranging from 0.0 to 27.8 % of urban areas in a 1060 m radius around each field. A total of eight nested spatial scales were thereafter 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 km² (1060 m). ArcGIS 9.3 was used for landscape analyses of regional land use maps, verified and ameliorated with aerial photographs to increase class discrimination accuracy. Along with the cover of urban areas we also quantified the cover of semi-natural habitats that included forest patches, hedgerows, tree lines, field margins and grasslands. Cover of urban areas and semi-natural habitats were not correlated ($R_s=0.16$, $P=0.37$). Preliminary analyses showed no effect of the proportion of semi-natural habitats at any scales on carabid community.

Carabid data

Carabids 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. We performed two consecutive sampling rounds of 10 days each between the 14th of May and the 4th of June 2014. The period was chosen according to the aphid phenology in order to sample the carabid communities that potentially could feed on aphid populations attacking winter cereals. Carabid beetles were preserved in 70% ethanol. Beetles were divided in morphogroups and then determined to species, except for 17 individuals that were determined to genus level (*Amara* and *Trechus* spp.). Based on literature, we selected five species traits of carabids in order to calculate functional richness: wing morphology, overwintering stage, feeding type, habitat preference and body size (Lindroth 1992; Lövei and Sunderland 1996; Ribera et al. 2001; Pilon et al. 2013; www.carabids.org - Homburg et al. 2014) (see Supplementary Information). These traits are expected to respond to both the landscape and local factors selected. Wing morphology of carabid beetles is important for dispersal ability (Gerisch, 2014). Each species was assigned to one of the following categories: macropterous (winged), brachypterous (wingless) and dimorphic (both forms can appear within a species). The overwintering stage is a trait expected to respond to soil management (Kromp 1999). We divided the species into three groups: carabids principally overwintering as larvae, as adults and carabids overwintering in both forms. They were also divided into predators, herbivores and omnivores according to their predominantly feeding traits during their entire life cycle. For habitat preference, we divided the species into forest species, species of open habitats and generalists. Moreover, according to literature, the average body size was assigned to each carabid species.

Data analysis

To investigate the effects of soil management (conservation vs. conventional tillage) and urbanization at different spatial scales on carabid beetle community, linear mixed effects models (Pinheiro *et al.*, 2013) were used in R (R Development Core Team, 2015). We first built full models and then we simplified them by removing one-by-one the non-

significant interaction terms. The normality of the distribution of the raw-dependent variables was assessed using QQ-plots. Data were ln-transformed where necessary. Crop type was initially included in all the models as fixed factor to test for potential effect of different crop species on carabid community. Since it did not influence any response variable, it was therefore removed from the models.

For the analyses of species richness and activity-density of the whole community, beetle data for each plot were pooled together (the total catch of the three pitfall traps for each sampling period). Fixed effects were tillage management and cover of urban areas, while the random effects were the field pair ID and the field ID. We also tested the same variables on species richness and activity-density for different functional traits i.e. feeding type and dispersal ability that were included as fixed effects in models. Further analyses on the other functional groups were not performed because of the high correlation between them: for example carabid feeding behaviour is known to be body size dependent (Muller 1985). Species richness and activity-density were standardized since values were very different between different groups of traits (e.g. predators, herbivores, omnivores).

Functional richness was calculated based on recent literature (Masonet al. 2005; Lepset al. 2006). Beetle data for the two sampling periods in each plot were pooled together in order to describe the functional richness of the whole carabid community present in each field. Fixed effects were tillage management and cover of urban areas, while the random effect was the field pair ID. The proportion of urban areas was included in all the models and the 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.

Results

Overall 9824 carabid beetles were collected and classified in 46 species, 19 genera and 12 tribes. We captured on average of 245 individuals per plot, with a minimum of 13 and a maximum of 881, while the average species richness was 11 with a minimum of 5 and a maximum of 22. Most of the individuals were predators (8217) and omnivores (1041)

and only 549 were herbivores. Concerning the dispersal ability, 5246 individuals were macropterous, 4273 were brachypterous and 288 were dimorphic.

