

# UNIVERSITY OF UDINE

# PhD IN AGRO-ENVIRONMENTAL SCIENCES (XXVII CYCLE)

#### PhD DISSERTATION

# ECOSYSTEM SERVICES AND BIODIVERSITY FOR AGRICULTURAL LANDSCAPE SUSTAINABILITY

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#### General introduction

#### 1. Agricultural landscape sustainability

In the near future demand of agricultural products will rapidly increase in order to satisfy a growing world population. The projections show that feeding a world population of 9.1 billion people in 2050 would require raising overall food production by some 70% between 2005 and 2050. It means almost the double in developing countries (FAO, 2009).

The growing demand for agricultural products has been met through agricultural intensification by increasing productivity per unit area. Intensive agriculture depends on high levels of external inputs (i.e. fertilizers, pesticides) and fossil fuels. Although this kind of agriculture system has been successful in increasing food production, it caused also several detrimental effects on the environment quality. For example, farm inputs contribute to soil and water quality degradation, to the increase of greenhouse gas emission and exposure to toxic agrochemicals (Kremer and Miles, 2012). Intensive agriculture has also led to an oversimplification of landscape structure due to crop monocultures and the fragmentation and elimination of natural habitats (Robinson and Sutherland, 2002). The loss of native habitats also affects agricultural production by degrading the services of pollinators and natural pest control (Kremen et al., 2002; Letorneau et al., 2011).

Therefore, the dependency of intensive agriculture on non-renewable resources (e.g. fossil fuels, fertilizers) and its environmental consequences make it unsustainable in the long run (Kimbrell, 2002). Humankind needs to meet the growing demand for food in a manner that is ecologically sustainable over the long term, i.e. for future generations. Sustainable agricultural practices together with ecological management of agricultural landscapes are seen as the way to address and mitigate the environmental problems associated with existing intensive farming systems.

A sustainable agricultural landscape maintains the resources upon which it depends, relies on a minimum of artificial inputs from outside the farm system, manages pests and diseases through internal regulation mechanisms, and is able to recover from the disturbance caused by cultivation and harvest (Edwards et al., 1990; Altieri, 1995). In the near future, agriculture needs to be more productive, stable, and resilient while minimizing environmental impacts (Foley et al., 2005).

In this context, ecological intensification has been proposed as a promising solution. Ecological intensification advocates to maintain or enhance agricultural production through the promotion of biodiversity and associated ecosystem services. Here, the challenge would be to reduce reliance on external inputs, while maintaining high productivity levels by fostering or reestablishing landscape ecosystem services and biodiversity. Therefore, ecological intensification may be defined as the development of optimal management of nature's ecological functions in an agricultural landscape. Ecological intensification can be reached by enhancing biodiversity and ecosystem services, to improve agricultural system performance and to reduce environmental impacts (Bommarco et al., 2013).

#### 1.1 Biodiversity conservation in agricultural landscape

Biodiversity conservation is important because biodiversity ensures ecosystems functions and therefore guarantees services which are crucial for man. These services encompass nutrient cycling, soil formation, filtering of water, soil and air, flood protection, genetic diversity, natural pollination, pest control by natural predators and stability of ecosystems (Daily, 1997; Altieri, 1999, Foley et al., 2005; MA, 2005; Schröter et al., 2005; Braat and ten Brink, 2008; Maes et al., 2012). Therefore, a decrease in biodiversity (through intensive agriculture and monoculture) reduces the self-sustainability of agro-ecosystems. As a consequence, farmers will be obliged to replace these functions e.g. by the application of expensive fertilizers and pesticides. Therefore, from an economic point of view, the risk is to need of expensive external inputs since the agroecosystem, depleted of the basic functional components, loses its ability to sustain its own fertility and functions (e.g. presence of natural predators of crop pests). Therefore, from an environmental point of view, the lack or erosion of biodiversity in agroecosystems requires constant human intervention, while agroecosystems managed with intact biodiversity benefit from free ecosystem services. According to Paoletti (1999), agroecosystem biodiversity is mainly influenced by the structure of the landscape mosaic: i.e. presence and fragmentation of semi-natural habitats, distribution and abundance of species. Extensively managed agroecosystems encompass also a variety of semi-natural habitats, such as hedgerows, woods and meadows, which increase biodiversity of several taxonomical

groups at different levels (Duelli and Obrist, 2003). For instance, the presence of these seminatural habitats can benefit insects and hence curb the spread of pests (Bianchi et al., 2006; Zhang et al., 2007). Moreover, the presence of semi-natural habitats can improve pollination (Klein et al., 2012), diversity of soil fauna (Vanbergen et al., 2007), leading for instance to an increase in soil fertility. Finally, such semi-natural structures can be ecological corridors (Shibu, 2009), that allow the passage and the movement of wild species among the different elements of the landscape. Landscape management aimed to increase such elements, contributes to enhance biodiversity and therefore helps the agroecosystem to fulfill its ecological functions and to become more sustainable.

Agriculture intensification contributes to the decrease in biodiversity (Matson et al., 1997) but facilitates, at the same time, the invasion of exotic plant species. Most exotic species prefer disturbed habitats with high availability of resources, such as light and nutrients (Alpert et al., 2000; Davis et al., 2000). From this point of view, agroecosystems are suitable to support a high number of exotic species (Lonsdale, 1999; Chytry' et al., 2005; Vila et al., 2007; Pyšek et al., 2009). Several authors recognize a negative effect of exotic species on native plant communities and their biodiversity (Williamson, 1996; Weber, 2003; Hulme, 2007; Kowarik, 2010; Shibu et al., 2013). These effects are mainly attributable to substitution mechanisms and the spread of exotic species, and hence damage ecosystem (Mack et al., 2000; Pimentel et al., 2005) and loss of biodiversity.

#### 1.2. Ecosystem services in agricultural landscape

Ecosystem services are the benefits that people obtain from ecosystems (Costanza et al., 1997). The concept of ecosystem services, therefore, links ecology and society. Ecosystem services are both goods (e.g. food, water, genetic resources, raw materials) and functions produced by ecosystems (e.g. nutrient cycling, soil formation, purification of water and air) and processes generated by ecosystems (e.g. erosion protection, flood control, water quality, pollination). The Millennium ecosystem Assessment (MA, 2005) grouped ecosystem services into four categories:

- Provisioning services, which include natural resources, such as food, fibers, fuel, water, and generic resources;
- Regulating services, which include the benefits obtained by the regulating effects of ecosystem processes, such as climate regulation, flood regulation, water purification, pest control, pollination;

- Cultural services, which include the non material benefits through recreation, aesthetic and spiritual enjoinment;
- Supporting services, that are the services necessary to the production of all other ecosystem services such as nutrient cycling, soil formation and primary production.

Ecosystem services research has become a major issue in ecology and policy. As a consequence, literature about ecosystem services has increased exponentially (Fisher et al., 2009). In recent years, several authors and projects started to develop numerous methods for classifying, identifying, quantifying and mapping ecosystem services in order to integrate the concept into decision making processes (de Groot et al., 2002; MA, 2003; MA, 2005; Chan et al., 2006; de Groot, 2006; Troy and Wilson, 2006; Egoth et al., 2008; Nelson et al., 2009; Burkhard and Kroll, 2010; de Groot et al., 2010; Koschke et al., 2012). However, the concept is used in different contexts and it is important to distinguish between services in form of goods and benefits that man obtains directly from ecosystem (Costanza et al., 1997) and functions and processes that just benefits man (Daily, 1997). In the agroecosystem context and for ecological intensification purposes, it makes sense to focus on ecosystem services in form of goods (i.e. crop production), but even more on ecosystem services in form of functions and processes which support the final services (i.e. crop yield). For ecological intensification, the primary interest is in managing the processes that mediate yield levels (Bommarco et al., 2013). In fact, agroecosystems are primarily a source of provisioning services (e.g. food), but they also contribute to supply other ecosystem services, some of which support the crop production itself (e.g. pest control, pollination, weed control, disease control, soil quality).

#### 2. Research purposes

The aim of the present research project was to study the contribution of the ecological intensification concept to the sustainability of agricultural landscape. Therefore, we analyzed the sustainability of agricultural landscapes by assessing the impacts of agricultural intensification on (i) biodiversity and (ii) ecosystem services.

Because agroecosystem is one of the most human shaped system, assessing how different cultivation systems and landscape structures affect biodiversity and ecosystem services is essential in order to guide the definition of solutions aimed to enhance agricultural landscape sustainability through ecological intensification.

Specifically, we addressed the following questions:

- How does biodiversity affect the functional quality of agricultural landscape?
- How do the structural characteristics of agricultural landscapes affect their own functional quality?
- How do agricultural intensification and the associated disturbances affect the spread of exotic plant species in different habitats?
- How do conservation tillage and landscape complexity affect different ecosystem services?

Ecosystems do not exist as isolated units, but represent different parts of a continuous landscape. The ecosystems forming a landscape are interactive, therefore landscape can also be considered as the highest level of biological complexity. The landscape scale approach allows to understand complex functions and interactions between different ecosystems and provides insights into both landscape and species diversity. These elements can suggest theoretical and practical basis for conservation planning (Roy and Behera, 2002). We studied how the structure and diversity of agricultural landscapes affect both species richness and the spreading of exotic plant species. Exotic plants are widely considered as one of the major threats to biodiversity caused by agricultural intensification itself.

In agroecosystems, the supply of ecosystem services is mainly influenced by human management throughout cultivation systems (Power, 2010). In particular, soil management may affect important supporting services, which sustain numerous other ecosystem services (Smukler et al., 2012). For example, the increase of soil organic matter (SOM) influences nutrient cycling and storage (Lal, 2006) and net primary productivity (Smith, 2007). Conservation tillage (CT) is a soil management practice combining the non-inversion of the soil with other management techniques, such as cover cropping, surface incorporation of crop residues or crop rotation. It brings benefits to the environment (Holland, 2004) and is, therefore, expected to contribute to the increase of ecosystem services. Along with local management, several ecosystem services are strongly governed by the quantity and spatial arrangement of non-agricultural land (Bommarco et al., 2013). Therefore, we want to focus on the effects of conservation tillage and landscape structure on the multiple ecosystem services supporting agricultural production.

#### 3. Outline of the thesis

The thesis consists of four research papers. The first paper (Chapter 2) focuses on the functional quality of agricultural landscapes as defined by biodiversity and resilience or sensitivity to disturbance. Relationships between the structural characteristics and the functional qualities of agricultural landscapes have been explored and insights for their ecological and sustainable management have been obtained. The aims of this part of the study were: (1) to develop a method for assessing the functional quality of agricultural landscapes considering its components, biodiversity and sensitivity, and (2) to study the relationship between functional quality and structural pattern (composition, configuration) of agricultural landscapes.

The second paper (Chapter 3) examines how agricultural intensification may affect the spread of exotic plant species (i.e. archaeophytes and neophytes) in different semi-natural habitats. The aims were: (1) to evaluate how disturbance in the landscape affects the spread of exotic plant species within semi-natural habitats, and (2) to evaluate whether alien plant species are less dispersal limited than native species by testing distance-decay of similarity for the different semi-natural habitats for both alien and native species.

The third paper (Chapter 4) focuses on the effects of conservation tillage on multiple ecosystem services supporting cereal production. The ecosystem services considered in the experiment fall into different categories, specifically: provisioning services (i.e. production), regulating services (i.e. weed control, aphid pest control) and supporting services (i.e. soil quality). In addition, we examined whether the complexity of the landscape affects the supply of these services (i.e. pest control and weed control), which depend on the composition of non-agricultural land in the surrounding landscape. Finally, we also examined whether or not intensity of fertilization had a significant impact on the production and the weed control service.

The fourth paper (Chapter 5) focuses on a single ecosystem service namely natural aphid pest control. The paper deals with the interactions of soil management (i.e. conservation tillage), landscape complexity and natural enemy communities with the biological control service provided by different guilds of natural enemies in winter cereal crops (birds, flying insects/vegetation-dwelling predators and ground-dwelling predators). The aims were: (1) to evaluate if conservation tillage enhances the biological control service, (2) to assess how the natural enemy guilds influence the control of aphid populations in the agricultural field, (3) to

verify if the level of biological control services increase with an increasing complexity of the landscape and, if so, if this acts additively or synergistically with local tillage management.

Finally, Chapter 6 provides the overall conclusions of the research project.

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# Conservation planning of agricultural landscapes: biodiversity patterns and habitat sensitivity<sup>1</sup>

#### **Abstract**

Agricultural landscapes managed with the aim at maintaining or enhancing biodiversity leads to qualitatively more functional landscapes. The biodiversity of agricultural landscapes is strongly shaped by human disturbance. We propose that landscape functional quality is the combination of biodiversity supported by the landscape and the specific disturbance sensitivity of the same landscape. The purpose of this study is to provide novel insights for a sustainable management of agricultural landscapes. We developed a method to assess the functional quality of agricultural landscape utilizing InVEST model. Subsequently, we performed a multivariate analysis (Canonical discriminant analysis-CDA) to better understand the order of magnitude of landscape metrics (of composition and configuration), which affect landscape functional quality. The purposed method was applied to 20 agricultural landscapes in Friuli Venezia Giulia plain - North-Eastern Italy. We found landscape functional quality to increase with high percentage of fairly large and not too isolated semi-natural areas and a high level of landscape heterogeneity. In addition, our analysis underlined the importance of specific semi-natural habitat types such as meadows and woods, rather than others (e.g. hedgerows) for improving functional quality of agricultural landscapes.

#### **Keywords**

Agricultural landscape, biodiversity, sensitivity, ecological sustainable planning

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<sup>&</sup>lt;sup>1</sup> De Simone S., Boscutti F., Sigura M., 2014. *Conservation planning of agricultural landscape: biodiversity patterns and habitat sensitivity.* Manuscript in preparation.

#### 1. Introduction

The importance of nature and biodiversity conservation in order to take theirs ecological, social and economic benefits for current and future generations is widely recognized (MA, 2003; MA, 2005; MA, 2005b). In particular, biodiversity promotes the capacity of ecosystems to provide services and functions (Daily, 1997; Altieri, 1999, Foley et al., 2005; MA, 2005; Schröter et al., 2005; Braat and ten Brink, 2008; Maes et al., 2012) and to provide man with food, timber, fiber, energy and raw materials, contributing in a fundamental way the world economy (MA, 2005). Biodiversity ensures important services of which man benefit, e.g. the regulation of the hydrological cycle and biogeochemical cycles of carbon and nitrogen, the filtering function of many natural environments for quality of water, soil and air, flood protection, the availability of genetic diversity in agriculture, natural pollination and stability of ecosystems (Swift and Anderson, 1993; Schröter et al., 2005).

Even agroecosystems can provide a range of services to human communities, such as flood control, water quality control, carbon storage, disease regulation and waste treatment (e.g. nutrients, pesticides). For example, an agricultural landscape characterized by a high floral diversity (i.e. presence of semi-natural habitats) can attract natural enemies of pests and crop pollinators and therefore leads to an increase in natural pest control and pollination services (Kremen et al., 2002; Letorneau et al., 2011). An agroecosystem managed through biodiversity conservation may contribute a variety of services which support agroecosystems themselves and surrounding ecosystems (Daily, 1997).

Agriculture is the human activity that more affects land use composition, affecting large parts of terrestrial areas (FAO, 2009) and hence leads to changes of natural ecosystems. Agroecosystem biodiversity is mainly influenced by the configuration and composition of the landscape mosaic (Paoletti, 1999), e.g. presence and fragmentation of semi-natural habitats influence distribution and abundance of species.

Extensively managed agroecosystems consist of both agricultural and semi-natural habitats, such as hedgerows, woods and meadows, which increase landscape biodiversity (Duelli and Obrist, 2003). For instance, the presence of semi-natural habitats can benefit insects which, in turn, often improves pollinators (Klein et al., 2012) and curbs the spread of pests (Bianchi et al., 2006; Zhang et al., 2007). Moreover, the presence of semi-natural habitats may lead to an increase in soil fertility by increasing the diversity of soil fauna (Vanbergen et al., 2007). Finally, such semi-natural structures can be ecological corridors (Shibu, 2009), that facilitate the movement of wild species among the different elements of a

landscape. On the other hand, intensively managed agroecosystems usually entail to a general simplification of landscape structure due to (i) the spread of crop monocultures and (ii) the fragmentation or complete elimination of natural habitats (Robinson and Sutherland, 2002). This can directly affect biodiversity, as the larger the area under monoculture the lower the viability of natural plant and animal population (Altieri, 1999). Increased biodiversity helps agroecosystems to better fulfill their ecological functions which, in turn, reduce the need of farm inputs such as fertilizers, herbicides, pesticides and fungicides. Farm inputs contribute to soil degradation, water eutrophication, increase of greenhouse gas emission (Kremer and Miles, 2012).

In recent years, the concept of ecological intensification has been introduced. It may be defined as the development of optimal management of nature's ecological functions, also through biodiversity enhance, to improve agricultural system performance and to reduce environmental impacts (Bommarco et al., 2013). Therefore, agricultural landscapes managed according to the ecological intensification concept are ecologically more functional and more sustainable.

