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Biodiversity management for integrating conservation and production in modern agricultural systems

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Alla mia famiglia
e a chi mi ha spronato

Abstract

The conservation of biodiversity is one of the primary concerns when dealing with agro-ecosystems, which other than providing food and fodder resources, can benefit of high levels of ecological functioning. The multifunctionality of agro-ecosystems has to be assessed in a multidisciplinary way and new methodologies should be developed to tackle it, at different spatial and temporal scales. The multi-scale approach is useful to delineate an overall view of the ecosystem and productive services of agricultural areas. This manuscript presents three researches dealing with the conservation of biodiversity, with a view to agricultural production, that were carried out in three different contexts of interest. Such a choice was intended to permit the application of new techniques and to adapt existing ones to different spatial and temporal scales.

The first context was chosen to delineate a new methodology for conserving semi-natural water resources in an highly fragmented landscape scenario, the district of Milan. In particular, one of the key elements of the territory is that of *fontanili*, semi-natural plain springs that represent both strong water resources for agriculture and the remnants of ancient green zones, but are more and more affected by urbanization rates and intensification of agricultural practices. They are listed as habitats for some endangered species, mainly aquatic, and conservation strategies are needed to preserve their ecosystem services. We chose to rely on landscape ecology analyses, which are able to assess how ecological corridors are structured between sources and sinks of biodiversity, represented by *fontanili*. We developed a new indicator, called Fuzzy Functionality Index (FFI), that for the first time collimates two of the more common types of analysis: the structural analysis of landscape fragmentation, and the assessment of species-specific permeability to movement. The index, resulting from a participative process, was at the basis of the spatial assessment of ecological corridors between *fontanili* and has proven to be highly effective and very flexible. It permits one to assemble geographic data, the knowledge of a multidisciplinary team and open source software to obtain a simple-to-read, mapped index at virtually no cost, eliding the issues of the traditional methods.

The second context is that of a rural livestock farming district at high altitudes, in the Alps of Northern Italy. Alpine meadows have been exposed to profound management shifts in modern times: changes in plant species composition and biodiversity losses are widespread issues. The aim of the work was to inspect how the variability of meadows could be explainable by the environment they depend on and by the management strategies applied. We analyzed the plant species composition, biodiversity and forage value of meadows in the

context and their relationships with environmental and management variables, collected among the farmers. The management variables explained a small amount of variance: only the number of cuts per year remarkably explained the plant species composition and biodiversity. The number of cattle and the field applied nitrogen only described the most intensively managed communities. The environmental variables better described the variability of responses: in particular, an increase of the Landolt Nutrient Index was associated with an increase of the forage value opposite to a decrease of the Shannon Index. The negative correlation between the two responses highlights a known dilemma referring to high altitude meadow communities, which are subjected to important environmental constraints. Some *taxa* as *Anthriscus sylvestris*, *Heracleum sphondylium*, *Rumex acetosa* and *Polygonum bistorta* were found to critically unbalance the species composition thus the overall biodiversity. This is certainly the most critical finding, explainable by the late first cuts commonly adopted and by long-term intensive management choices. Homologated management strategies could not explain the wide ecological variability investigated, but indeed they made possible to understand how the system should be deeply revised, in respect to limiting environmental constraints and fodder capabilities at high altitudes.

The third context is that of an agronomic field experiment carried out over a long period. The work compares biomass, Milk Feed Units (MFU) and Crude Proteins (CP) yields, over a period of 21 years (1986-2006), referring to five fodder cropping systems: (i) a one-year double-crop rotation (R1) of autumn-sown Italian ryegrass + spring-sown silage maize; (ii) a three-year rotation (R3) of grain maize (first year), autumn-sown barley + silage maize (second year), and Italian ryegrass + silage maize (third year); (iii) a six-year rotation (R6) of Italian ryegrass + silage maize (years 1,2,3) + mixed meadow of white clover and tall fescue (years 4,5,6); (iv) a continuous grain maize (CM); and (v) a permanent meadow (PM). All cropping systems were subjected to two levels of agronomic inputs: high (A), indicating the amounts of fertilizers and herbicides normally applied by farmers in the region, and low (B) consisting in reduced amounts of fertilizers (-30%) and herbicide rates (-25%) compared to A. We found R1 > R3 > R6 > PM > CM in terms of biomass yields, with a slightly different trend for MFU yields, whereas R6 > R1 > PM > R3 > CM regarding CP yields. The two treatments always resulted A > B. The five cropping systems significantly varied between the 21 years of experiment: all of them showed decreasing performances except for PM, improving in biomass, MFU and CP over time. The three rotations (R1, R3, R6) appeared the most stable cropping systems over time. These findings suggest the importance of complex cropping systems, which could provide high quality of fodder besides guaranteeing a remarkable agricultural diversification.