The results from LMM showed strong effects of both tillage management and urbanization on carabid beetles community displaying more rich and more abundant communities in the fields managed under conservation tillage and in the landscapes characterized by low cover of urban areas (Table 4). Moreover, for the analyses of species richness, activity-density and functional richness we found an interaction between tillage system and the proportion of urban areas in the landscape (750 m scale), i.e. the negative effect of urbanization was more evident in the fields managed under conventional tillage while in the fields managed under conservation tillage the species richness, activity-density and functional richness remained stable or declined less strongly along the urbanization gradient (Fig. 12). These effects were maintained from 530 to 1060 m around fields.

Table 4 Results of the mixed model testing the effect of soil management (conservation vs. conventional) proportion of urban areas (750 m) and functional traits (feeding type and dispersal ability) on carabid community.

(a) Species richness	df	F	P
Tillage	1, 7	16.587	0.0047
% Urban areas (750 m)	1, 7	10.552	0.0141
Tillage x % Urban areas (750 m)	1, 7	5.7283	0.0479
(b) Activity-density			
Tillage	1, 7	6.1203	0.0427
% Urban areas (750 m)	1, 7	1.7728	0.2248
Tillage x % Urban areas (750 m)	1, 7	7.9703	0.0257
(c) Species richness (feeding type)			
Tillage	1, 7	14.199	0.0070
% Urban areas (750 m)	1, 7	12.557	0.0094
Feeding	2, 92	0.0000	1.0000
Tillage x % Urban areas (750 m)	1, 7	1.3486	0.2836
Tillage x Feeding	2, 92	1.7603	0.1777
% Urban areas (750 m) x Feeding type	2, 92	0.3070	0.7364
Tillage x % Urban areas (750 m) x Feeding	2, 92	3.2734	0.0423
(d) Activity-density (feeding type)			
Tillage	1, 7	10.651	0.0138
% Urban areas (750 m)	1, 7	4.6787	0.0673
Feeding	2, 98	2.2035	0.1158
Tillage x % Urban areas (750 m)	1, 7	6.6840	0.0362
(e) Species richness (dispersal ability)			
Tillage	1, 7	4.5764	0.0697
% Urban areas (750 m)	1, 7	10.663	0.0138
Dispersal	2, 96	0.0000	1.0000
Tillage x % Urban areas (750 m)	1, 7	5.3494	0.0540
Tillage x Dispersal	2, 96	9.6700	0.0001
(f) Activity-density (dispersal ability)			
Tillage	1, 8	3.5120	0.2538
% Urban areas (750 m)	1, 8	3.0562	0.1355
Dispersal	2, 96	0.2680	0.7655
Tillage x % Urban areas (750 m)	1, 8	9.7715	0.0167
% Urban areas (750 m) x Dispersal	2, 96	2.9451	0.0524
(g) Functional richness			
Tillage	1, 7	0.2808	0.6125
% Urban areas (750 m)	1, 7	49.271	0.0002
Tillage x % Urban areas (750 m)	1, 7	16.502	0.0048