Since the biodiversity of agroecosystem is strongly shaped by human impact and the associated disturbance, the assessment of disturbance is crucial for assessing the quality of agricultural landscapes. In agricultural landscapes, disturbance is mainly caused by crop management treatments, which vary in function of crop type (e.g. perennial vs. annual crops) and intensity of cultivation practices (e.g. industrial crops vs. organic crops). Disturbance is also linked with the concept of landscape sensitivity. In fact, Allison and Thomas (1993) reviewed various definitions of landscape sensitivity and the nowadays commonly accepted ecological definition is "susceptibility of a system to disturbance". According to Zacharias and Edward (2005) sensitivity is how habitats respond to stress factors, defined as disturbance-caused deviations of environmental conditions beyond the expected range. At landscape scale, therefore, the term landscape sensitivity identifies the potential and the probable magnitude of change within a physical system in response to disturbance (e.g. agriculture) and the ability of this system to resist the change (i.e. resilience) (Allison and Thomas, 1993). According to Jackson et al. (2007), the resilience of a landscape and its biodiversity will increase with increasing habitat diversity and structural complexity.

Although a simple assessment of landscape biodiversity produces meaningful results in order to evaluate landscape functionality, the assessment of landscape sensitivity to disturbance could depict a more comprehensive knowledge on agricultural landscape

regulating functions. Combine the assessment of both components (i.e. biodiversity and sensitivity) could be helpful to assess a real representation of landscape functional quality. Therefore, we propose that landscape functional quality can be assessed as combination of biodiversity supported by the landscape and the specific disturbance sensitivity of the same landscape. Many studies on the planning of sustainable and ecological landscapes focused on landscape function analysis and on the definition of indicators reflecting the state of landscape functionality (see Tongway and Ludwig, 2011). Usually, the indicators proposed are used to assess a single process or single a function. In our study, by contrast, landscape function is not subdivided into a single functions or processes, but it is defined as the capacity of landscape to maintain a high level of biodiversity which, in turn, will ensure a variety of landscape functions and regulatory services. In addition to biodiversity, the notion of landscape functionality proposed in this paper also considered landscape sensitivity. Therefore, we strongly believe that the proposed notion of landscape functional quality is particularly well suited to describes the capacity of a landscape to provide regulation services in highly disturbed agricultural landscapes.

The general purpose of the present study was to contribute to the ongoing debate on landscape functionality by providing novel insights into the ecological and sustainable management of agricultural landscape. Specifically, the objectives of the study were:

- i) to develop a method for assessing the functional quality of agricultural landscape considering both biodiversity and sensitivity;
- ii) to elucidated the relationship between functional quality and structural characteristics of agricultural landscapes (composition, configuration) in order to derive practical conclusions for an ecologically sustainable planning and management of agricultural landscapes.

#### 2. Materials and methods

#### 2.1 Methodological approach

The methodological approach used encompassed three steps (Fig.1). (1) In a first step we developed a method to assess the functional quality of agricultural landscapes taking into account both biodiversity and sensitivity. We used the biodiversity evaluation module in the InVEST model, which produces landscape habitat quality maps based on spatial information on land use. Habitat quality is a function of habitat (i.e. land use) suitability to biodiversity

(i.e. the potential biodiversity of a habitat) and the presence of threats to biodiversity. The impact of threats on habitats is mediated by four factors: (i) the relative impact of each type of threat, (ii) the impact of the threat across space, (iii) its decay in the space mode (i.e. linear or exponential distance-decay function to describe how a threat decays over space) and (iv) the relative sensitivity of each habitat type to each kind of threat (Tallis et al., 2010). (2) The second step included the analysis of landscape patterns (composition and configuration) by landscapes metrics calculated with FRAGSTATS ® 4.1 software (McGarigal and Ene, 2012). (3) Finally, in the third step, we performed a multivariate analysis to define the importance of the different landscape metrics (of composition and configuration) for the functional quality of agricultural landscapes.

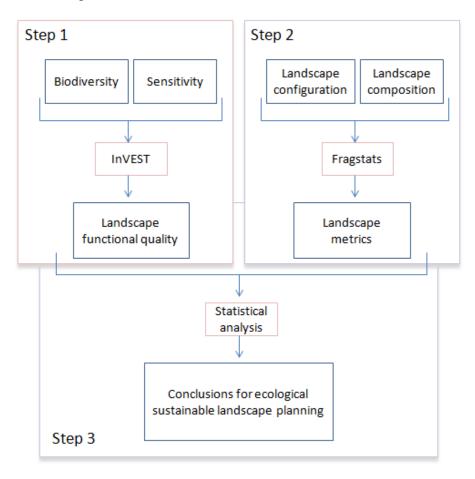


Fig.1. Flowchart of the methodological approach.

#### 2.2 Study area and sampling design

The proposed method was applied to the Friuli Venezia Giulia plain in North-Eastern Italy (46°10′08″N, 12°57′44″E to 45°46′18″N, 13°30′56″E). The area is characterized by different types of agricultural landscapes, ranging from most intensively cultivated areas, to areas where a more extensive type of agriculture is still present and which are, therefore, rich in

semi-natural elements, such as woods, meadows and hedgerows. We randomly selected 20 agricultural landscapes of 4 km<sup>2</sup> (2 km x 2 km) according to UTM grid (Universal Transverse Mercator). We discarded quadrats with less than 40% agricultural land use, and/or more than 20% urban land use, as non-agricultural landscapes. For each of the 20 selected quadrats we established by photo-interpretation a land use/land cover (LULC) map (scale of 1:2000). We distinguished seven types of LULC, which represent the different habitat, namely woods, hedgerows, meadows, annual crops, perennial crops, anthropic areas (i.e. built areas, infrastructure) and natural water bodies.

#### 2.3 Assessment of the functional quality of the landscape

We used vascular plants as indicator in order to identify inputs required by InVEST model. In fact, plant communities highly characterize the habitats structure and are the basic resource for many other organisms (Weibull et al., 2003). Vascular plant diversity was used as indicator for the biodiversity of each habitat type distinguished (i.e. LULC type), because of its recognition as one of the most reliable predictors of the overall biodiversity in semi-natural ecosystems such as urban and agricultural landscapes (Simonson et al., 2001; Sauberer et al., 2004; Bräuninger et al., 2010).

In agroecosystems, we considered the threats as a generalized disturbance extended to all habitats caused by agricultural activities. This kind of threat is represented mainly by agricultural land use and by the intensification of agriculture. Effects of agricultural intensification are a general decline in native species (Sutcliffe and Kay, 2000; Robinson and Sutherland, 2002; Baessler and Klotz, 2006; Hyvonen, 2007) and an increase in both invasive exotic plants (i.e. neophytes) (Hobbs and Humphries, 1995) and in species typical of disturbed environments (i.e. therophytes) (McIntyre et al., 1995; Debussche et al., 2009). From this point of view, the presence of exotic and therophyte species is a good indicator of agricultural intensification. Depending on the presence of exotic and therophyte species, each habitat (i.e. crops and semi-natural habitats), may represent a source of threat (Sigura et al., 2014; unpublished data). Therefore, we assumed each habitat to be a source of both biodiversity and threat.

In our study, we defined required model inputs as follows: (1) habitat sensitivity was defined as the habitat's susceptibility to the exotic and therophyte species, (2) habitat suitability to biodiversity as defined as floristic diversity, (3) the threat to biodiversity was represented by the spread of exotic and therophyte species among different habitats in the

landscape. The relative impact of the threat was defined by the quantity of exotic and therophyte species present in each type of habitat and the impact of the threat across space was represented by the maximum dispersal distance of exotic and therophyte species and their decay in the space mode (i.e. linear or exponential distance-decay function to describe how a threat decays over space).

In order to obtain the necessary input data, in 4 of 2 km x 2 km quadrats, we analyzed plant community data ("Multifarm" project, L.R. 26/05 art. 17), using sample plots proportionally distributed in the different habitat (i.e. woods, hedgerows, meadows, annual crops and perennial crops). The size of the plot sampling was defined according to minimum area concept established for a phytosociological *relevé* (Westhoff et al., 1973; Chytrý et al., 2003; van der Maarel, 2005), i.e. 11 m² for crops, 25 m² for meadows and hedgerows and 100 m² for woods. Within each plot all vascular plant species and their estimated cover values were recorded. Cover values were estimated using the Braun-Blanquet scale (Braun-Blanquet, 1964) as modified by Pignatti (1953). Nomenclature and taxonomy followed Poldini et al. (2001) and Pignatti (1982). On the whole, 138 plots were studied: 21 in woods, 29 in hedgerows, 50 in meadows, 28 in annual crops and 10 in perennial crops. The overall number of sample plots within each landscape has been distributed randomly and proportionally to both number of patches of each habitat type and total area occupied by it in the landscape.

#### Habitat sensitivity

Hemeroby Index (HI) (Sukopp, 1972; Poldini, 2009) was used to quantify habitat sensitivity to the threat. This index is a measure of the level of degradation of the plant community and indicates the deviation from natural conditions. The magnitude of this deviation is quantified by comparing the number of species of therophytes and neophytes compared with the total number of species (Eq.1).

$$HI = \frac{(N+T)}{S} \tag{1}$$

where N is the number of neophyte species, T is the number of therophyte species and S is the total number of species in the sample plot. The Hemeroby Index was calculated for each of the 138 plots. Afterward, for each habitat type, the mean HI was calculated and then, as required by InVEST model, the means were transformed to a scale from 0 to 1. In order to assign the relative sensitivity of each habitat type to each source of threat (i.e. other habitats),

we used to propagule pressure theory, that is a measure of the number of individuals released into a region to which they are not native (Carlton, 1996). The colonization of a habitat by exotic species (especially neophytes) is strongly influenced by both propagule pressure (Lockwood et al., 2005; Colautti et al., 2006) and the susceptibility to invasion of the habitat in question which, in turn, increases with increasing disturbance. The importance of propagule pressure can vary based on local conditions (Lockwood et al., 2005) and therefore high levels of disturbance will increase the spread of neophytes by creating suitable habitats for colonization and establishment (Theoharides and Dukes, 2007). Because they are likely to contain a greater pool of exotic species, disturbed habitats also represent a source of exotic propagules to neighboring habitats (Timmins and Williams, 1991; Searcy et al., 2006). Therefore, we defined the relative sensitivity of each habitat type to each source of threat (i.e. every other type of habitat) through mean hemeroby of habitat, considering the diffusion to occur unidirectionally along an ecological gradient, from habitat with greater to those with smaller mean hemeroby, while habitats with smaller hemeroby do not affect habitats with greater hemeroby.

#### Biodiversity suitability

Shannon diversity index (H) was calculated for each of 138 plots studied (Shannon and Weaver, 1949) in order to estimated floristic diversity as an indicator of biodiversity suitability. Afterwards, for each of the seven habitat types distinguished, the mean Shannon diversity index was calculated and then transformed to a scale from 0 to 1. Each habitat was characterized by a certain magnitude of floristic diversity but also by a certain magnitude of hemerobic species. Therefore, in order to obtain habitat suitability to biodiversity input, InVEST model has been run separately for each habitat type considering each habitat to be threatened by its own level of hemeroby. In this way we obtain the value of suitability to biodiversity of each habitat balanced with its level of hemerobic species.

#### Threats to biodiversity, their impact across space and their decay in the space mode

In order to obtain the relative impact of each threat, a weight  $(w_r)$  was assigned to each habitat, which is a measure of the relative destructiveness of a threat source to all habitats with respect to each other. The weight ranging from 0 to 1, where 1 is the greatest possible impact. The mean value of hemeroby in each habitat converted in a scale from 0 to 1 was used to identify the weights for each habitat, where 1 correspond to the maximum value of hemeroby recorded in the habitats and correspond to the habitat that is the greater threat.

Finally, in order to quantify the dispersal distance and its decay in the space mode, hemeroby value of each plot had been put in relation with the distance to the closest source of hemeroby, i.e. to the closest annual crops. We used linear mixed models (LMMs) to estimate (i) the distance of dispersal, (ii) the decay of hemeroby in space and (iii) the random effect of geographic location of the plots studied. Prior to analysis, the hemeroby index value were square-root- transformed to obtain normal distribution and stabilize the variance (Sokal and Rohlf, 2012) and distance of dispersal data were log+1 transformed. We used diagnostic plots of the model residuals to make sure that the underlying assumptions of linear mixed model were met. The calculations were made in R statistical software (R Core Team, 2013) with the "nlme" package (Pinheiro et al., 2013).

#### 2.4 Landscape structure analysis

Landscape configuration and composition were analyzed by a set of landscape metrics and indices. In order to characterize the composition of the landscape, for each of the 20 selected 2 km x 2 km quadrats we calculated the percentages of surface area covered by (i) woods, (ii) meadows, (iii) hedgerows, (iv) all the semi-natural areas combined (woods + hedgerows + meadows), as well as the average patch size (m²) of the semi-natural elements (woods, hedgerows and meadows) (Tab.1).

In order to characterize the configuration of the landscape, we calculated (i) the Landscape Division Index (LDI) (McGarigal and Ene, 2012), which represent landscape fragmentation, and (ii) the Euclidean Nearest Neighbor distance (ENN) (McGarigal and Ene, 2012), which is a measure of patch isolation (see supplementary materials, p.45). The LDI was calculated for the entire landscape, for all the semi-natural areas combined, and separately for the woods, the hedgerows and the meadows, respectively. The ENN was calculated for the woods, the hedgerows and the meadows as well as for all semi-natural areas combined (Tab.1).

Finally, for each of the 20 sampled 2 km x 2 km-quadrats, the Index of Landscape Conservation (ILC) (Pizzolotto and Brandmayer, 1996) was calculated as synthetic index to include information of both composition and configuration (Tab.1). ILC index is a measure of landscape heterogeneity but, it takes also into account their contribution to biodiversity of the different land-use types (see supplementary materials, p.45).

**Tab.1.** The 14 landscape metrics used to describe the functional quality of the landscape in the 20 quadrats of 2 km x 2 km studied calculated in each landscape.

	Acronym	Landscape metrics
	pW	Percentage of woods
	pM	Percentage of meadows
Composition	рН	Percentage of hedgerows
Composition	pNAT	Percentage of semi-natural areas
	AREA_NAT	Average size (m <sup>2</sup> ) of semi-natural patch
	LDI	Landscape division index for the entire landscape
	LDI_NAT	Landscape division index for all the semi-natural areas combined
	LDI_W	Landscape division index for the woods
	LDI_H	Landscape division index for the hedgerows
Configuration	LDI_M	Landscape division index for the meadows
	ENN_W	Euclidean nearest neighbor distance for woods
	ENN_H	Euclidean nearest neighbor distance for hedgerows
	ENN_M	Euclidean nearest neighbor distance for meadows
	ENN_NAT	Euclidean nearest neighbor distance for semi-natural areas
Composition + Configuration	ILC	Index of Landscape Conservation

#### 2.5 Statistical analysis

The application of the InVEST model to the 20 quadrats yielded 20 grid maps of the functional quality, ranging from 0 to 1. By calculating the mean of all the grid cells in a 2 km x 2 km-quadrat, we obtained an overall value of the functional quality of the landscape for each of the 20 quadrats studied.

Afterward, four groups (*a, b, c, d*) of landscape functional quality were created by quartiles in order to ensure a sufficient number of elements in each group; where *a* comprised the five smallest mean values and *d* the five greatest values. One-way analysis of the variance (ANOVA) was performed to verify statistical differences among the four groups (p-value < 0.05). Previously, normal distribution was tested by Shapiro-Wilk test and post-hoc test (i.e. Tukey HSD test) were conducted to assess pairwise comparisons. Analysis of variance was done using R statistical software (R Core Team, 2013).

A Canonical Discriminant Analysis (CDA) was performed in order to identify the landscape metrics, which separate best the four groups of landscape functional quality distinguished. CDA analysis was performed using the "candisc" package (Friendly and Fox, 2013) in R statistical software (R Core Team, 2013). CDA determines how a set of quantitative variables may differentiate among several known classes. CDA obtains linear functions of quantitative variables that maximally separate two or more groups, while minimizing variation within groups (Rencher, 1992).

#### 3. Results

#### 3.1 Assessment of the functional quality of the landscape

#### InVEST model inputs

As expected, in the seven habitat types studied the values for (i) sensitivity and (ii) suitability for biodiversity were negatively correlated. (Tab.2). For anthropic areas and natural water bodies, biodiversity and hemeroby data were not surveyed, so both sensitivity and suitability values were estimated. We attributed the sensitivity and suitability values both equal to 0 for anthropic areas, whereas for natural water bodies we attributed the sensitivity value equal to 0 and suitability value equal to 1. Annual crops sensitivity was not indicated, since annual crops were not threatened by other habitats.

**Tab.2.** Mean values of sensitivity and suitability for the habitat types distinguished. Annual crops have no value of sensitivity because they are not threatened by other habitats.