Riassunto

La conservazione della biodiversità è uno degli aspetti prioritari nei riguardi degli agro-ecosistemi, che, oltre a garantire la produzione di risorse alimentari e foraggere, beneficiano di alti livelli di funzionalità ecologica. La multifunzionalità degli agro-ecosistemi dev'essere analizzata attraverso metodologie multidisciplinari e comunque moderne, allargate alle differenti scale spaziotemporali. L'approccio multi-scala è utile nel delineare una visione d'insieme delle prerogative di conservazione e produzione delle aree agricole. Questo manoscritto vuole presentare un'analisi congiunta della conservazione della biodiversità e della produzione agraria in tre contesti di interesse, scelta, questa, accompagnata dall'applicazione di tecniche d'indagine a scale spaziali e temporali diversificate.

Il primo contesto è stato scelto per delineare una nuova metodologia applicata alla conservazione della biodiversità delle risorse idriche semi-naturali, in provincia di Milano, paesaggio fortemente frammentato. Uno degli elementi chiave del territorio è quello dei fontanili, risorgive semi-naturali della pianura, fonti di acqua irrigua ed elementi residuali del verde, sempre più colpiti dall'urbanizzazione e dalle attività agricole intensive. I fontanili sono, oltretutto, habitat di specie a rischio, principalmente acquatiche, perciò necessitano di azioni conservazionistiche molto forti e indirizzate. Abbiamo scelto di operare nell'ambito della *Landscape ecology*, disciplina capace di indagare i corridoi ecologici esistenti tra "fonti" e "pozzi" di biodiversità. Abbiamo sviluppato un nuovo indice chiamato *Fuzzy Functionality Index* (FFI) capace di collimare l'analisi della frammentazione territoriale e quella, prettamente specie-specifica, della permeabilità al movimento delle specie. L'indice è basato su un processo partecipativo ed ha permesso di studiare l'allocazione dei corridoi ecologici tra fontanili in modo estremamente flessibile. Esso permette, infatti, di unire dati cartografici, le conoscenze di un gruppo multidisciplinare di esperti e programmi *open source* per restituire un quadro paesaggistico realistico a costo zero, limitando notevolmente le problematiche dei metodi tradizionali del settore.

Il secondo contesto in analisi è quello di un distretto agricolo rurale ad alta quota, nelle Alpi dell'alta Valtellina. I prati Alpini sono stati teatro di cambiamenti radicali nei tempi moderni, mostrando cambiamenti nella composizione specifica e conseguente perdita di biodiversità. Necessità primarie per garantire la continuità dell'attività agricola nelle zone marginali sono indubbiamente la conservazione dei cotici e la loro gestione sostenibile. L'obiettivo dello studio era quello di analizzare una grande varietà di prati condotti da allevatori bovini ed indagare l'effetto delle variabili ambientali e gestionali sulla loro composizione specifica, biodiversità e capacità produttiva. Le variabili gestionali, ottenute attraverso questionari, non sono risultate idonee nello spiegare la varianza dei prati, in