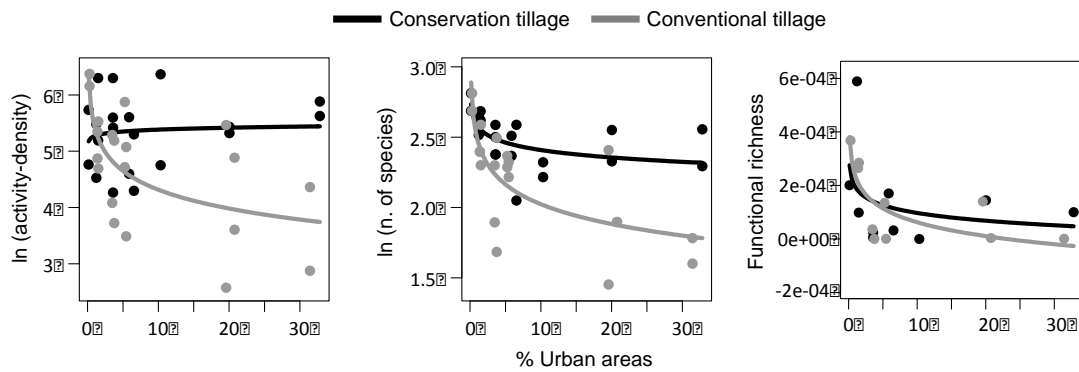


Figure 12 Effect of tillage system (black line and dots, conservation tillage; grey line and dots, conventional tillage) and the proportion of urban areas in the landscape (750 m radius) on carabid \ln -transformed activity density, \ln -transformed species richness and functional diversity. Dots correspond to model predictions.

Regarding the analyses of the feeding trait, we found a similar interaction between tillage system and urbanization, clearly evident only for the species richness of herbivores, while for omnivores and predators that interaction was less evident (Fig. 13). The activity-density of different feeding groups was not affected by either tillage management or urbanization. For the dispersal ability analyses, we found the activity-density of different groups to be affected by tillage management and the species richness to be affected by urbanization. In both cases, macropterous carabids were more influenced than the other groups by the fixed factors (Fig. 14).

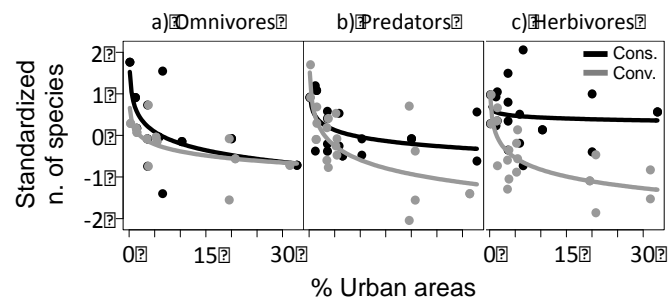


Figure 13 Effect of tillage system (black line and dots, conservation tillage; grey line and dots, conventional tillage;) and the proportion of urban areas in the landscape (750m) on the standardized number of species of (a) omnivore, (b) predator and (c) herbivore carabids. Dots correspond to model predictions.

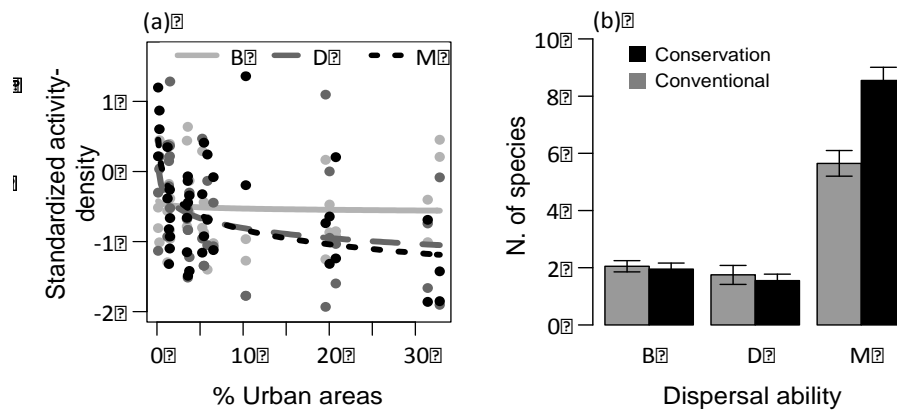


Figure 14 (a) Effect of the proportion of urban areas in the landscape (750m) on the standardized activity-density of brachipterous (B), dimorphic (D) and macropterous (M) carabids. Dots correspond to model predictions. (b) Effect of tillage system (black bars, conservation tillage; grey bars, conventional tillage) on the number of species of brachipterous (B), dimorphic (D) and macropterous (M) carabids.

Discussion

Our study explored the combined effect of tillage management and urbanization on carabid beetle communities in winter cereal crops. We found that processes at the local and at the landscape scale interacted: urbanization strongly decreased species richness, activity-density and functional richness only in fields managed under conventional tillage, while conservation tillage support more diverse (both taxonomically and ecologically) and abundant beetle communities also in highly urbanized landscapes. We also found that different functional groups differently responded to tillage management and landscape composition.