Habitat	Suitability	Sensitivity
Meadows	0.96	0.13
Woods	0.87	0.14
Hedgerows	0.57	0.26
Perennial crops	0.34	0.50
Annual crops	0.10	-
Natural water bodies	1	0
Anthropic areas	0	0

The impact of threats (weights -  $w_r$ ) corresponded to sensitivity values, except for annual crops, which assumed value equal to 1, since it is the habitat with the strongest threat, and natural water bodies and anthropic areas assumed values equal to 0, since they are not considered as threats.

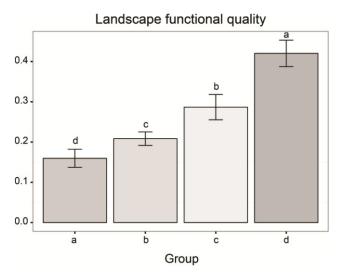
Regarding the impact of the invasion threat across space, results of LMM for hemeroby index (HI) and distance from annual crops revealed a negative correlation between hemeroby and distance from annual crops (Tab.3). An increasing distance from annual crops was accompanied by an exponential decrease of hemeroby, up to a distance from annual crops of 100 m. Beyond a distance of 100 m, no further decrease in hemeroby was observed. For this reason we chose 100 m as limit for both the invasion threat across space and the exponential decay in the space mode.

**Tab.3.** Result of LMM on the hemeroby index and the distance from annual crops. The model residuals followed a normal distribution (p-value = 0.72). Intercept, standard error, degrees of freedom (DF), t-value and p-value (\*\*\* = < 2.2e-16) are reported. The level of significance of the model was set at p-value = 0.05.

	Value	Std.Error	DF	t-value	p-value
Intercept	0.6853	0.046	147	14.96	***
log (distance from annual crops (m) + 1)	-0.0901	0.008	147	-11.05	***

#### Landscape functional quality

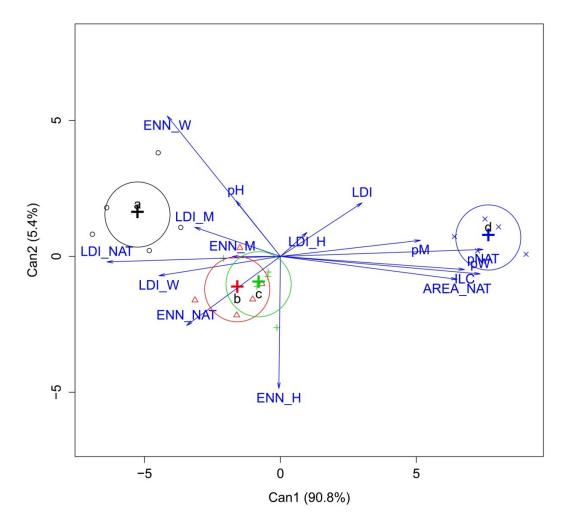
The values for the functional quality of the landscape, resulting from InVEST ranged from 0.1278 to 0.4479. The result of analysis of variance for the four groups of landscape functional quality revealed significant differences ( $F_{3,16} = 90.93$ ; p-value < 0.0001) and post-hoc pairwise comparisons showed significant differences between all possible pairs. Regarding the functional quality of the landscape, the means for the four quartiles were 0.16, 0.21, 0.29 and 0.43, respectively (Fig.2).



**Fig.2.** Mean functional quality of the landscape by groups as calculated by the InVEST model. Different letters on the histogram bars indicate significantly differences.

#### 3.2 Conclusions for ecological sustainable landscape planning

The 14 landscape metrics listed in Tab. 1 were used in the Canonical Discriminant Analysis (CDA), where three statistically significant canonical functions discriminated among the four groups of functional quality (Fig.3, Tab.4). The first two canonical axes explained 90.81% and 5.44 % of the multivariate dispersion. In Fig. 3 the centroids of the four groups of functional quality of the landscape are basically arranged along axis 1 with the poorest group on the far left and the best group on the far right. Towards the right hand side of the first axis we observed increasing percentages (i) all of semi-natural habitats combined (pNAT in Fig. 3) as well as (ii) of woods (pW) and (iii) meadows (pM), respectively. Moreover, along axis 1 also (iv) the average patch size of any type of semi-natural area (AREA\_NAT) increased as well as (v) the Index of Landscape Conservation (ILC). Towards the left hand side of axis 1, by contrast, we observed increasing degrees of fragmentation of (i) any type of semi-natural area (LDI\_NAT), (ii) the woods (LDI\_W) and (iii) the meadows (LDI\_M), as well as (iv) an increasing of isolation of meadows patches (ENN M).



**Fig.3.** Canonical Discriminant Analysis (CDA) among the four groups of landscape functional quality with a = smallest functional quality and d = greatest functional quality. Centroids (crosses) and confidence interval (0.95, line around crosses) of each landscape functional quality group are shown. The abbreviations of the metrics used for describing the composition and the configuration of the landscape (= blue arrows) are explained in Tab. 1.

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**Tab.4.** Summary of Canonical Discriminant Analysis (CDA) for the four groups of functional quality of the landscape in the 20 quadrats of 2 km x 2 km studied. The abbreviations of the metrics used for describing the composition and the configuration of the landscape (= blue arrows) are explained in Tab.1.

	Can1	Can2	Can3
CanRsq	0.96538	0.62555	0.53544
Eigenvalue	27.8814	1.6706	1.1526
Difference	26.211	26.211	26.211
Percentage	90.81	5.44	3.75
Cumulative	90.81	96.25	100.00
p-Value	4.366e-13 ***	1.959e-05 ***	0.0005573 ***
Composition of the	landscape		
pM	-6.7968e+06	2.3457e+06	2.7105e+06
рН	-3.5005e+06	1.2081e+06	1.3960e+06
PW	-5.3524e+06	1.8472e+06	2.1345e+06
pNAT	6.8569e+06	-2.3665e+06	-2.7345e+06
AREA_NAT	-1.2695	-0.15124	1.1722
Configuration of the	e landscape		
LDI	0.66234	0.54513	0.27054
LDI_W	-2.86	0.57719	1.8499
LDI_H	-4.3514	2.6631	1.8163
LDI_M	-1.337	-0.02653	1.0700
LDI_NAT	3.0982	-1.5983	-1.8034
ENN_W	0.21672	0.60864	0.044517
ENN_H	-1.5559	-0.20393	1.0624
ENN_M	0.57707	-0.23763	0.37707
ENN_NAT	0.94362	-0.56568	-0.79644
Composition + conf	iguration of the landscape		
ILC	0.055266	0.48411	0.11403

The first axis represented a gradient of increasing landscape functional quality associated with a high percentage of fairly large and not too isolated semi-natural areas and a high level of landscape heterogeneity (Tab.5).

The second axis explained only (5.44%) of the multivariate dispersion depicting towards the top of Fig. 3 an increase in (i) the percentage occupied by hedgerows (pH), (ii) the isolation of wooded patches (ENN\_W), (iii) the general fragmentation of the landscape (LDI), which is characteristic for the groups a and d. Toward the bottom of axis 2: the isolation of (i)

semi-natural areas (ENN\_NAT) and (ii) hedgerows (ENN\_H) increases, which is typical for the groups b and c. The gradient of the second axis was more associated to an increase of isolation metrics and general fragmentation of landscape.

**Tab.5.** Mean values of landscape functional quality and landscape metrics for each group. The abbreviations of the metrics used for describing the composition and the configuration of the landscape (= blue arrows) are explained in Tab. 1.

Group	a (worst)	b	С	d (best)			
Landscape functional quality (0-1)	0.16	0.21	0.29	0.43			
Composition of the landscape: percentage cover and patch size (AREA_NAT)							
pW	1.8641	8.1298	12.3335	22.9062			
pM	2.9208	5.4594	5.2611	15.3855			
рН	4.4612	1.6671	4.0089	2.9105			
pNAT	9.2462	15.2563	21.6036	41.2022			
AREA_NAT	0.8157	2.0234	3.4149	5.0862			
Configuration of the landsca	pe: fragmentation (l	andscape division In	dex LDI)				
LDI	0.8029	0.6899	0.8851	0.9538			
LDI_NAT	0.9992	0.9926	0.9830	0.9671			
LDI_W	0.9999	0.9964	0.9956	0.9918			
LDI_H	0.9998	0.9999	0.9997	0.9999			
LDI_M	0.9999	0.9985	0.9993	0.9975			
Configuration of the landsca	pe: isolation (Euclide	ean Nearest Neighbo	ur ENN)				
ENN_W	574.7635	101.9580	115.9255	50.1290			
ENN_H	56.4589	112.5701	124.0567	71.1731			
ENN_M	130.9474	76.6954	156.1839	42.2002			
ENN_NAT	49.3773	58.7599	47.1179	32.2020			
Composition + configuration	of the landscape (In	dex of Landscape Co	nservation)				
ILC	0.2419	0.2913	0.3263	0.4428			

#### 4. Discussion and conclusions

Assessment of the functional quality of the landscape

The analysis carried out on floristic community to find model inputs confirmed both (i) different support to the biodiversity and (ii) different sensibility to disturbance of habitats composing cultivated landscape. Annual and perennial crops were characterised by minimum values of diversity (Manhoudt et al., 2005), since these habitats are characterized by simplified plant communities, oriented to production. At the same time, they presented high value of hemeroby, due to management practices, such as ploughing, mowing, pesticide applications (Boutin and Jobin, 1998; Kleyer, 1999; Aavik and Liira, 2010). Perennial and annual crops were confirmed as the highest sources for the spread of hemerobic species (i.e. source of threat) and, at the same time, habitats that support lower level of biodiversity. Regarding semi-natural habitats, meadows represented a hot spot of biodiversity (Wilson et al., 2012), followed by woods and hedgerows. Their sensitivity showed an opposite trend when compared with diversity. Our results confirmed that greater level of biodiversity are associated to greater resistance to spread of hemerobic species and therefore to disturbance (Naeem et al. 1994, Tilman and Downing 1994, Tilman et al., 2006).

The analysis of distance to dispersal of hemerobic species revealed a direct relationship between abundance of hemerobic species and proximity of disturbance source (i.e. annual crops). As demonstrated in other studies, focused on neophyte species, the degree of spread increased with the proximity of source of disturbance (Forman and Deblinger, 2000; Watkins et al., 2003; Hansen and Clevenger, 2005).

InVEST model allowed to integrate both components of landscape functional quality, namely biodiversity and sensitivity to invasion by hemerobic species. Several studies claim that landscape structure influences both biodiversity (Honnay et al., 2003; Duelli and Obrist, 2003; Walz, 2011; Walz and Syrbe, 2013) and dispersal (propagation) of exotic species (With, 2002; Kumar et al., 2006). Therefore, the InVEST model was revealed to be a good tool for correctly assessing the functional quality of agricultural landscapes. In addition, InVEST model was able to discriminate between different structures of the landscape, creating groups of landscape functional quality that showed significant differences between all possible pairs.

Conclusions for the planning of ecologically sustainable agricultural landscapes

The proposed approach is a useful tool for assessing the functional quality of agricultural landscapes and, therefore, for the planning of ecologically sustainable agricultural landscapes (Botequilha Leitão and Ahern, 2002). The aim of the study was to determine which landscape metrics (of composition and configuration) were particularly suited for assessing the functional quality of agricultural landscapes in order to make simple but effective recommendations for ecologically sustainable management and land use schemes.

We found that the functional quality of the landscape was positively correlated with presence of semi-natural areas, woods and meadows. It is well known, that the presence of semi-natural habitats in agricultural landscapes leads to greater biodiversity (Paoletti, 1999; Duelli and Obrist, 2003). In particular, meadows and woods with low levels of hemerobic species proved to be hot spots of biodiversity in agricultural landscapes.

Also high heterogeneity of the landscape (i.e. landscape conservation index) and low fragmentation of the semi-natural areas, in particular, of the woods and meadows, were positively correlated with the functional quality of the landscape. The relationship between landscape heterogeneity and biodiversity has been extensively studied (Deutschewitz et al., 2003; Honnay et al., 2003; Kumar et al., 2006). In our study, it should be considered that heterogeneity corresponded to the availability of semi-natural areas, in fact both heterogeneity and percentage of semi-natural areas followed the same trend (Tab.5) and both led to an increase in the functional quality of the landscape. According to the literature, it is well known, that the effect of landscape heterogeneity on plant species richness is generally much greater than the effect of fragmentation (Honney et al., 2003). However, many small patches of different habitat types usually contain more plant species than few large patches, indicating that the effect of patch size is less important than diversity of habitats (i.e. landscape heterogeneity) (Honnay et al., 1999). However, in our approach for assessing the functional quality of agricultural landscapes, which takes into account the sensitivity of habitat beside diversity, small patches of semi-natural habitats were presumed to be more likely to be invaded by hemerobic species than large patches. This can be is easily explained by the edge effect, i.e. by the ratio of perimeter to surface area. Consequently, the lack of large patches of semi-natural habitat increases the probability of invasion by hemerobic species. This was confirmed by our results, where along the increasing gradient of landscape functional quality an increase in the patch size of semi-natural areas was found (Tab.5).

Further, we found functional quality of the landscape to be negatively correlated to the spatial isolation of meadows. Other studies found that distance to suitable habitats (i.e. isolation) influences plant diversity (Grashof-Bokdam, 1997; Butaye et al., 2001). Less isolated habitats are generally richer in species because they can be colonized by new individuals preventing local extinction caused by demographic and environmental coincidences (Shaffer, 1981; Honnay et al., 2003).

The second gradient revealed by the Canonical Discriminant Analysis, which was associated with isolation of semi-natural areas, hedgerows and woods as well as with the general fragmentation of the landscape. Since the first gradient was much more important than the second, however, landscape fragmentation and the isolation of semi-natural habitat patches are far less important the planning of ecologically sustainable agricultural landscapes than the total amount of semi-natural areas and the general heterogeneity of the landscape.

Walts (2011) in a recent review on the relationships between landscape structure and species diversity of different taxa, concluded that landscape characteristics such as a high proportion of semi-natural habitats, large patch size, high habitat diversity, high structural diversity and high connectivity have generally a positive effect on biodiversity. However, some of these landscape characteristics are mutually exclusive (Walts, 2011). In fact, a landscape element or trait that is beneficial for a specific *taxon* can be disadvantageous for another. In our study, the same relationships between landscape structure and species diversity highlighted by Walts (2011) were found for plant *taxa*. However, our findings highlighted that these relationships act together with the increase of landscape functional quality considering both biodiversity and sensitivity in disturbed landscapes such as agricultural landscapes.

In addition, our results underline the importance of meadows and woods in agricultural landscape. Promoting the presence and the structural complexity of these habitats instead of others such as e.g. hedgerows will increase functional quality of agricultural landscapes.

As a conclusion, we can claim that our method for the assessment of landscape quality in terms of its own potential functionality (i.e. landscape functional quality), integrating landscape biodiversity assessment with landscape sensitivity assessment, obtained a real representation for a highly disturbed landscape as the agricultural landscapes. This method allowed us to derive several indications evidences for the ecological planning of agricultural landscape in order to enhance sustainability.



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## SUPPLEMENTARY MATERIALS

Description of the landscape metrics used in the analysis.

1. Landscape Division Index (LDI) represents the landscape fragmentation and is equal to 1 minus the sum of patch areas (m<sup>2</sup>) divided by total landscape area (m<sup>2</sup>), quantity squared, summed across all patches of the corresponding patch type (1).

$$LDI = \left[1 - \sum_{j=1}^{n} \left(\frac{a_{ij}}{A}\right)^{2}\right] \tag{1}$$

where  $a_{ij}$  = area (m<sup>2</sup>) of the patch ij and A= total landscape area (m<sup>2</sup>).

2. Euclidean Nearest Neighbor distance (ENN) is a measure of patch isolation. Nearest neighbor distance is defined using euclidean geometry as the shortest straight-line distance between the patch and its nearest neighbor of the same class (hedge to hedge) (2).

$$ENN = h_{ii} \tag{2}$$

3. Landscape Conservation Index (ILC) index is a measure of landscape heterogeneity but, at the same time, weights the different land-use typology. The index is calculated following the formula (2):

$$ILC = 1 - \frac{A}{A_{max}} \tag{3}$$

where  $A = \sum_{i=1}^{n} c_i$ 

where  $A = \sum_{i=1}^{n} c_i$  where n is the number of land-use typology and  $c_i$  is the cumulative relative value of the  $i^{th}$  land-use. To obtain cumulative relative value, each land-use were ordered according to their importance in the contribute to biodiversity from the less (i = 1) to the mayor (i = n). In this way, land-use with a higher degree of biodiversity have greater weight. In fact, if  $x_{l...}$   $x_n$  are the relative areas occupied by the classes, the cumulative values are:

$$C_1 = x_1, C_2 = x_1, + x_2, ..., C_n = x_1 + ... + x_n$$
 (4)

and

$$A_{max} = (n-1) \tag{5}$$

The index ranges between 0 and 1. Values close to 1 indicate landscapes characterized by high heterogeneity and rich in semi-natural habitats.