generale. Soltanto il numero di tagli per anno ha potuto esprimere parte della variabilità floristica e della biodiversità dei cotici. La consistenza zootecnica e l'azoto al campo hanno spiegato invece la variabilità delle comunità eutrofizzate, mentre le variabili ambientali sono risultate significative nella maggior parte dei casi. In particolare, un aumento dell'indice dei nutrienti di Landolt è associato a un aumento del valore foraggero e, all'opposto, ad un decremento dell'indice di Shannon. La relazione negativa tra valore foraggero e indice di Shannon è comune nelle tipologie di prato analizzate e indica una nota contrapposizione tra la capacità produttiva e quella conservativa, tipica delle comunità vegetali di alta quota, essendo esse soggette a vincoli ambientali molto restrittivi. Alcuni *taxa* come *Anthriscus sylvestris*, *Heracleum sphondylium*, *Rumex acetosa* e *Polygonum bistorta* sono stati rilevati con forti abbondanze, che pregiudicano il bilanciamento delle specie nel cotico e la biodiversità stessa. Questo fenomeno è certamente interessante e conseguenza della procrastinazione del primo taglio e di scelte gestionali intensive. Le variabili gestionali analizzate, pur non avendo spiegato in modo soddisfacente la variabilità floristica, hanno permesso di capire come l'intero sistema rurale in esame debba essere rivisitato, in virtù dei severi vincoli ambientali a cui è soggetto questo contesto ad alta quota.

Il terzo ed ultimo contesto è quello di un esperimento agronomico di lunga durata a scala di campo, nella pianura lodigiana. Il lavoro compara le rese in biomassa, unità foraggere latte (UFL) e proteine grezze (PG) di cinque sistemi colturali foraggeri, in un lasso temporale di 21 anni. I sistemi colturali sono così formulati: (i) una rotazione annuale di loiessa e mais insilato (R1); (ii) una rotazione triennale di mais da granella, orzo + mais insilato, loiessa + mais insilato (R3); (iii) una rotazione sessennale di loiessa + mais insilato (primi tre anni), prato di festuca e trifoglio bianco (ultimi tre anni) (R6); (iv) monocoltura di mais da granella (CM) e (v) prato permanente. Tutti i sistemi colturali sono stati sottoposti a due trattamenti agronomici, il primo (A) con dosi di fertilizzanti e fitofarmaci tipici per la zona, il secondo (B) con dosi decurtate di fertilizzanti (-30%) ed erbicidi (-25%) rispetto ad A. Abbiamo riscontrato R1 > R3 > R6 > PM > CM in termini di resa in biomassa, con un andamento leggermente diverso per le UFL. Per quanto concerne le PG, R6 > R1 > PM > R3 > CM indica una produzione di proteine molto apprezzabile per il prato permanente. Le rese sono sempre state maggiori per il trattamento A rispetto a B, senza eccezioni. Gli andamenti nel lungo periodo indicano *performances* decrescenti per tutti i sistemi colturali tranne il prato permanente, l'unico con rese sempre migliori nel tempo. In accordo con un'analisi di tipo AMMI, le tre rotazioni (R1, R3, R6) si sono dimostrate le più stabili negli anni, mentre il prato permanente ha mostrato un cambiamento nella composizione specifica. Questi dati suggeriscono l'importanza dei sistemi colturali complessi, che garantiscono la produzione di foraggi di qualità e la diversificazione di prodotto.

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1. Introduction

The biological and agronomical studies as regards biodiversity conservation, among others, have become of great interest in the last decades parallel to the prominent human population growth and, consequently, to the need of exploiting more and more natural resources. It is commonly demonstrated that a higher gain of diversity leads to higher levels of functioning of the ecosystem (Cardinale et al., 2012) and, in parallel, diversified ecosystem models achieve a multitude of services at high levels, that is defined as multifunctionality (Isbell et al., 2011). Guaranteeing the exploitation of natural resources and their conservation for the future relies on the more common definition of ecosystem services, which can be summarily marked as the benefits that humans derive from ecosystems (Lavorel et al., 2015). Those are mined by uncontrolled agriculture intensification among other causes, which has led to a nearly 7-fold increase in nitrogen fertilization in the second half of the 20th century, among the other nutrients and beside terrific impacts on aquatic ecosystems (Tilman, 1999). The anthropogenic global change we observe in the everyday life has led to tangible effects and it affects the “real-world” ecosystem services (Cardinale et al., 2012). In fact, soil fertility, pest control, pollination, hydrological flows and water quality are profoundly affected by biodiversity losses among others and the need to maintain (and improve) such ecosystem services appears obvious (Gillison et al., 2016). However, our knowledge about clearly defined conservative strategies is sometimes insufficient (Bennett et al., 2015; Gillison et al., 2016). As an example from Bennett et al. (2015), the human intervention of restoring a pond *via* an engineered filtering system could lead to profoundly different consequences in respect to the natural, ecological cleaning processes of a similar pond. That means, the lack of knowledge in any intervention driven to maintain and restore ecosystem services can lead to a change of the emerging properties of such ecosystems (Gordon et al., 2008; Ingegnoli, 2015) and some ecological “surprises” (using a term by the cited Authors) may be concealed behind the process!