As expected, we found conservation tillage to increase activity-density and species richness of the entire carabid beetle community. Reduced tillage has been widely shown to have a positive effect on soil arthropods (Ball et al. 1998; Soane et al. 2012). Lower arthropod populations under conventional tillage are likely attributable to both direct and indirect effects of more intense ploughing: directly, agricultural machineries mechanically kill or injure individuals; indirectly, higher soil disturbance decreases soil environment quality and resource availability (Ball et al. 1998; Holland 2004; Thorbek & Bilde 2004). Reduced tillage is in fact characterized by a constant soil cover (either cover crops or crop residues) that increases soil habitat stability and structure, providing shelter, optimal humidity, temperature and higher food availability (Soane et al. 2012).

Nevertheless, in the case of carabid beetle, different authors reported different community changes under non-conventional tillage systems (Holland and Luff 2000), from increased diversity and abundance (e.g. Ball et al. 1998; Hatten et al. 2007; Menalled et al. 2007), shifts in species composition (e.g. Boscutti et al. 2015) to no effect found (e.g. Belaoussoff et al. 2003). However, local soil type, crop type, climate and concomitant farming practices seem to play a major role in shaping the potential benefits of non-conventional tillage on carabid beetle abundance and diversity (Holland and Luff 2000). We also found tillage management to have a different impact on different groups of functional traits. Conservation tillage increased species richness of herbivores, whereas it had little and no effect on predators and omnivores, respectively. This might be explained by the fact that fields managed under conservation tillage have been often shown to harbor more abundant and diverse plant and prey communities (Murphy et al. 2006) that, in turn, could have sustained a more diverse community of herbivores and, with a lesser extent, predators. Omnivores might be able to efficiently exploit resources under both tillage managements. Furthermore, tillage affected the beetle community depending on wing morphology: conservation tillage increased the number of species of macropterous beetles, whereas it did not affect brachipterous or dimorphic beetles. This might be caused by the fact that both brachipterous and dimorphic groups were present with a considerably low number of species with respect to macropterous beetles. The effect of tillage management might thus have been hidden for both groups.

Our results clearly showed that the amount of urban areas in the landscape surrounding arable fields significantly affects carabid beetles. The effect of urbanization on arthropods has been largely investigated, presenting different responses by different taxonomic groups (e.g. Alaruikka et al. 2002). Although biodiversity can be surprisingly high in extremely urbanized areas, carabid beetle diversity and abundance are not favored by urbanization. Several studies reported decreased carabid diversity and abundance with increasing urbanization in Canada, Finland (Niemela et al. 2002; Venn et al. 2003), Japan (Ishitani et al. 2003) and UK (Sadler et al. 2006). Moreover urbanization seems to favor more generalist species that are able to survive in lower quality, human dominated habitats (Gaublomme et al. 2008). In line with these findings,

we found high proportion of urban areas to have a stronger effect on herbivore and predatory beetles than on omnivores. Moreover, we found urbanization to markedly constrain carabid functional richness - meaning the occupancy of niche space in the community - indicating that highly urbanized areas offer less ecological niches for the beetle community. In our study area carabid beetles always best responded to landscape complexity at 750 m scale. Our findings are in line with previous studies showing ecologically effective impacts of the surrounding landscape on carabid assemblages occurring within this range (Purtauf et al. 2005).

Interestingly, we found local and landscape processes affecting carabid communities to interact: the negative effect of urbanization on species richness, activity-density and functional richness was either stronger (activity-density and functional richness) or visible (species richness) only in fields managed under conventional tillage. These results suggest that conservation tillage may improve within-field habitat quality, locally supporting more diverse (both taxonomically and ecologically) and abundant beetle communities also in highly urbanized landscapes. Fields under conventional tillage may instead offer a less favourable habitat for beetle persistence. This process is particularly evident for more specialized carabid groups i.e. herbivores and predators, whereas omnivores showed to be affected more by the landscape content than by the soil management.