3

# Landscape disturbance and susceptibility to invasive plant species in contrasting agricultural landscapes<sup>2</sup>

#### **Abstract**

Agricultural landscapes are composed by a mosaic of both disturbed arable lands and less disturbed semi-natural habitats. Arable land can provide a source of invasive exotic plants to neighboring semi-natural areas. We want to analyze how agricultural landscape intensification can affect the spread of exotic plant species (i.e. archaeophytes and neophytes) in different semi-natural habitats and to evaluate whether exotic plants are less dispersal limited than native species by testing distance-decay of similarity for the different semi-natural habitats. We analyzed five habitats, viz. woods, hedgerows, field boundaries, meadows and crops, in four agricultural landscapes of Friuli Venezia Giulia – North-Easter Italy, along a gradient of landscape intensification (% cover of arable land). Alpha-diversity of neophytes, archaeophytes and native plant species have been related with degree of disturbance (% of crops) and proximity to disturbance (crop distance). Beta-diversity was related to geographical distance (Euclidean distance) in order to investigate the potential different distance-decay of similarity between native and exotic species. At the local scale, we found differences among habitats on their susceptibility to be invaded by exotic plants changed according to the proximity to the source or amount of disturbance in the landscape. The presence of exotic plant species (both neophytes and archaeophytes) leads to a greater homogenization of the flora in different semi-natural habitats across the landscape.

## **Keywords**

Exotic plant species, agricultural landscape, semi-natural habitats, neophytes, disturbance

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<sup>&</sup>lt;sup>2</sup> De Simone S., Boscutti F., Sigura M., Marini L., 2014 (Submitted). *Landscape disturbance and susceptibility to invasive plant species in contrasting agricultural landscapes*. Biological invasions.

## 1. Introduction

Agricultural landscapes are characterized by large degree of human disturbance and high availability of resources that are frequently implicated in the spread of invasive exotic plants (Hobbs and Humphries, 1995; Lonsdale, 1999; Alpert et al., 2000; Davis et al., 2000; Chytry' et al., 2005; Vilà et al., 2007; Pyšek et al., 2009). However, agricultural landscapes are composed by a mosaic of both disturbed arable lands and semi-natural habitats such as meadows, hedgerows or woods. While arable lands, along with ruderal and urban sites, are considered one of the most invaded habitat, semi-natural habitats present generally higher resistance to exotic plant invasion (Chytry' et al., 2005, 2008; Pyšek et al., 2009). The resistance to invasion is expected to be strongly influenced by both local conditions (e.g. native species composition as well as intensity and frequency of management treatments) and propagule pressure (Lockwood et al., 2005, Colautti et al., 2006). In this light, highly disturbed habitats, such as arable crops, can provide a source of propagules to neighboring natural areas (Timmins and Williams, 1991; Searcy et al., 2006). Hence, propagule pressure can be related to landscape changes (e.g. increase in arable lands), which can enhance exotic spread by creating suitable habitats for colonization and establishment (Theoharides and Dukes, 2007). Investigating how disturbed habitats in the landscape can affect invasion of exotic plants into semi-natural habitats is of utmost importance.

Understanding how the spatial distribution of resources, populations or habitats affect various stages of the invasion process requires a landscape approach (Vilà and Ibáñez, 2011). For instance, there is evidence that, proximity to a source of dispersing individuals of exotic species will increase the probability that the invasive species will expand its geographical range (Rouget et al., 2003). Therefore, landscape structure will determine the interaction of local-scale population dynamics with local spread, in a way that favors invasion or reduces the resistance of communities to invasion (With, 2002).

In this paper, we want to analyze how agricultural landscape intensification can affect the spread of exotic plant species (i.e. archaeophytes and neophytes) in different semi-natural habitats. First, we want to evaluate how the level of disturbance in the landscape affects the invasion of exotic plant species into semi-natural habitats. We hypothesize that increasing cover of arable land and decreasing distance between source and sink habitats will increase invasion of exotic species into the most common semi-natural habitats. Second, we want to evaluate whether exotic plants are less dispersal limited than native species by testing

distance-decay of similarity for the different semi-natural habitats for both alien and native species (Nekola and White, 1999).

## 2. Materials and methods

# 2.1 Study area and sampling design

The study area was located in the Friuli Venezia Giulia Region of North-Eastern Italy (45°51′24"N, 13°00′33"E to 46°01′28"N, 13°03′01"E). The region is characterized by an average annual temperatures of 13°C and an average annual rainfall of between 1100 mm and 1600 mm (Stefanuto, 2003). The most frequent crops are maize, soybean, winter cereals (mostly barley and wheat) and grapes. We selected four quadrats, 2 km x 2 km in size, in the agricultural landscape of Friuli Venezia Giulia along a gradient of agricultural intensification, as expressed by the % cover of arable land (range 53.4 - 96.6%) ("Multifarm" project, L.R. 26/05 art. 17). For each quadrat, detailed maps (1:2,000) of habitats were established by means of both photo-interpretation and field surveys. Physiognomically homogeneous areas were assigned to one of five habitat types, viz. (i) woods, (ii) hedgerows, (iii) field boundaries, (iv) meadows and (v) crops, respectively. In each quadrat, these habitats were then surveyed using stratified sampling. The plots within each site was defined according to the number of semi-natural patches (woods, hedgerows, meadows). Subsequently, the overall number of sampling plots was distributed proportionally to both number of patches and the total area occupied by each type of habitat. Field boundaries were not included in the calculation because of their small surface, and hence, they were coupled with crops. In each type of habitat, the plots were randomly distributed. In total, 152 plots were investigated, 25 in woods, 29 in hedgerows, 50 in meadows, 24 in crops and 24 in field boundaries. Floristic data were collected during the growing season of 2010. All surveys were conducted in the period of maximum vegetative growth (i.e. woods and hedgerows in spring-time, meadows in late spring-time, crops and field boundaries in summer-time). The size of the plot sampling was defined according to minimum area values established for studied plant communities (Westhoff et al., 1973; Chytrý et al., 2003; van der Maarel, 2005), i.e. 5 m<sup>2</sup> for field boundaries, 11 m<sup>2</sup> for crops, 25 m<sup>2</sup> for meadows and hedgerows and 100 m<sup>2</sup> for woods. Within each plot all vascular plant species and their estimated cover values were recorded. Cover values were estimated using the Braun-Blanquet scale (Braun-Blanquet, 1964). Nomenclature and taxonomy followed Poldini et al. (2001) and Pignatti (1982). Exotic or native *status* was classified according to the inventory of the alien flora of Italy (Celesti et al., 2009). For exotic *taxa* we further distinguished two groups, following the classification based on the time of immigration (Pyšek, 1995): (i) archaeophytes, introduced before 1500, and (ii) neophytes, introduced after 1500.

# 2.2 Plant community

Alpha-diversity

Alpha-diversity was the number of plant species found within each plot (species richness). Alpha-diversity was calculated for all the plant groups considered (i.e. natives, neophytes and archaeophytes). As plot size was different among the different habitats, the analyses on alpha-diversity were performed separately for each type of habitat.

# Beta-diversity

Analysis of beta-diversity can provide information on the mechanisms of spread and organization of exotic species groups (Leprieur et al., 2009). In order to understand how the composition of the landscape affects the distribution of exotic and native species in different habitats, beta-diversity was separately calculated for each of the plant groups considered, i.e. for natives, exotics as well as for archaeophytes and neophytes. Beta-diversity was calculated in terms of floristic (dis)similarity, using the Bray-Curtis dissimilarity index (Bray et al., 1957):

$$\beta = \frac{(A+B-2*J)}{(A+B)}$$

where A and B are the numbers of species present exclusive on the two plots in question, and J is the number of species present on both plots. Prior to analysis of beta-diversity, plant cover estimates were transformed using the ordinal transformation (van der Maarel, 2005, 2007).

# 2.3 Landscape indices

In order to understand how landscape disturbance affects the spread of exotic plants inside semi-natural habitats, alpha-diversity of neophytes, archaeophytes and native plant species has been related with two landscape variables (i) percentage of arable land in the surrounding 250 m and (ii) distance from the main source of disturbance, i.e. distance from the closest arable field. Percentage of arable land was calculated in circle-shaped buffer with a radius of

250 m around each plot (i.e. crop percentage), whereas distance to closest source of disturbance was calculated as the euclidean distance between each plot and the closest arable field (i.e. crop distance). Crop percentage represented the degree of disturbance in the landscape. The Pearson correlation ( $\rho$ ) between crop percentage and crop distance was -0.76; within habitat: Woods  $\rho$  = -0.81; Hedgerows  $\rho$  = -0.79; Meadows  $\rho$  = -0.67).

# 2.4 Data analysis

# Alpha-diversity analysis

We used multi-model inference within an information theoretic framework to evaluate the influence of the landscape variables on the alpha-diversity of archaeophytes, neophytes and natives, respectively, within the different habitats (Burnham et al., 2002). We used linear mixed models (LMMs) to estimate model parameters as model residuals approximated a normal distribution. Models included species richness as response variable and alien status (i.e. archaeophytes, neophytes or natives), landscape variables and their interaction as fixed effects. The random effects of geographical position (i.e. location of the study quadrats) and sub replicates for the status (i.e. plot id) were included. Alpha-diversity values have been transformed to obtain normal distribution and stabilize the variance (i.e. logarithmic transformation), and to standardize the number of species of different plant status (i.e. Zscore) (Sokal et al, 2012). The two variables were modeled separately for all habitats with the exception of crops and field boundaries, where the variable "crop distance" was discarded. Given the non-linear relationship between independent variable and dependent variables, the model was linearized by logarithmic transformation. Multi-model inference compared the fit of all possible models obtained by the combination of the variables. We used Akaike's information criterion (AIC) to choose the best fitting model. AIC is a measure of the relative quality of a model dealing with the trade-off between the goodness of fit and the complexity of the model. The best fit is indicated by the lowest AIC value (AIC MIN). In a set of models each model i can be ranked using its difference in AIC score to the best-fitting model ( $\Delta$  AICi = AICi- AICi MIN). A model in the set can be considered plausible if its  $\triangle$ AIC is below 2 (Burnham et al., 2002). We also derived the Akaike's model weight (wi) which is the probability that the model i is the best fitting model if the data were collected again under identical circumstances (Burnham et al., 2002). We also calculated the relative importance of the variables using Akaike's model weight. The multi-model inference based on AIC was executed using R statistical software (R Core Team, 2013) with the 'MuMIn' package (Barton, 2013). The linear mixed models were applied using the "nlme" package (Pinheiro et al., 2009).

## Beta-diversity analysis

Beta-diversity was related to geographical distance (euclidean distance) in order to investigate the potential different distance-decay of similarity between native and exotic species. To perform the analysis on beta-diversity we used regression on distance matrices (MRM) (Lichstein, 2007). MRM makes a regression between two matrices. The matrices contained distances or dissimilarities among all the pairwise combinations of natives, archeophytes and nephytes in each plot. The response matrix was the beta-diversity matrix (Bray-Curtis dissimilarity index) and the explanatory matrix was the geographical distance matrix (distance in meters between each pair of plots). Beta-diversity matrices were calculated separately for native species, archaeophytes, neophytes and archaeophytes and neophytes together). MRMs were conducted for each response variable separately using both a linear model with and without including a quadratic term to account for a possible non-linear relationship. Tests of statistical significance were performed by permutation (n=999). The MRM analyses were conducted using R statistical software (R Core Team, 2013) with "MRM" function in the "ecodist" package (Goslee and Urban, 2007). R<sup>2</sup> values were used to enucleate the variance explained by the model.

## 3. Results

## 3.1 Disturbance effects on exotic and native species distribution

In total 402 plant species were recorded, of which 61 were exotic species (34 neophytes, 27 archaeophytes) and 341 native species.

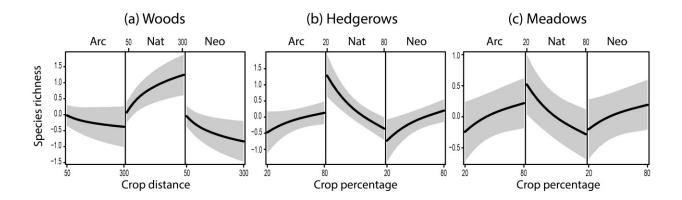
For woods, the multi-model inference showed that only two models were supported (Tab.1). The models included crop distance and species status and their interaction. Neophytes and archaeophytes were negatively associated with crop distance, whereas native species were positively associated with crop distance (Fig.1). Both models explained c. 30% of the total variation in species richness.

For hedgerows, the multi-model inference showed that there were three plausible models, which included crop percentage and status and their interaction. As the crop percentage

increased, exotic species richness increased, whereas native species tended to decrease (Fig.1). The models explained c. 40% of the total variation in species richness.

For meadows, we found three plausible models that included crop percentage and status, explaining between 12 and 13% of the total variation in species richness. The model including only crop percentage and status variables showed that exotic species were positively associated with crop percentage, whereas native species were negatively associated with the variable (Fig.1).

For field boundaries and crops, the multi-model inference showed that the best model was the null model, indicating that no variable explained the species richness (Tab. 1).



**Fig.1.** Relation between species richness (log-transformed and standardized) of native, archaeophytic and neophytic species and landscape variables, calculated with the best fitting model chosen within Multi-model inference for woods (a), hedgerows (b), and meadows (c). Crop distance stands for the distance to the closest arable field, crop percentage for proportion of area covered by crop fields in circle with a radius of 250 m around the sampling plot. Confidence intervals (0.95) is also shown.

**Tab.1.** List of plausible models performed with multi-model inference. There are reported: the intercept (Int), the variables considered in each model (CD = crop distance = the euclidean distance between each plot and the closest arable field; CP = crop percentage = percentage of surface area occupied by crops in circle with a radius of 250 m around the sampling plot); St = status of the species, i.e. native species, archeophyte, neophyte), their relative importance,  $R^2$ , AIC,  $\Delta$ AIC and model weight (wi). n.a. = not applicable. The variable crop distance and the interaction between the two variables crop distance and status were not included in the analyses for the habitats field-boundaries and crops.

Int	CD(a)	CP	St	CD:St(a)	CP:St	$\mathbb{R}^2$	AIC	ΔΑΙC	W
Woods									
0.80	-0.209		+	+		0.30	201.4	0.00	0.60
1.41	-0.285	-0.091	+	+		0.30	203.0	1.69	0.26
relative	variables	importanc	e						
	0.99	0.40	1	0.99	0.14				
Hedgerows									
-4.47	0.316	0.875	+		+	0.38	222.2	0.00	0.39
-1.84		0.449	+		+	0.37	222.2	0.05	0.38
-6.78	0.593	1.248	+	+	+	0.40	223.2	1.06	0.23
relative	variables	importanc	e						
	0.62	1	1	0.23	1				
Meadows									
-1.28		0.341	+		+	0.12	421.6	0.00	0.59
-1.84	0.079	0.416	+		+	0.12	423.2	1.56	0.27
-3.10	0.261	0.581	+	+	+	0.13	425.3	3.63	0.10
relative	variables	importanc	e						
	0.38	0.97	0.96	0.10	0.96				
Field-bound	laries								
0.00	n.a.			n.a.		0.00	209.3	0.00	0.43
-13.6	n.a.	3.020	+	n.a.	+	0.12	209.8	0.50	0.33
0.60	n.a.	-0.135		n.a.		0.00	211.3	1.99	0.16
relative	variables	importanc	e						
	n.a.	0.51	0.41	n.a.	0.33				
Crops									
0.00	n.a.			n.a.		0.04	206.3	0.00	0.60
-3.92	n.a.	0.870		n.a.		0.05	207.9	1.59	0.27
relative	variables	importanc	e						
	n.a.	0.32	0.13	n.a.	0.01				

# 3.2 Distance decay of similarity at landscape scale

For native plant species, we found a positive correlation between geographical distance and beta-diversity for each of the four semi-natural habitats studied, i.e. woods, hedgerows

meadows and field-boundaries. With increasing geographical distance between two sampling plots of the same habitat type, their composition of native species became more dissimilar (Tab.2). For exotic species, by contrast, we found only very weak relationships between geographical distance and beta-diversity for hedgerows ( $R^2 = 0.03$ ) and field boundaries ( $R^2 = 0.07$ ) and no relationship at all for meadows and crops. Only woods presented a significant relationship ( $R^2 = 0.18$ ). Neophytic species had the same behavior as all exotic species combined, whereas archaeophytes did not show any significant relationship except for a weak relationship in hedgerows ( $R^2 = 0.01$ ).

**Tab.2.** Model regression results between beta-diversity and geographical distance for native species, exotic species, neophytes and archaeophytes separately. There were reported the linear and quadratic effects of geographical distance matrix on dissimilarity species composition matrix, slope and R<sup>2</sup>.

	Native species		Exotic species		Neophytes		Archaeophytes	
	Slope	R <sup>2</sup>	Slope	R <sup>2</sup>	Slope	R <sup>2</sup>	Slope	R <sup>2</sup>
Woods								
Linear	9.66e-06	0.60	9.11e-06	0.17	9.11e-06	0.18	-	-
Quadratic	2.20 -0.77	0.67	2.08 -0.40	0.18	2.07 -0.40	0.18	-	-
Hedgerows								
Linear Quadratic	3.39e-06 0.88 0.03	0.10 0.10	4.09e-06 -	0.03	3.79e-06 -	0.03	5.58e-07 0.144 -0.10	0.01 0.01
Meadows								
Linear Quadratic	5.27e-06 2.43 -0.06	0.20 0.20	- -	-	-	-	- -	-
Crops								
Linear Quadratic	- -	-	- -	-	- -	-	- -	-
Field boundaries								
Linear Quadratic	3.39e-06 0.65 -0.69	0.12 0.25	- -0.25 -0.63	- 0.07	- 0.14 -1.45	0.14	- -	-

## 4. Discussion and conclusions

Disturbance and spread of exotic plant species

The results showed that landscape disturbance, expressed as crop percentage and crop distance, affected different semi-natural habitats were differently with regard to their vulnerability to invading exotic plant species. In particular woods, meadows and hedgerows were more strongly affected by disturbance, whereas field boundaries and crops seemed not to be influenced by changes in the degree of disturbance. Crops and field boundaries did not respond to the crop percentage, therefore there was no influence on their floristic composition according to the context in which they are included (i.e. landscape disturbance). Both habitats were strongly affected by the local disturbances due to the agricultural management treatments. This was confirmed by several authors, which pointed out the strong influence of agricultural practices (e.g. fertilizers, pesticides, mechanical disturbance) on the flora composition (Marshall and Moonen, 2002; Hovd and Skogen, 2005; Aavik and Liira, 2010) rather than landscape variables.

On the other hand, the two landscape variables tested were of different relevance for the less-disturbed semi-natural habitats. Woods were affected only by crop distance but not by the total cover of arable land. With increasing crop distance, the number of exotic species tended to decrease both for archaeophytes and neophytes. Similar results have been observed in other studies (Forman and Deblinger, 2000; Honnay et al., 2002; Watkins et al., 2003; Hansen and Clevenger, 2005), which claim that the degree of invasion increases within the proximity of the source of disturbance. This shows a direct relationship with the source of spread of exotic plant species, highlighting at the same time a significant attenuation with increasing distance from the source. Such type of habitat susceptibility could be considered as "local" rather than "diffuse". On the other hand, meadows and hedgerows were influenced by crop percentage, i.e. the proportion of land occupied by arable fields. In these habitats, the diffusive spread of exotic species was probably linked to a higher endogenous disturbance, due to management practices (e.g. cleaning, mowing, fertilizing). These disturbs lead to a higher susceptibility of these habitats (Alpert et al., 2000; Chytrý et al., 2008). It could explain a comparatively small homeostasis of hedgerows and meadows, making them more sensitive to disturbance diffusion across the agricultural landscape (i.e. crop percentage). In all habitat the spread of both groups of exotic plants (i.e. archaeophytes and neophytes) was similar.

In conclusion, we showed that disturbance at the landscape level affects different seminatural habitats in different ways, due to their intrinsic ecological traits. Woods are more sensitive to the proximity to source of disturbance, i.e. crop distance, while hedgerows and meadows are more influenced by the overall amount of disturbance, i.e. the percentage of landscape occupied by crop fields.

# Distance decay of similarity at landscape scale

Variation in distance-decay similarity for different plant groups, i.e. native plant species, exotic, archeophytes and neophytes, allowed us to understand if different groups have different dispersal limitations (Nekola and White, 1999). We put in relation the beta-diversity of natives and exotics, and more specifically archaeophytes and neophytes, with geographical distance, in order to determine the differences in terms of diffusion of species among different habitats. Biological similarity typically decreases with geographical distance, and is expected to be driven by different mechanisms (Soininen et al., 2007). In our study, we found that floristic composition of semi-natural habitats showed a strong relationship between geographical distance and floristic dissimilarity. The floristic similarity of both native and exotic species on two crop fields, by contrast, did not depend on the geographic distance between the two fields. This indicate that severity of management treatments (e.g. tillage, fertilizers, pesticides) create a rather homogenous habitat, with low variability of species composition. Within semi-natural habitats, the strongest relationship was found in woods. This habitat is probably influenced by local ecological characteristics (i.e. soil type, water table depth, climate), while the other habitats were more homogeneous across space, because they are subjected to stronger management practices (i.e. mowing, pesticide applications). This management probably tends to favor stress tolerant species and thus to homogenize species composition of both plant and animal communities (Chytrý et al., 2008). This is especially true for hedgerows and field boundaries, which have simplified linear structures. Regarding exotic plant species, many authors have suggested that the biogeography of exotic species is primarily a consequence of human processes, and for this reason, exotic species are likely to be less dispersally limited than native species due to human-assisted introductions (Olden, 2006; Blackburn et al., 2008; Leprieur et al., 2009). In fact, in our study, we found for all the habitats considered that neophytes and archaeophytes tended to be more homogeneously distributed across space than native species. Only in the woods, exotic plant species, and in particular neophytes, showed a different pool of species according to geographical distance, while for the other habitats there were a lower (hedgerows, field boundaries) or no relationship (meadows).

In conclusion, at the local scale, we found differences among habitats on their susceptibility to be invaded by exotic plants changed according to the proximity to the source or amount of disturbance in the landscape. Moreover, in agricultural landscapes pool of exotic species are generally homogeneous across localities, depleting the overall variability of vascular flora.

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4

# Effects of conservation tillage on multiple ecosystem services supporting cereal production<sup>3</sup>

#### **Abstract**

We analyzed the effects of conservation tillage, in contrast to conventional tillage, on the supply of multiple ecosystems services supporting cereal production. The ecosystem services considered in the experiment were (i) production, (ii) weed control, (iii) natural aphid pest control and (iv) soil quality (SOM quantity). In addition, we examined whether landscape complexity affects the supply of natural pest control and weed control and whether intensity of fertilization influences production service and weed control. The experiment was undertaken in 15 pairs of fields (conventional tillage vs. conservation tillage) of winter cereals (i.e. wheat and barley) located in Friuli Venezia Giulia Region in North-Eastern Italy, selected along a gradient of landscape complexity. The results showed that tillage system, landscape complexity and fertilization had different influences on ecosystem services. Production did not differ between conservation and conventional tillage, whereas fertilization increased production. Conservation tillage decreased weed control services and favored a greater diversity of weeds. Landscape complexity and fertilization did not show any influence neither on weed control services nor on weed diversity. We found a greater effect of natural pest control by ground predators under conservation tillage. On the other hand, parasitism on aphids was not affected by tillage system, but increased with landscape complexity. Finally conservation tillage positively affected the soil quality.

# **Keywords**

Ecosystem services, conservation tillage, winter cereal crops, landscape complexity

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<sup>&</sup>lt;sup>3</sup> De Simone S., Tamburini G., Boscutti F., Sigura M., Marini L., 2014. *Effects of conservation tillage on multiple ecosystem services supporting cereal production*. Manuscript in preparation.

## 1. Introduction

Ecosystem services are the benefits that people obtain from ecosystems (MA, 2005a, b). Agricultural ecosystems, which cover nearly 40% of the terrestrial surface of the Earth (FAO, 2009), provide food but they contribute also to other ecosystem services, some of which support crop production itself (e.g. pest control, pollination, weed control, disease control, and soil quality). The supply of several ecosystem services is, therefore, influenced by agricultural management (Power, 2010). For this reason, it is important to understand whether agricultural management aimed at maximizing crop yield in the short-term can affect negatively other ecosystem services which, in turn, may have a negative feed-back on yield in the long-term.

In this context, soil management can modify important supporting services that sustained the provisioning of food (Smukler et al., 2012). For example, the increase in soil organic matter (SOM) influences nutrient cycling and storage (Lal, 2006) as well as net primary productivity (Smith, 2007). Conservation tillage (CT) is a soil management practice consisting in the non-inversion of soil combined with other management techniques such as cover crops, surface incorporation of crop residues and complex crop rotation. CT is expected to bring benefits to the environment (Holland, 2004) and is therefore expected to contribute to the increase of several ecosystem services. CT can benefit soil quality (e.g. soil fertility, reduce surface run-off, mitigated leaching of nutrients, reduce disruption of soil structure and erosion, and improve water quality), and can possibly also benefit biodiversity (Holland, 2004; Trewavas, 2004; Aina, 2011). Even though several studies examined the benefits of CT on environment and soil, up to now, only little is known about the benefits of CT on ecosystem services *sensu stricto*.

In addition to conservation tillage, also mineral nitrogen fertilization can counteract soil degradation. Mineral fertilizers are largely used to maintain soil nutrient levels depleted by crop production, and sustain productivity (Sheldrick et al., 2002). Nitrogen fertilization can increase soil organic matter (Paustian et al., 1992), however the influence of this effect depends on management treatments (Alvarez, 2005) and the availability of crop residues or other organic fertilizers. Therefore, the interaction of these parameters can influence the supply of ecosystem services such as production and weeds control.

In addition to the local management, the effects of tillage systems on multiple ecosystem services are expected to depend on the landscape matrix (Tscharntke et al., 2005). Several ecosystem services are strongly governed by the quantity and spatial composition of non-agricultural land use at landscape scale (Bommarco et al., 2013). On the other hand,

agroecosystems are characterized by simplified landscapes due to increase of cultivated matrix and loss of natural habitats. Simplified landscapes cause reduction in diversity of natural enemies (Radford et al., 2005; Maron et al., 2012) and therefore natural pest control can be compromised (Flynn et al., 2009; Tilman et al., 2001; Tscharntke et al., 2005, 2007). In this context, it is necessary to investigate the effects of management system, landscape effects and their interaction on ecosystem services. Albeit several studies were focused on specific ecosystem services and specific agroecosystem management practices (see Kremen and Miles, 2012), little is known about interactions between soil management, landscape complexity and their impact on multiple ecosystem services.

The aim of this study was to analyze the effects of conservation tillage, as compared to conventional tillage, on the supply of multiple ecosystems services supporting cereal production. The ecosystem services considered in the experiment fall into different categories, specifically: provisioning services (i.e. production), regulating services (i.e. weed control, aphid pest control), and supporting services (i.e. soil quality). In addition, we examined whether the complexity of the landscape affects the supply of these services (i.e. pest control and weed control), which depend on the composition of non-agricultural land in the surrounding landscape. Finally, we also examined whether intensity of fertilization influences production service and weed control.

## 2. Materials and methods

# 2.1 Study area and sampling design

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

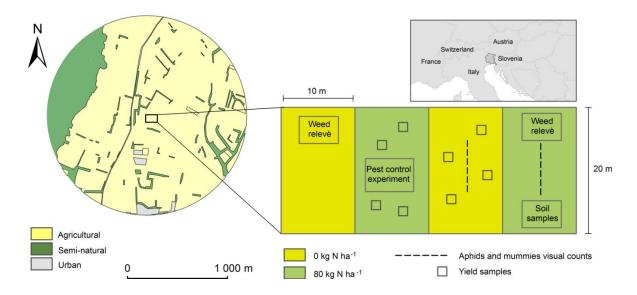
The experiment was carried out in 15 pairs of fields (i.e. 30 fields) of winter cereals (i.e. 7 pairs of barley and 8 pairs of wheat). In each pair, one field was subjected to conservation tillage (CT) and one to conventional tillage (CoT). Conservation tillage was characterized by non-inversion of the soil for at least 5 years (range=5-20 years, mean=9.8 years). In the study area, CT was always coupled with cover crops between harvests, generally consisting of grass

species. On the other hand, conventional tillage included fields where the seedbed was prepared by deep primary tillage with inversion of the top soil (30 cm), followed by one or two tills for seedbed preparation.

The pairs were selected along a gradient of landscape complexity, varying from simple to complex landscapes. Simple landscapes were characterized by a high percentage of agricultural land and/or urban settlements and a low percentage of semi-natural habitats, i.e. woods, meadows, field margins and hedge rows. Complex landscapes, by contrast, were characterized by a high percentage of semi-natural habitats and low percentage of agricultural land and/or urban settlements (see supplementary materials p.81). The maximum distance between the two fields of a pair was 1,200 m. Landscape complexity was assessed by photo-interpretation in a circle around the center of each field with a radius of 1 km. We made sure that the percentages of agricultural land, urban settlements and semi-natural habitats did not differ between CT and CoT (Linear Mixed Models for agricultural land use p-value = 0.48; for semi-natural habitats use p-value = 0.70; for urban land use p-value= 0.76).

In order to assess the influence of landscape complexity on each field, we calculated the percentages of (i) cropland, (ii) urban settlements and (iii) semi-natural habitats, i.e. woods, meadows, hedge-rows. In order to cover different spatial scales, the above-mentioned variables were calculated for circular plots around each field with a radius of 95, 135, 190, 265, 375, 530, 750 and 1000 m, respectively.

The experiment was conducted from April to June 2014. In each field, we reserved a 20 m x 60 m strip at the edge where no pesticides and herbicides were applied. Within each pair, the 20 m x 60 m strips bordered with semi-natural edge habitats of similar structure and composition. Each 20 m x 60 m strip was subdivided six plots, 10 m x 20 m in size, of which the two outermost ones were considered as buffer zones. Among the four remaining ones, two were fertilized with 80 kg ha<sup>-1</sup> of ammonium nitrate. Data collection was performed in the different plots as described below (Fig.1).



**Fig.1.** Sampling design for one of the 30 selected fields. On the left hand side, result of the photo-interpretation on a circular plot around the fields. On the right hand side, the subdivision of the pesticide-free strip, subdivided into four  $10 \text{ m} \times 20 \text{ m}$  plots where data were collected.

# 2.2 Data collection

## Production service

To measure production we harvested four randomly selected 0.25 m<sup>2</sup> quadrats (Fig. 1) in each field and in each treatment plot (tillage by fertilization). The dry weight (kg) of the grain was measured.

## Weed control service

Regarding weed control, we sampled the species composition for both tillage (i.e. CT and CoT) and fertilization treatments. *Relevés* were conducted in a 2 m x 5 m rectangular plot of 10 m<sup>2</sup> (Fig. 1). In each 2 m x 5 m plot, we recorded the number of weed species, i.e. all the non-crop vascular plant species, present and estimated their cover in percent. Overall weed cover was defined as the cumulative cover of all the weed species present. Overall number of species (species richness) was used for calculating the plant diversity index. All vegetation data were collected at the maximum weed development stage, during the 3<sup>rd</sup> decade of May.

#### Pest control service

We examined the biological control of aphids by both ground predators and parasitoids. Aphid pest control by ground predators was analyzed with a predator exclusion treatment. The experimental design consisted of (i) a total exclusion treatment, where all natural enemies were excluded and (ii) an open treatment, where terrestrial enemies had access to the plants. For each treatment, suitable cages were created, i.e. a total exclusion cage, where all natural enemies were excluded and an open cage, which permit the entrance of ground predator. Cages were placed at the center of one of the fertilized plot of each field. The total exclusion cage consisted of a plastic cylinder (0.3 m in diameter, 0.25 m in height) covered by a net with a mesh size of 1 mm, which was inserted into the ground for at least 10 cm. Open cages were composed only by the thin meshed network, in order to exclude flying insect and vegetation-dwelling predators, while the cylinders were absent in order to permit the access to ground predators. To avoid data bias due to differences in the initial aphid abundance, we inoculated field plants with aphids reared in the lab. 10 days before the inoculation, seven tillers of crop plant were selected for each cage in order to standardize the plant material available to the aphids. The selected plants were therefore covered by non-woven fabric domes to exclude natural enemies that were removed by hand from each dome prior to the start of the experiment. Aphid material (Sitobion avenae) was provided by Katz Biotech AG® and directly placed on the plants (c.150 aphids per cage, both adults and nymphs). Inoculation was done at the heading stage of the cereals (BBCH50-55) during good weather conditions (absence of precipitation and strong wind, minimum air temperature 18 °C). After 5 days, the established aphids were counted and plants were re-inoculated where needed (cages with less than 15 aphids). 10 days after the first inoculation (= time 0), aphids were counted and exclusion treatments started removing the non-woven fabric domes from each cage. Aphids were recounted visually in each cage 5 and 10 days, respectively, after the start of the exclusion experiment in order to record the predation rate over time (i.e. suppression of aphids due to natural enemies). The predation rate was calculated for each field, as the average proportion of aphids growth in the close cages compared with aphid predated in the open cage, as follows (Eq.1):

$$Predation \, rate = \frac{N_{treatment 5}}{R_{close} \times N_{treatment 0}} \tag{1}$$

where  $N_{treatment 5}$  is the number of aphids in the open cage after 5 days,  $R_{close}$  is the number of aphid in the close cage after 5 days and  $N_{treatment 0}$  is the initial number of aphids in the open cage at the beginning of the 5 day period. This metric ranged from 0 to 1, where 0 indicates no net loss of aphids in the open cage and 1 indicates that 100% of aphids were predated (Gardiner et al., 2009). Just before the onset of the experiment, a local storm event damaged the cages in 3 field pairs (6 fields) compromising aphid establishment. The analyses regarding the predation rate experiment were thus based on data from 12 field pairs (24 fields) only. We did not sample the unfertilized control plots because we did not expect any effect of short-term N fertilization on the predation rate.