Our results emphasize the importance of considering both local management and landscape composition when evaluating biodiversity response to human disturbance in agro-ecosystems. Our study confirms that more urbanized areas harbor significantly poorer carabid beetle communities. In these systems the adoption of conservation tillage may locally improve beetle diversity and abundance mitigating the negative effects of landscape urbanization, probably sustaining the provision of key ecosystem services to crop production provided by this arthropod group, such as biological pest control. Moreover, the endorsement of tillage systems able to increase in-field habitat quality in highly urbanized environment might guarantee high level of carabid beetle diversity rather than the more difficult task of altering landscape composition, consequently promoting biodiversity in agro-ecosystems.

Supplementary information List of the species collected pooled in different functional groups (Lindroth 1992; Lövei and Sunderland 1996; Ribera et al. 2001; Pilon et al. 2013; www.carabids.org - Homburg et al. 2014).

Tribe	Species	Wing morphology	Overwintering stage	Feeding type	Habitat	Size (mm)
Pterostichini	<i>Abax parallelepipedus</i>	brachypterous	imago/larva	predator	forest	19
Platynini	<i>Agonum muelleri</i>	macropterous	imago	predator	generalist	7.5
Platynini	<i>Agonum sexpunctatum</i>	macropterous	imago	predator	generalist	7.5
Platynini	<i>Agonum sordidum</i>	macropterous	imago	predator	generalist	7.5
Zabrini	<i>Amara aenea</i>	macropterous	imago	herbivore	generalist	7
Zabrini	<i>Amara similata</i>	macropterous	imago	herbivore	generalist	8.5
Platynini	<i>Anchomenus dorsalis</i>	macropterous	imago	predator	generalist	6.5
Harpalini	<i>Anisodactylus binotatus</i>	macropterous	imago	herbivore	open	10.5
Harpalini	<i>Anisodactylus signatus</i>	macropterous	imago	omnivorous	open	12
Bembidiini	<i>Asaphidion flavipes</i>	macropterous	imago	predator	open	3.5
Licinini	<i>Badister bullatus</i>	macropterous	imago	predator	generalist	5
Brachinini	<i>Brachinus crepitans</i>	macropterous	imago	predator	open	8
Brachinini	<i>Brachinus ejaculans</i>	macropterous	imago	predator	open	8
Brachinini	<i>Brachinus elegans</i>	macropterous	imago	predator	open	7.5
Brachinini	<i>Brachinus explodens</i>	macropterous	imago	predator	open	5.5
Brachinini	<i>Brachinus nigricornis</i>	macropterous	imago	predator	open	7.5
Brachinini	<i>Brachinus plagiatus</i>	macropterous	imago	predator	open	8.5
Brachinini	<i>Brachinus psophia</i>	macropterous	imago	predator	open	7
Brachinini	<i>Brachinus sclopeta</i>	macropterous	imago	predator	open	5.5
Sphodrini	<i>Calathus fuscipes</i>	dimorphic	larva	predator	generalist	12
Carabini	<i>Carabus coriaceus</i>	brachypterous	larva	predator	generalist	37
Carabini	<i>Carabus germarii</i>	brachypterous	larva	predator	generalist	30
Carabini	<i>Carabus granulatus</i>	dimorphic	imago	predator	open	21.5
Chlaeniini	<i>Chlaenius nitidulus</i>	macropterous	imago	predator	open	15
Anisodactylini	<i>Diachromus germanus</i>	macropterous	imago	herbivore	open	9
Sphodrini	<i>Dolichus halensis</i>	macropterous	larva	predator	open	16.5
Harpalini	<i>Harpalus affinis</i>	macropterous	imago/larva	herbivore	open	10
Harpalini	<i>Harpalus anxius</i>	macropterous	imago	herbivore	open	7
Harpalini	<i>Harpalus dimidiatus</i>	macropterous	imago	herbivore	open	12.5
Harpalini	<i>Harpalus distinguendus</i>	macropterous	imago	herbivore	open	9
Harpalini	<i>Harpalus flavicornis</i>	macropterous	imago	herbivore	open	8
Harpalini	<i>Harpalus latus</i>	macropterous	imago/larva	herbivore	generalist	9.5
Harpalini	<i>Harpalus oblitus</i>	macropterous	imago	herbivore	open	9.5
Harpalini	<i>Harpalus pygmaeus</i>	macropterous	imago	herbivore	open	6