Further, in each of the 30 fields we assessed the parasitism rate by a visual count of the ratio between the numbers of parasitized aphids (mummies) and unaffected aphids. In each field, the numbers of parasitized mummies and unaffected aphids were collected on 50 crop plants randomly selected along the centerline in both fertilized and unfertilized plots. Count was repeated twice, the first time during the growth stages of stem elongation of the crop species and the second during fruit development.

# Soil quality service

As indicator of the quality of the top soil, we used Soil Organic Matter (SOM). In each field, we randomly selected one of the two fertilized 10 m x 20 m-plots, where with an auger we randomly took five soil cores with a diameter of 3 cm and length of 15 cm. Prior to analysis, the five cores were combined to one soil sample. We did not sample the control plot without fertilization because we did not expect any effect of short-term N fertilization on SOM.

## Disease incidence

Prior to the start of the experiment, we made sure that the incidence of fungal diseases did not differ among the fields studied. In each field, we randomly selected one unfertilized and one fertilized 10 m x 20 m-plot. In each of the selected plots, we randomly selected 50 individuals of the crop plant and, on each crop plant, we randomly selected one leaf, which we inspected for fungal infections, i.e. Rust, Leaf Spot, Mildew and Fusarium. The investigations were carried out twice, once during stem elongation of the crop plant and a second time during fruit development. A generalized linear mixed model GLMM of the Poisson family was used to analyze the incidence of fungal diseases. Number of diseased leaves was the response variable, whereas tillage system and fertilization were included as fixed factors, and crop

biomass as independent variable. Type of crop, time of investigation, pair identification number and field identification number were considered as random factors. Results of the GLMM showed that there were no significant differences among the fields neither with regard to the incidence of fungal diseases nor with regard to any of the other variables considered in the model (p-value> 0.05).

## 2.3 Data analysis

Linear mixed models LMMs were used to test the main effects on ecosystem services of the different tillage systems (i.e. CT vs. CoT), landscape complexity and their interaction. As random effects, we included type of crop, pair ID and field ID. We verified that underlying assumptions of LMM with help of diagnostic plots of model residuals. Calculations were made with the "nlme" package (Pinheiro et al. 2009) in R (R Core Team 2013).

Regarding production service, we used grain yield as response variable, whereas tillage system, fertilization and their interactions were considered as fixed factors..

Regarding weed control service, we used both weed cover and species richness as response variables, whereas landscape complexity, tillage system, fertilization and their interaction (tillage system and fertilization) were included as fixed factors. In order to fulfill model assumptions, the variable weed cover was log transformed (x+0.01) prior to analysis.

Aphid control by ground predators (i.e. predation rate) and parasitoids (i.e. parasitism rate) was analyzed with two models, considering as response variables predation rate and parasitism rate, respectively. To meet model assumptions, parasitism rate was log transformed (x+0.01) prior to analysis. In both models, tillage system was used as fixed factor and landscape variables were considered as additional independent variables, whereas type of crop, counts, pair ID and field ID were considered as random effects. For the parasitism rate model, fertilization treatment was added as random factor.

Finally, soil quality was assessed considering SOM as response variable and tillage system as fixed factor.

By introducing years of CT as fixed factor into all the models, we avoided potentially associated biases.

## 3. Results

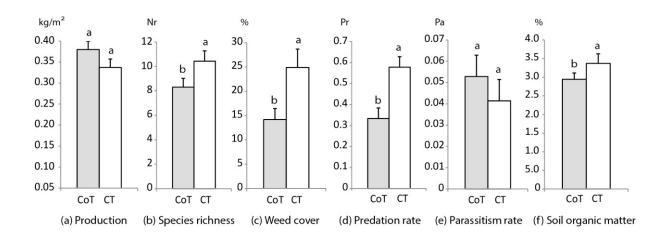
The results of LMM for provisioning services showed that grain production was significantly affected by the fertilizer treatment but not by the tillage system (Fig.2, Tab. 1). On fertilized

plots, 38% more grain was produced than on unfertilized plots (0.44 vs 0.27 kg/m<sup>2</sup> of dry weight of grain).

Regarding weed control service, a total of 91 weed vascular plant species were recorded, 63 in CoT and 76 in CT fields, respectively. The result of LMMs showed that tillage system had a significant impact on weed species cover and weed species richness. Under CT, species richness and cumulative cover of weed species were greater by 20.4% and 42.97%, respectively, than under CoT. (Fig.2). Landscape complexity at the different spatial scales considered and fertilization, by contrast, had no effect on weed species cover and weed species richness.

Regarding pest control by ground predators there was a strong effect of predators on aphid populations, which was greater under conservation tillage than under conventional tillage (Tab.1, Fig.2). Landscape complexity at the different spatial scales considered, by contrast, had no effect on aphid predation (Tab.1). Regarding the rate of aphid parasitism, however, the opposite was true. We found a significant positive impact of landscape complexity at the 1 km-scale, whereas the tillage system had no significant effect (Tab.1, Fig.2).

Finally, the results of LMM for soil quality service indicated a weak effect of tillage system on organic matter in the top soil to the advantage of conservation tillage (Tab.1) (Fig.2).



**Fig.2.** Impacts of conservation tillage (CT) vs. conventional tillage (CoT) on different ecosystem services. Mean and standard errors are given. Production service is expressed as dry weight of grain  $(kg/m^2)$  (a); weed control service as number of weed species (Nr) (b) and their cumulative cover (%) (c); pest control service is represented by predation rate (Pr) (d) and parasitism rate (Pa) (e), finally soil quality service is expressed by percent of soil organic matter (SOM) (f). Different letters above the paired columns indicate that CT and CoT are significantly different (p <0.05).

**Tab.1.** Results of LMMs. Given are the variables, the main effects and their interaction (if considered), the degrees of freedom (DF), F-value and p-value of each model. The variables of landscape complexity were presented only if significant.

Ecosystem service	Variable	Main effects	DF	F-value	p-value
Production	Grain	Tillage	12	2.52772	0.1378
		Fertilization	28	74.13785	<0.0001
Weed control	Cumulative weed cover	Tillage	13	4.98717	0.0437
		Fertilization	28	0.21914	0.6433
		Tillage:Fertilization	28	0.20097	0.6574
	Weed species richness	Tillage	13	5.33301	0.038
		Fertilization	28	1.88431	0.1807
		Tillage:Fertilization	28	3.05228	0.0916
Pest control	Aphid predation rate	Tillage	21	8.84051	0.0073
	Aphid parasitism rate	Tillage	25	0.04859	0.8273
		Percent of Crop	25	6.25005	0.0193
		Tillage:Percent of Crop	25	0.29065	0.5946
Soil quality	Organic matter in top soil SOM	Tillage	14	4.37271	0.0552

## 4. Discussion and conclusions

Conservation tillage had a positive effect on multiple ecosystem services demonstrating that there was no negative trade-off between provisioning service and various supporting ecosystem services. Analyzing the interactions between agricultural management and multiple ecosystem services provided useful information to find optimal synergies in the context of ecological intensification. Ecological intensification entails the environmentally friendly replacement of anthropogenic inputs and/or enhancement of crop productivity by fostering regulating and supporting ecosystem services (Bommarco et al. 2013). Our study elucidated the relationships between tillage system, landscape complexity and yield-related key supporting and regulating ecosystem services such as weed and pest control.

Regarding the provisioning services, grain yield was affected only by the fertilization treatments but not by the tillage system. It is well known that fertilizer applications increase production (Campbell et al., 2011) and that nitrogen is the most common nutrient used to

increase yield (Raun and Johnson, 1999). The effects of conservation tillage on crop yield have been extensively studied, and the studies produced conflicting results depending on conditions of soil, climate, type of conservation tillage and association with other agricultural practices (Edwards et al., 1988; Nyborg et al., 1995; López and Arrúe, 1997; De Vita et al., 2007). In our study, we found crop yield to be the same under conservation tillage as under conventional tillage did.

Regarding the regulating services, weed and pest control, respectively responded differently to the two tillage systems studied. Weed control was negatively affected by conservation tillage, i.e. cumulative cover and species richness of weed species were significantly greater than under conventional tillage. These findings are in line with other studies (e.g. Tolimir et al. 2006, Murphy et al. 2006, Demjanová et al. 2009). Recent studies have showed that floristic diversity may have a positive impact on the functioning of agroecosystems (Albrecht, 2003; Franke et al., 2009), such as pollination service (Gabriel and Tscharntke, 2007), e.g. by providing habitats for natural enemies of crop pests (Schellhorn and Sork, 1997) and by reducing the impact of diseases on the crop plants (Ratnadass et al., 2012). In our study, the fact that the cumulative cover of weed species was significantly greater under conservation tillage than under conventional tillage did not affect grain yield, even if no herbicides were applied. In contrast to the tillage system, landscape complexity and fertilization with nitrogen did not show any influence on the number and cumulative cover of weed species. Regarding landscape complexity, the same was found by other authors (e.g. Rew et al., 1996; Bischoff and Mahn, 2000), which explained their findings by suggesting that seeds of many arable weeds can spread only over small distances. In addition, they suggested that species richness and cumulative cover of weed species primarily depend on the local seed bank and on the management treatments rather than on the complexity of the surrounding landscape. According to the literature, fertilization with nitrogen generally fosters not only the growth of crop plants but also of weeds (e.g. Dhima and Eleftherohorinos, 2001). In our study, by contrast, we observed no such effect on the fertilized plots. This may be explained by the fact that nitrogen availability may be able to reduce weed interference with crops (Di Tomaso, 1995; O'Donovan et al., 2001). Natural pest control provided by ground predators and parasitoids presented contrasting results. This service is expected to be influenced by both crop management and landscape complexity (e.g. Tscharntke et al., 2007; Rush et al., 2010; Rush et al., 2013). In this study we found that aphids pest control by ground predators was much greater under conservation tillage than under conventional tillage. This is

consistent with the literature. Kendall (2003) and Holland (2004), for instance, report that abundance and diversity of ground-dwelling organisms tends to increase with decreasing tillage intensity, presumably because reduced disturbance renders the environment more stable (Altieri, 1999). Parasitism rate, on the other hand, was in our study significantly affected by the complexity of the surrounding landscape but not by the tillage system. Parasitism rate decreased significantly with decreasing landscape complexity, i.e. percentage of arable land in the surrounding landscape. Any increase in arable land leads to a reduction of natural and semi-natural habitats and the associated floristic diversity, which represents a key resource for parasitoids (e.g. Olson and Wäckers, 2007). Schmidt et al. (2003) demonstrated that both parasitism and predation by ground-dwelling organisms are very important to control aphid populations in crop fields. Our results support the hypothesis that natural pest control in agricultural ecosystems is supported by landscape complexity (Chaplin-Kramer et al., 2011; Rush et al., 2013), in particular, if combined with conservation tillage.

Regarding soil service, we found that conservation tillage affected soil organic matter SOM in the top 15 cm of the soil. This is in line with the literature. Some authors, however, report that reduced tillage systems may indeed lead to more SOM in the top soil but that this increase is accompanied by a decrease in SOM in the deeper soil layers (Puget and Lal, 2005; Baker et al., 2007). Several studies found that minimum and no tillage had more SOM only in the uppermost five centimeters of the soil, probably due to the effect of residue retention on the soil surface (Lal et al., 1990; Wanniarachchi et al., 1999; Puget and Lal., 2005).

In addition to the environmental also economic aspects of the different tillage systems should be taken into account since tillage is one of the most costly agricultural treatments with regard to both energy and labor. Therefore, a reduction in tillage intensity may lead to a better economic efficiency reducing the time and energy required for seedbed preparation (Kepner et al., 1978; Bonari et al., 1995; Košutić et al., 2005; Tabatabaeefar et al., 2009). In modern agriculture, it is necessary to identify and quantify the trade-offs between provisioning services (i.e. crop production) and regulating and supporting services such as pest and weed control and soil quality. In our study, conservation tillage, together with landscape complexity, proved to be a triple win type of management. First, the yield was the same as under conventional tillage. Second, it is cheaper than conventional tillage because it reduces the costs for fuel, labor and pesticides. Third, it is better for the environment because it improves the quality of the top soil as well as the floristic and faunistic diversity which, in

turn, are key for a variety of agriculturally relevant ecosystem services. Our findings, thus, strongly support the idea that conservation tillage is sustainable, both ecologically and economically. For generalized conclusions and recommendations, however, additional studies are needed, addressing other crops and other ecosystem services.

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## SUPPLEMENTARY MATERIALS

Pair identification number (id), tillage system (CT = Conservation tillage, CoT = Conventional tillage), distance between paired fields (m), percent of urban, semi-natural (i.e. woods, hedge-rows, meadows) and agricultural land use calculated in within a radius of 1 km from the field center of the 30 studied fields are showed.

Pair id	Tillage system	Years of CT	Field distance (m)	Urban	Semi-natural	Agricultural
1	СоТ	-		10.55	14.54	74.91
	CT	15	8	9.23	16.61	74.16
2	СоТ	-	6	29.72	10.87	59.41
	CT	20		37.94	10.22	51.83
3	СоТ	-	90	3.78	11.05	85.16
	CT	15		6.86	11.64	81.50
4	СоТ	-	4	18.78	5.82	75.40
	CT	10		19.31	5.89	74.80
_	СоТ	-	222	1.00	4.07	94.93
5	CT	7	320	0.15	14.54 16.61 10.87 10.22 11.05 11.64 5.82 5.89	96.11
	СоТ	-	222	10.79	3.33	85.89
6	CT	10	220	8.30	3.01	88.69
7	СоТ	-	500	1.29	5.58	93.13
	CT	10		6.12	4.48	89.40
8	СоТ	-	4000	2.73	44.95	52.32
	CT	5	1000	9.04	22.42	68.54
9	СоТ	-	1200	15.02	10.48	74.50
	CT	5	1200	1.57	17.10	81.32
10	СоТ	-	0	2.33	13.70	83.97
	CT	5	8	2.75	11.92	85.33
	СоТ	-	0	2.80	20.54	76.66
11	CT	5	3	3.33	20.54 20.85	75.82
12	СоТ	-	60	27.78	7.77	64.45
12	CT	10		28.23	7.69	64.08
13	СоТ	-	245	11.34	1.54	87.12
	CT	10		1.71	1.25	97.04
14	СоТ	-	422	1.47	2.32	96.21
	CT	10	430	1.88	3.66	94.46
15	СоТ	-	750	4.44	1.48	94.08
	СТ	10	750	0.74	7.62	91.64

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# Conservation tillage enhances natural pest control in winter cereals<sup>4</sup>

#### Abstract

Biological control (BC) of pests is an important ecosystem service and insects as natural enemies of pests have been estimated to be responsible for the 50-90% of the pest control in winter cereals. We conducted a field exclusion experiment in order to examine the relative importance of tillage system and landscape complexity on the BC of aphids provided by three different guilds of natural enemies in winter cereal crops, namely birds, flying insects/vegetation-dwelling predators and ground-dwelling predators. The experiment was undertaken on 15 pairs of fields (conventional tillage vs. conservation tillage) of winter cereals located in Friuli Venezia Giulia Region in North-Eastern Italy, selected along a gradient of landscape complexity. We hypothesized that (1) conservation tillage will enhance BC services as compared to conventional tillage; (2) each of the natural enemy guilds studied will influence the control of aphid population in winter cereals; (3) the level of BC services will increase with increasing landscape complexity. We found that conservation tillage supports greater overall BC of aphids in winter cereals than conventional tillage. Primarily this was due to ground-dwelling predators (carabids and arachnoids), whereas some of the other natural enemy guilds studied contributed little to aphid population control. Landscape complexity had a positive effect on parasitism only under conventional tillage but not under conservation tillage, mainly thanks to vegetation-dwelling predators and BC was higher in complex than in simple landscapes. Our results emphasize the importance of (i) considering both the tillage system and the landscape complexity when planning strategies for maximizing natural pest control in agroecosystems and of (ii) adopting a functional guild approach to reveal the hidden processes behind the provision of ecosystem services.

## **Keywords**

Natural pest control, conservation tillage, landscape complexity, winter cereal.

<sup>&</sup>lt;sup>4</sup>Tamburini G., De Simone S., Boscutti F., Sigura M., Marini L., 2014. *Conservation tillage enhances natural pest control in winter cereals*. Manuscript in preparation.

#### 1. Introduction

Biological control (BC) of pests is an important ecosystem service estimated to have an annual value of at least \$400 billion per year worldwide (Costanza et al., 1997). The insect natural enemies have been estimated to be responsible for the 50-90% of the pest control occurring in crop fields (Pimentel, 2005) saving \$4.5 billion per year in agricultural crops in the United States alone (Power, 2010).

A large body of evidences suggests that agricultural intensification is threatening natural pest control (Kleijn et al., 2009; Geiger et al., 2010; Winqvist et al., 2011; Bommarco et al., 2013). During the past 50 years, worldwide estimated crop losses to animal pests have increased significantly despite a steady increase in the use of chemical pesticides (Oerke, 2006). In a future where agriculture will face severe environmental, economic and social challenges (Foley et al., 2005; MEA, 2005), natural pest control could offer ecologically and economically very promising solutions.

Biological control depends on multiple factors acting from the field to the landscape scale (Tscharntke et al., 2007; Rusch et al., 2010). Various farming practices have been found to affect natural enemy communities and the associated pest control service. Organic farming, for instance, has often been shown to locally support greater BC than more intensive farming practices (Crowder et al., 2010; Wingvist et al., 2011). Little is known, however, how other key agricultural practices affected natural pest control (Rusch et al., 2010). In particular, little is known about the mechanisms linking agricultural soil management (e.g. crop rotation, soil tillage) and above-ground ecosystem services such as BC (Rusch et al., 2013).

Conservation tillage (CT) is a farming practice that includes all the techniques characterized by the non-inversion of soil combined with other management techniques such as cover crops, surface incorporation of crop residues and complex crop rotation. Globally, CT is practiced on about 45 million ha, mainly in North and South America but it is increasingly adopted in Europe and other parts of the world (Holland, 2004). CT is expected to minimize the negative impacts of farming operations and to improve soil structure, hydrology and biodiversity (Kladivko, 2001; Holland, 2004; Collette et al., 2011; Soane et al., 2012). Up to now, however, little is known about (i) how CT does really affect natural pest control and (ii) how landscape complexity does support the presumed positive effects of CT in that respect.

Landscape complexity is a key factor shaping natural enemy communities because complex landscapes with large proportions of semi-natural habitats provide a more stable environment than annual crops. Semi-natural habitats are crucial for (i) maintaining populations of alternative hosts and preys for parasitoids and predators, (ii) protecting natural enemies populations against crop disturbance, (iii) offering additional nectar resources during the vegetation period and (iv) shelter during winter (Denys and Tscharntke, 2002; Bianchi et al., 2006). A growing number of meta-studies have shown how complex landscapes support more diverse and abundant communities of natural enemies (Bianchi et al., 2006; Chaplin-Kramer et al., 2011). Only recently, scientists explored the effect of landscape complexity and farming practices on the effective BC provided by natural enemy communities (Winqist et al., 2011; Chaplin-Kramer and Kremen, 2012; Rusch et al., 2013). Up to now, however, the combined effects of tillage system and landscape complexity on BC in winter cereals have not been studied in detail.

In a field exclusion experiment, we examined the relative importance of tillage system and landscape complexity on the BC of aphids in winter cereals, taking into account three different guilds of natural enemies, namely: birds, flying insects/vegetation-dwelling predators and ground-dwelling predators. In winter cereals, the food web interactions contributing to the BC of aphid population include specialized aphid-suppressors such as parasitoids (*Hymenoptera*, *Parasitica*), larvae and adults of ladybirds (*Coccinellidae*), larvae of hoverflies (*Syrphidae*), larvae of lacewings (*Chrysopidae*), and more generalist predators, such as carabid beetles (*Carabidae*), rove beetles (*Staphylinidae*), web and cursorial spiders (*Araneae*) (Brewer and Elliott, 2004). The role of birds in controlling aphid pest populations, or in constraining BC by interguild predation, has been studied for several systems (Railsback and Johnson, 2014) but never for winter cereals.

This study is among the first experimental studies linking tillage system, landscape complexity, natural enemy communities and BC service provided by different guilds to aphid control in winter cereals. Using a design where landscape complexity and tillage system (conservation vs. conventional tillage) were orthogonal factors, we wanted to test three hypotheses. First, conservation tillage will enhance BC service and, in particular, BC provided by ground-dwelling predators. Populations of generalist predators such as carabid beetles, spiders and rove beetles, are expected to benefit from conservation tillage because the combination of reduced soil disturbance, increased surface residues and greater weed diversity provides a more suitable environment at multiple life stages (Ball et al., 1998; Kendall, 2003; Thorbek and Bilde, 2004; Soane et al., 2012). Second, all the natural enemy guilds considered will influence the control of the aphid population in the field. Third, BC

services will increase with increasing landscape complexity, which will act additively to or synergistically with the local tillage system.

#### 2. Materials and methods

## 2.1 Study area

The study was conducted between April and June 2014 on 30 winter cereal fields located in the agricultural landscape of the Udine province, NE Italy (46°10′08″N, 12°57′44″E to 45°46′18″N, 13°30′56″E). This region is an extensive lowland area, c. 615 km² in size, characterized by temperate climate with a mean annual precipitation of 1371 mm and a mean annual temperature of 13°C. Our sampling design consisted of 15 pairs of neighboring winter cereal fields. Within each pair, one field was subject to conservation tillage and the other to conventional tillage (distance between the two fields: 0-1,200 m). Field pairs were separated by at least 1 km except for two that were only 300 m apart. In autumn 2013, eight of the 15 field pairs were planted with winter wheat and seven with barley. On the fields under conservation tillage, the soil had not been converted for at least five years (mean: 9.8 years, range: 5 to 20 years). Conservation tillage management also coupled with cover crops between harvests, generally consisting of grass species. Under conventional tillage the seedbed was prepared by 30 cm deep moldboard plowing followed by one or two tills.

In each field, we identified a 60 m x 20 m strip adjacent to a semi-natural edge habitat of more or less identical structure and composition. Each strip was subdivided into six 10 m x 20 m plots, of which the outermost ones were considered as buffer zones. Among the four remaining plots, we selected randomly two non-adjacent plots for the exclusion experiment and the sampling of natural aphid enemies. Nitrogen fertilizer was applied to two plots following farming recommendations (80 kg/ha in two applications). No chemical pesticides and herbicides were applied.

## 2.2 Exclusion experiment

Exclusion treatments consisted of cylindrical cages (height: 1.5 m, diameter: 0.3 cm) designed to exclude combinations of three different guilds of natural enemies, namely (i) flying insects and vegetation-dwelling predators (F) (parasitoids, flying beetles, larvae of ladybirds, hoverflies, lacewings and web spiders), (ii) ground-dwelling predators (G) (carabid beetles,

cursorial spiders and rove beetles) and (iii) birds and other vertebrates (B). Flying insects and vegetation-dwelling predators were excluded using a fine polyester net (mesh size: 1 mm), birds and other vertebrates with an anti-bird net (mesh size: 1.5 cm).

In treatments excluding ground-dwelling predators, a plastic ring 0.3 m in diameter and 0.25 m in height was dug 10 cm deep into the soil and an 8 cm-wide band of insect glue was applied along the perimeter (SI Methods). One live pitfall trap was placed inside each plastic ring, which was regularly checked and emptied as needed during the experiment, i.e. between April and June 2014.

In the remaining exclusion treatments, access of ground-dwelling predators was guaranteed by fixing the nets 5 cm above the soil surface. An opening at the side of the cages, sealed with blinder clips, was used to examine plant material during the experiment. A total of six exclusion treatments were installed and randomly located within one of the fertilized 10 m x 20 m plots in each field.

Natural colonization of crop plants by aphids can be very irregular both in time and in space. Preliminary surveys showed comparable low densities of naturally occurring aphids among the fields, well below the economically relevant threshold. To avoid data bias due to differences in the initial aphid abundance, we inoculated field plants with aphids reared in the lab. 10 days before the inoculation, seven tillers of plant crop were selected for each treatment in order to standardize the plant material available to the aphids. The selected plants were therefore covered by non-woven fabric domes to exclude natural enemies that were removed by hand from each dome prior to the start of the experiment. Aphid material (Sitobion avenae) was provided by Katz Biotech AG® and directly placed on the plants (c.150 aphids per treatment, both adults and nymphs). Inoculation was done at the heading stage of the cereals (BBCH50-55) during good weather conditions (absence of precipitation and strong wind, minimum air temperature 18 °C). After 5 days, the established aphids were counted and plants were re-inoculated where needed (treatment with less than 15 aphids). 10 days after the first inoculation (= time 0), aphids were counted and exclusion treatments started. Aphids were recounted visually in each treatment 5 and 10 days, respectively, after the start of the exclusion experiment. In each field, for all the 6 exclusion treatments (-G, -B, -F-B, -G-B, -F-G-B and an open control O) and for each 5 day period, suppression of aphids due to natural enemies was quantified as the proportion of aphids predated in the exclusion treatment compared with the aphid population growth in the total exclusion treatment (-F-G-B), calculated following the methodology of Gardiner et al. (2009):

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

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

## 2.3 Sampling natural aphid enemies

In each field, the second fertilized plot was used for the sampling of natural aphid enemies. Vegetation-dwelling predators were visually monitored once, 3 days after the onset of the experiment. The sampling was conducted along two 20 m transect inspecting 50 randomly chosen tillers of the crop plant. The combined number of flying beetles, web spiders, larvae of hoverflies, ladybirds and lacewings, respectively, was expressed as total number of individuals per 100 tillers of the crop plant. Ground-dwelling predators were caught with three plastic pitfall traps in fertilized 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 and protected against rain by plastic roofs fixed with nails to the soil. The first sampling period coincided with the 10 days of the exclusion experiment, the second lasted for the following 10 days. Invertebrates were preserved in 70% ethanol. Per field and sampling period, the catches of the three pitfall traps were combined and the numbers of individuals of carabid beetles, rove beetles and cursorial spiders were summed-up. During the second sampling period, the pitfall traps in one field were destroyed. The analyses regarding the

pitfall catches were, therefore, based on data from 30 and 29 fields for the first and second sampling period, respectively.

## 2.4 Analysis of landscape complexity

Landscape complexity was assessed based on regional land use maps aerial photographs with help of ArcGIS 9.3. We distinguished six types of land use, namely (i) arable fields, (ii) urban settlements and the semi-natural types (iii) woods, (iv) natural water bodies, (v) meadows and (vi) hedgerows. Around each of the 30 fields studied, landscape complexity was assessed for eight concentric circular plots with radii of 95, 135, 190, 265, 375, 530, 750 and 1000 m, respectively. As mentioned above, field pairs were selected along a gradient of landscape complexity. Regarding the circles with a radius of 1000 m, percentage of semi-natural habitats, i.e. woods, natural water bodies, meadows, hedgerows, ranged from 1.25% to 44.95%. Tests with Linear Mixed Models showed that the percentages of arable fields, semi-natural areas and urban settlements did not differ between the two tillage systems considered (p-values = 0.48, 0.70 and 0.76, respectively).

## 2.5 Data analysis

BC index, aphid parasitism, predator number and the relationship between BC index and predator number were analyzed using general linear mixed-effects models (lme, 7 models) and generalized mixed linear model (glmer, 1 model) (Tab.1). We first built full models and simplified them subsequently by removing variables one-by-one, starting with interactions, followed by not-significant terms. We used traditional analysis based on p-values due to the very low colinearity among our variables.

**Tab.1.** Characteristics of models used in the analyses (exclusion treatment, tillage system; counting (or sampling), field pair, plot, crop type.

n.	Transf.	Model distribution	Response variables	Explanatory variables	Random effects
1	-	Normal	BC index	Exclusion treatment	Counting
				Tillage system	Crop type
				% semi-natural habitats	Pair
					Plot
2	log (y+0.01)	Normal	Parasitism	Tillage system	Crop type
				% semi-natural habitats	Treatment
				n. of vital aphids	Pair
					Plot
3,4,5	log (y+1)	Normal	Ground-dwelling	Tillage system	Sampling
			predators number: - carabid beetles	% semi-natural habitats	Crop type
			<ul><li>cursorial spiders</li><li>rove beetles</li></ul>		Pair
6	-	Poisson	Vegetation-dwelling	Tillage system	Crop type
	predators number		% semi-natural habitats	Pair	
7	-	Normal	BC index (Vegetation-	Predator : prey ratio	Crop type
	dwelling predators)			Treatment	
8	-	Normal	BC index (Ground- dwelling predators)	Predator : prey ratio	Crop

BC index (model 1) was calculated for each 5-day-period of the exclusion experiment (from time 0 to the 5th day and from the 6th to the 10th day) for each exclusion treatment. Two outliers, were excluded from the analysis. Tukey multiple comparison test was applied to determine significant differences among exclusion treatments.

Prior to analysis, parasitism and number of ground-dwelling predators were log-transformed to achieve normal distribution of residuals. Number of carabid beetles, rove beetles and cursorial spiders were analyzed, separately (models 3, 4 and 5, respectively). Because of the large amount of zeros in the data, records of ladybirds, hoverflies and web spiders were pooled to "vegetation-dwelling predators" and analyzed with a generalized mixed linear model with a poisson distribution (model 6). To test the relationship between

number of natural enemies and the BC recorded during the exclusion experiment, we calculated the total BC index (from time 0 to the 10th day) and the predator: prey ratio (Thies et al., 2011). For vegetation-dwelling predators (model 7) the ratio was calculated as the number of predators recorded divided by the number of live and parasitized aphids counted in each treatment at the end of the exclusion experiment. BC index data came from both -G and -G-B exclusion treatments since we found no significant difference in aphid predation between the two (see results). For ground-dwelling predators (model 8) the ratio was calculated as the number of predators caught in the pitfall traps after 10 days divided by the number of aphids counted in each treatment (-F-B). For both vegetation- and ground-dwelling predators the predator: prey ratio was log-trasformed (Brose et al., 2006). Where the variable landscape complexity was included in the models, analyses were performed at all spatial scales considered. In the results, however, we presented only the spatial scale with significant main effects and interactions. The analyses were performed using the "nlme" and "lm4" packages (Pinheiro et al., 2013) implemented in R Statistical Software 3.1.1 (R Development Core Team, 2013).

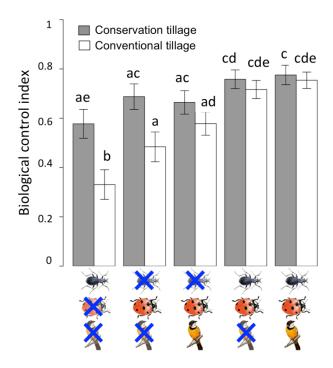
### 3. Results

An average of  $57 \pm 49$  aphids successfully colonized the plants in each treatment. At the beginning of the experiment (time 0), aphid numbers were the same in all the combinations of exclusion treatments and tillage systems. Contributions of natural enemy guilds to BC were found to be affected by both tillage system and landscape complexity (Tab.2).

**Tab.2.** Results mixed effects models relating BC index, parasitism, predators number, the relationship between BC index and predator number, respectively, to explanatory variables. Explanatory variables are exclusion treatment (five levels of natural enemy exclusion), tillage system (conservation or conventional) landscape complexity (% semi-natural habitats in a radius around fields) and their interactions. dDF = denominator degrees of freedom; nDF = numerator degrees of freedom.

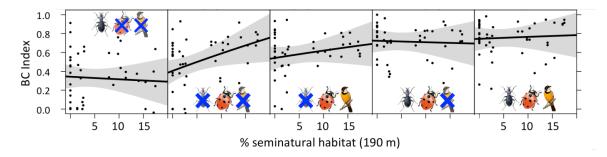
Models and explanatory variables	nDF	dDF	Test	P-value
BC index (model 1)			F	
Exclusion treatments	4	174	18.98	< 0.001
Tillage system	1	20	10.14	0.004
% semi-natural habitats (190 m radius)	1	20	1.95	0.177
Exclusion treatment x tillage system	4	174	2.61	0.036
Exclusion treatment x % semi-natural habitats (190 m radius)	4	174	2.56	0.039
Parasitism (model 2)			F	
nr. of aphids	1	33	2.86	0.099
Tillage system	1	33	7.75	0.008
% seminatural habitats (750 m radius)	1	33	0.54	0.466
Tillage system x % semi-natural habitats (750 m radius)	1	33	4.72	0.037
Ground-dwelling predators abundance			F	
carabid beetles (model 3)				
Tillage system	1	26	5.98	0.021
cursorial spiders (model 4)				
Tillage system	1	26	10.28	0.003
rove beetles (model 5)				
Tillage system	1	26	0.13	0.713
Vegetation-dwelling predators abundance (model 6)			Deviance	
Tillage system	1	-	-2.81	0.004
BC index (vegetation-dwelling predators) (model 7)			F	
Predator: prey ratio	1	39	6.30	0.001
			2.00	
BC index (ground-dwelling predators) (model 8)			F	
Predator : prey ratio	1	10	8.21	0.003

Overall predation of aphids was 11% higher under conservation tillage than under conventional tillage. Exclusion of birds and other vertebrates did not affect BC. In the fields under conventional tillage, the exclusion treatments -B, -G, -B-G and -F-B lead to reductions in the BC index by 1.3%, 11.5%, 8.2% and 20.5 %, respectively, than in the associated control treatments -O (Fig.1).