Harpalini	<i>Harpalus rubripes</i>	macropterous	imago/larva	herbivore	generalist	10
Harpalini	<i>Harpalus tardus</i>	macropterous	imago	predator	generalist	9
Nebriini	<i>Leistus rufomarginatus</i>	dimorphic	larva	predator	forest	8.75
Nebriini	<i>Nebria brevicollis</i>	macropterous	larva	predator	forest	11.5
Bembidiini	<i>Philochthus lunulatus</i>	macropterous	imago	predator	open	3.5
Pterostichini	<i>Poecilus cupreus</i>	macropterous	imago	omnivorous	open	11
Pterostichini	<i>Poecilus koyi</i>	brachypterous	imago	predator	generalist	12
Harpalini	<i>Harpalus griseus</i>	macropterous	larva	omnivorous	open	9.5
Harpalini	<i>Harpalus rufipes</i>	macropterous	larva	herbivore	open	13.5
Pterostichini	<i>Pterostichus melanarius</i>	dimorphic	larva	predator	open	15
Pterostichini	<i>Pterostichus melas</i>	brachypterous	larva	predator	open	16
Pterostichini	<i>Pterostichus niger</i>	dimorphic	larva	predator	generalist	18.5

Chapter 5

General conclusions

The aim of this study was to investigate potential effects of landscape structure (semi-natural habitats) and local land-use (on-farm management) on ecosystem services in conventional agriculture. Our work considered some ecosystem services crucial to crop production and different management practices expected to affect them.

We showed that in Veneto region there was a considerably efficient pollination service irrespective of landscape composition and margin type. Even the simple landscapes were able to support species that provided efficient pollination. However, margin type had a significant effect on the abundance and diversity of pollinators, with higher species diversity in grass margins compared to hedgerows. The presence of higher diversity of herbaceous species in the grassy margin compared to hedgerows was the main cause of the observed increase in pollinators (Chapter 2).

We found yield loss due to the reduction of fertilizer inputs to decrease with SOC when no insecticide was applied, indicating indirect effects of pest pressure. Furthermore, we show that landscape complexity enhanced some natural enemies of aphids (Chapter 3). An appropriate field management can ameliorate many of the negative impacts of agriculture, while largely maintaining provisioning services, especially on-field management practices can enhance the ecosystem services provided by agriculture. If future agriculture is to maintain sufficient production levels while minimizing negative impacts on the environment and climate by reducing agro-chemical inputs, our study points to the dual, and probably ecologically coupled, benefit of supporting SOC and biological control services to this end (Chapter 3). Our results indicated a general positive effect of natural pest control by generalist predators from boosting SOC, and we have found few other cases that demonstrate such ensuing effects of SOC on pest control in agriculture. Synthetic fertilizers can shift the balance in the plant toward growth and reproduction, while compromising defense capabilities of affected plants and making them more palatable for herbivores. Our findings demonstrated that mineral nitrogen input, even at a local, within-field scale, can strongly influence above-ground arthropod food webs presumably due to its effects on plants and soil fauna that has low dispersal ability.

We explored the combined effect of tillage management and urbanization on ground beetle communities and we demonstrated that conservative management of agricultural soil

contributes to the maintenance of diversity and richness of ground beetle communities (Chapter 4).

Our results highlight the key importance of the surrounding landscape context, along with local factors, to ecosystem services' delivery. A general conclusion from our four studies is that the benefits of implementing single local intervention such as hedgerows (off-field) or conservation tillage (on-field) is higher in landscapes with low cover of existing semi-natural habitats. The thresholds to define a landscape 'simple' may vary drastically between regions and a single figure cannot be provided. Future agri-environment schemes to foster biocontrol and pollination in simple landscapes should focus conservation tillage while hedgerow effects is still not clear.

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