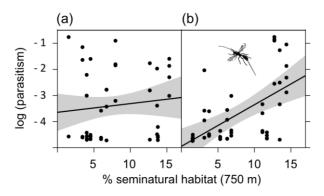
**Fig. 1.** Effects of the exclusion of different guilds of natural enemies on the BC index (mean  $\pm$  1 SE) in combination with conservation tillage (grey bars) and conventional tillage (white bars), respectively. Columns sharing the same latter are not significantly different (adjusted P values < 0.05). Crossed-out symbols indicate the exclusion of corresponding guild. Guilds of natural enemies are: flying insects and vegetation-dwelling predators (ladybird symbol); ground-dwelling predators (beetle symbol); and birds and other vertebrates larger than 1.5 cm (bird symbol).

In the fields managed with conservation tillage, BC index was 4.2%, 16.9%, 24.6% and 43.0 % lower in the treatments -B, -G, -B-G and -F-B respectively, compared to -O. Only the BC provided by ground-dwelling predators (-F-B) significantly differed between tillage systems, being 22.6% higher in fields under conservation tillage. Vegetation-dwelling predators showed only an indication of higher predation in conservation tillage fields (p=0.105, from Tukey multiple comparison test). BC index best responded to landscape complexity within a radius of 190 m. From simple to complex landscapes, BC index increased only in treatments excluding ground-dwelling predators (interaction exclusion treatment x % semi-natural habitats, Fig.2). This indicates that the BC was higher in complex than in simple landscapes mainly for vegetation-dwelling predators, for which the BC index increased from  $\sim$  0.4 in simple landscapes to  $\sim$  0.7 in complex landscapes. For ground-dwelling predators, on the contrary, BC index did not show significant variation along the gradient of landscape complexity. Regarding the BC index we found no significant interaction between tillage system and landscape complexity.



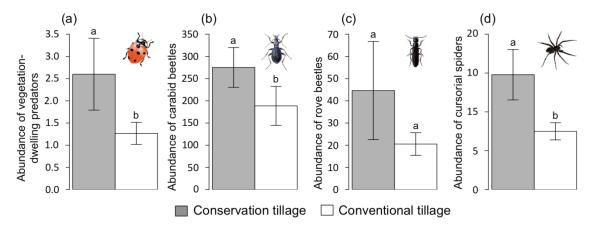
**Fig. 2**. Effects of excluding natural enemy guilds along a gradient of landscape complexity (% of semi-natural habitats in a circle with a radius of 190 m) on BC index. Interaction of exclusion treatments and % semi-natural habitats was significant (Tab.2) Confidence intervals (0.95) is also shown (grey-shaded area).

Parasitism was found to be significantly higher in fields under conservation tillage than under conventional tillage. Regarding landscape complexity, we found a significant interaction between tillage system and % semi-natural habitats. Under conventional tillage, parasitism increased with increasing landscape complexity (in a radius of 750 m), whereas no effect was observed under conservation tillage (Fig.3).



**Fig. 3.** Effect of landscape complexity (% of semi-natural habitats in a 750 m radius) on log-transformed parasitism in fields under (a) conservation and (b) conventional tillage. Confidence intervals (0.95) is also shown (grey-shaded area).

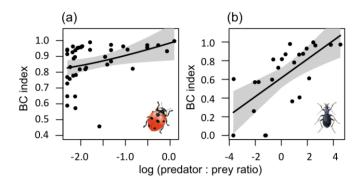
During the monitoring of vegetation-dwelling predators a total of 5 ladybirds, 3 larvae of hoverflies and 50 web spiders were recorded. The total number of vegetation-dwelling predators was significantly greater on fields under conservation tillage (an average of 2.6 individuals per 100 tillers, SE = 0.8) than on those under conventional tillage (1.2, SE = 0.2) (Fig. 4). Landscape complexity, however, did not affect the number of vegetation-dwelling predators.



**Fig. 4.** Number (i.e. abundance) of (a) vegetation-dwelling predators, (b) carabid beetles, (c) rove beetles and (d) cursorial spiders in response to tillage system (grey bars, conservation tillage; white bars, conventional tillage). Number of vegetation-dwelling predators was expressed as the total number of individuals flying beetles, web spiders, larvae of hoverflies and ladybirds observed per 100 tillers of the crop plant. Number of ground-dwelling predators referred to the two periods of pitfall trap sampling (10 days each). Different letters above the two columns of the graphs a, b, c and d indicate a significant difference between the two tillage systems at P < 0.05.

With pitfall traps a total of 13641 carabid beetles, 1910 rove beetles and 654 cursorial spiders were caught during the two 10-day sampling periods. The numbers of carabid beetles and cursorial spiders were significantly affected by tillage system. Under conservation tillage, their numbers were significantly greater than under conventional tillage (carabid beetles: 275.2 (SE = 44.7) vs. 188.9 (SE = 45.5); cursorial spiders: 14.8 (SE = 3.2) vs.7.5 (SE = 1.1) (Fig.4). Rove beetles, on the other hand, were not affected by the tillage system. Landscape complexity, however, did not affect the number of ground-dwelling predators.

The BC index correlated positively with the predator: prey ratios for both the vegetationand the ground-dwelling predators. Positive relation was steeper for ground-dwelling predators (Fig.5).



**Fig. 5.** Relationship between BC index and log-transformed predator: prey ratio for (a) vegetation-dwelling predators and (b) ground-dwelling predators. Confidence intervals (0.95) is also shown (grey-shaded area).

#### 4. Discussion and conclusions

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

Conservation tillage was found to support a higher overall BC (11% higher than under conventional tillage) and more abundant arthropod communities. In particular, both abundance and predation by ground-dwelling predators were significantly higher under conservation tillage (by 35 and 24% respectively) as compared to conventional tillage. Considering the significant differences observed for ground-dwelling predators (model 1, Tukey multiple comparison test, p = 0.004) and for the overall BC (model 1, principal effect of tillage system, p = 0.004), this simply indicates that small populations of aphids are equally well controlled under both conservation and conventional tillage management when they are accessible to all the guilds of natural enemy. Our results generally support the first hypothesis showing that a soil management that limits the detrimental effects of farming practices on soil enhances predator abundance and consequently the BC service provided.

Our findings confirm previous studies showing a response of ground-dwelling arthropods to within-field habitat quality in general and, specifically, to conservation tillage. For instance, more abundant ground beetle communities were found in fields under conservation tillage compared with conventional ones (Cárcamo, 1995; Kladivko, 2001). Conventional tillage was shown to affect carabid populations directly by mechanically injuring or killing individuals (Holland and Luff, 2000) and indirectly by degrading habitat quality and alternative prey availability (Hance, 2002; Holland, 2004). Moreover, surface residue cover is important for maintaining soil moisture and temperature conditions suitable for the survival and development of the numerous carabid species that spend their larval stage in the soil (Cochran et al., 1994). Holland and Reynolds (2003) showed that spiders are affected by tillage management as well. The compared to conventional tillage more stable soil environment and higher weed density associated with conservation tillage create a deeper

layer of litter and a structurally more complex vegetation, ideal for spiders (Rypstra et al., 1999; Holland, 2004). This could also explain why we found vegetation-dwelling predators to be more abundant in fields managed with conservation tillage since, in our study 86% of the vegetation-dwelling predator specimens were web spiders. Diehl et al. (2013) have also reported that web spiders respond negatively to tillage intensity and positively to vegetation complexity. In our study, the number of rove beetles was not affected by the tillage system. Kroos and Schaefer (1998) reported the same with regard to the overall abundance of rove beetles, even though they found tillage systems to affect species composition.

In accordance with previous studies that followed similar methodologies (Schmidt et al., 2003; Thies et al., 2011), we found vegetation-dwelling predators to be more effective for aphid control than ground-dwelling predators. Contrary to our expectations (second hypothesis), however, birds and other vertebrates did not contribute to control the aphid population since their exclusion did not lead to any significant differences in the BC index. In conclusion, therefore, our study suggests that birds and other vertebrate may not be important for aphid control in cereal crops, even though some bird species are known to consume aphids as alternative prey (Cowie and Hinsley, 1988; Snow and Perrins, 1998; Naef-Daenzer et al., 2000; Eeva et al., 2009). Birds have shown to play an important role in the provision of BC service in tropical agroecosystems (Karp et al., 2013; Railsback and Johnson, 2014). An explanation of our findings could be that the structure of these crops in North-Easternern Italy does not allowed birds to easily locate and hunt their prey.

In our study, the positive correlation between the BC index and the predator: prey ratio for both vegetation- and ground-dwelling predators strengthened the link between the predator sampling and the exclusion experiment, showing higher BC in those fields where predators were more abundant. Vegetation-dwelling predators provided high BC index also in fields where few predator individuals were sampled. Indeed many vegetation dwelling predators are commonly recognized as aphid specialists (e.g. ladybird and hoverfly larvae) able to control aphid populations even at low densities. On the contrary, the diet of ground-dwelling predators (e.g. carabid and rove beetles) comprises a much wider number of species leading to a less effective aphid control even if their populations are large (Brewer and Elliot, 2004). Furthermore, the efficiency of ground-dwelling predators is also constrained by their limited mobility (Winder et al., 2005). Web spiders in winter wheat fields, on the other hand, have been demonstrated to have a narrower diet compared to cursorial spiders (Nyffeler et al., 1999) and to greatly rely on aphids for their sustenance (Harwood et al., 2004).

In our study, landscape complexity positively affected the BC provided by the vegetationdwelling predators in a radius of 190 m. This supports the hypothesis that semi-natural habitats in the landscape benefit the BC by providing more mobile natural enemies, which is consistent with recent studies from different agroecosystems (Gardiner et al., 2009; Chaplin-Kramer et al., 2011; Holland et al., 2012; Rush et al., 2013). Surprisingly, we did not find any effect of landscape complexity on the abundance of vegetation-dwelling predators. This could be partially due to the sampling method used, underestimating the actual presence in the field of the more mobile predators such as ladybirds. Other authors have demonstrated that ladybirds respond positively to landscape complexity regarding both their abundance and their predation (Bianchi et al., 2004; Gardiner et al., 2009). In our study, neither abundance nor the BC by ground-dwelling predators were influenced by landscape complexity at any of the spatial scales considered. Several studies showed that landscape complexity is important for the populations of natural enemies (Chaplin-Kramer et al., 2011). Other authors, however, showed that different functional groups or species may respond differently to landscape complexity (Purtauf et al., 2005; Schmidt and Tscharntke, 2005; Shackelford et al., 2013). As regard BC, the effect of the landscape complexity on ground-dwelling predators may have been mediated by other factors such as seasonality or pest abundance. Östman et al. (2001), for instance, showed how both BC provided by ground-dwelling predators and aphid establishment depended on landscape complexity only early in the season.

In our study, parasitism was found to be enhanced by high proportion of semi-natural habitats in the landscape only on the fields under conventional tillage. On fields under conservation tillage, by contrast, the parasitism was significantly higher than under conventional tillage and consistently independent of landscape complexity. Parasitoids are known to profit from semi-natural habitats in the landscape owing to higher availability of (i) overwintering sites, (ii) shelters from disturbances caused by farming practices and (iii) more diverse and abundant food sources (Thies et al., 2005; Tscharntke et al., 2007; Thies et al., 2011; Rand et al., 2012). Moreover, floral nectar has been shown to be an important component of the diet of adult parasitoids, and that its availability in the landscape may influence parasitism (Lavandero et al., 2005; Rusch et al., 2010; Araj et al. 2011). This may explain why, in our study, fields under conservation tillage showed higher parasitism than on fields under conventional tillage. Several studies in fact reported non-conventional tillage (conservation, reduced or no-tillage) to increase weed abundance and diversity (Holland, 2004; Soane et al., 2012), which may provide an important within field food resource. This

may explain why landscape complexity in our study did not affect the parasitism on fields under conservation tillage.

In conclusion, our study provides evidence for the positive effects of both tillage system and landscape complexity on the biological control of aphids in cereal crops and on the contributions of different guilds of natural aphid enemies. We found conservation tillage to support more abundant arthropod communities and higher overall BC than conventional tillage. The impact of the tillage system was particularly pronounced with regard to the ground-dwelling predators, whereas landscape complexity proved to be particularly important for the vegetation-dwelling predators. Surprisingly, landscape complexity had a positive effect on parasitism on fields under conventional tillage but not on those under conservation tillage. Our results emphasize that it is important (i) to consider both tillage system and landscape complexity when planning strategies for maximizing BC service in agroecosystems, and (ii) to adopt a functional guild approach to reveal hidden processes behind the provision of ecosystem services.

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6

# **Overall conclusions**

The aim of this research was to provide a contribution to the study of agricultural landscape sustainability through ecological intensification concept. Ecological intensification advocates to maintain or enhance agricultural production through the promotion of biodiversity and associated ecosystem services. In our research the complexity wherewith these processes occur in the cultivated landscape was addressed through a multiscale approach considering both the landscape and the local (field) scale.

In particular, for biodiversity and exotic plant spread, landscape approach was used to obtain a comprehensive view of the processes. This provided insights into both landscape and species diversity and suggests a theoretical and practical basis for conservation planning. Field scale approach was applied for the assessment of tillage management on ecosystem services, considering also the effects of landscape context on several studied ecosystem services (i.e. weed control and natural pest control).

An important result was to define a profitable method for the assessment of landscape quality in terms of its own potential functionality (i.e. landscape functional quality) integrating landscape biodiversity assessment with landscape sensitivity assessment to obtain a real representation for a highly disturbed landscape as the agricultural landscape. Obviously the relation between biodiversity and ecosystem functions could not only depend on species richness of plant *taxon*. However, our approach to biodiversity assessment seem to be a good proxy for the assessment of landscape functional quality in agricultural landscape, because it assess the general capacity of landscape to guarantee several landscape functions also considering landscape sensitivity to disturbance. InVEST model was revealed to be a good tool to the presentment (model) of landscape functional quality at the landscape scale and CDA analysis gave us several evidences on relations between landscape functional quality and the coexistence of specific landscape pattern characteristics. These evidences consist in:

high abundance in semi-natural habitat types, large surface area of individual patches of seminatural habitats, high heterogeneity and high connectivity. The results highlighted the importance of particular semi-natural habitat types in agricultural landscape (i.e. meadows and woods): promoting presence and structure of these habitats respect others (e.g. hedgerows) can lead a greater improvement of agricultural landscape functional quality and therefore landscape sustainability.

Our study on exotic plant invasion confirmed the importance of composition and configuration of landscape. Different habitats reacted differently to landscape disturbance (i.e. quantity of disturbance and proximity to disturbance) in relation to spread of exotic plants. Therefore, in landscape planning, the choice of particular semi-natural habitats respect others may more contribute to enhance the biodiversity and reduce the threat given by the spread of exotic species. This is a further confirm that appropriate land use management can lead to a lower diffusion of exotic species.

Therefore, in the context of ecological intensification, the evidences emerged at landscape scale highlight the importance of landscape structure to improve landscape functionality due to biodiversity, to reduce exotic plant invasion and hence to promote sustainability.

However, further studies are needed to implement conservation planning strategies for cultivated landscapes. Our results supplied general evidences to promote biodiversity, but did not supply quantified guidelines by the definition of thresholds for habitat composition and configuration.

At local scale, regarding ecosystem services, our results showed that conservation tillage and landscape complexity affected ecosystem services in different ways. Production service did not show differences between tillage system, soil quality was favored under conservation tillage and pest control was enhanced in conservation tillage and in high landscape complexity context, whereas weed control services was penalized, but gained in weed diversity. In step with ecological intensification aims, we can claim that conservation tillage had proved to be win-win management practice oriented to the promotion of ecosystem services: performed as well as conventional tillage and, together with landscape complexity, enhanced pest control and weeds diversity with less use of pesticides and environmental benefits as results. It must be said that further studies should be focused on the integration of different types of agronomic practices and solutions of landscape organization and multiple ecosystem services are needed to reach a conclusion about the ecological and economic conditions that may lead to tradeoffs between agricultural production and ecosystem services.

In fact, management aimed at enhancing one ecosystem service may negatively affect other ecosystem services. For example, conservation tillage leads to enhance the natural pest control service, but at the same time decrease weed control service. It must also consider that an optimal management strategy could depend upon geographical location of the farm, cropping system, soil type and landscape structure. This gaps in knowledge are still largely unexplored and should be investigated.

In conclusion, agricultural landscape management finalized to the improvement of ecological intensification is possible, ensuring production and environmental sustainability. The research highlights the need to join the application of environmentally sustainable cultivation practices combined with a thoughtful landscape planning mindful of semi-natural habitat type and their configuration in the pattern.

Evidences provided by our research, can be a useful instrument for stakeholders and decision makers involved in the cultivated landscape planning, such as the Rural Development Programme (RDP) financed by The Common Agricultural Policy. In this context, our results can support the definition or the improvement of recommendations for the agro-environmental measures aimed to the increase of environmental value through the protection of landscape and biodiversity and to the reduction of pressure due to agriculture.

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