That said, it is of relevance that understanding and managing ecosystem services delivery is of key importance for human well-being (Millennium Ecosystem Assessment, 2005) beside solving the trade-offs that occur when preferring one strategic action in respect to one another (Allan et al., 2015). To this end, one of the most criticized aspects of biological conservation strategies nowadays lies in the difficulty to achieve ecosystem services’ conservation at a multifunctional level, simultaneously. In particular, considering the conservation of biodiversity at all levels together may conduce to misleading results, as it is difficult to collimate all the relationships between living entities and ecosystem phenomena in a single indicator or with a single methodology (Bradford et al., 2014). That may miss some useful information with practical negative consequences. On the other hand, it has become clear that the majority of interventions are unbalanced towards a small set of ecosystem services and they are not pointed to a multifunctional ecosystem as a whole. For example, agricultural land could be managed with regards to conservational purposes alone but missing productive ones, or livestock farmers may prefer to maximize hay production whereas other land managers would discard it, only focusing on the high visual attractiveness of semi-natural grasslands (Junge et al., 2015; Lindemann-Matthies et al., 2010). The same would happen for water resources, that are extremely well-linked to agricultural activities and to the environmental burdens deriving from agronomical and hydraulic processes (WFD – Water Framework Directive, 2000), but at the same time hosting several precious organisms often needing effective conservation strategies (Kløve et al., 2011). To maintain and improve the previously defined emerging properties of ecosystems (or, as *leitmotiv* for this PhD thesis, agro-ecosystems) it is undoubtedly important to consider all the concerns in a multifunctional optic by developing new and effective techniques and approaches. Such techniques and methodologies may tackle multifunctional ecosystem processes investigation in some of those contexts where either productive and conservative services should be equally focused on different scales in space and time. That is to say that it appears extremely important to carry out agro-ecosystems analysis at least at three spatial levels (cropping-farming-regional levels) and, consequently, at different time scales (year and multi-year levels). In the scientific literature, few papers are facing these emerging issues.

This PhD project aimed at filling these theoretical and methodological gaps by proposing biodiversity analysis and management strategies for integrating conservation and production, applying novel tools for describing the modern agricultural systems. This topic was chosen to answer to the utmost agro-ecological paradigm of coordinating the productive capability of agricultural land and the preservation of its biological diversity for future generations. Such an ideal agro-ecosystem should be devoted to the sustainable production of ever-growing demanded goods and services whilst guaranteeing rural livelihood and preserving the integrity of its environment in time, in a multifunctional optic. Three contexts were chosen for the application of novel and traditional analyses to the territory of Northern Italy, considering either (i) a large, intensive agricultural district of a typical plain environment, (ii) a rural livestock farming district in an Alpine context at high altitudes, and (iii) an agronomic field experiment with the availability of long term data related to different cropping systems and rotations. Such contexts served as an indication of how varied can be the ecological processes at different spatial and temporal scales and, as a consequence, how diverse should be the methods to investigate productive and conservative issues of modern agricultural systems given these premises.

The first context we analyzed was that of the Po plain springs, also called *fontanili*, typical quasi-natural springs of Northern Italy interspersed in a highly fragmented landscape. On the one hand, the district of Milan is known as one of the most fragmented territories of the whole European Union, where streets and urban centers are surrounded by a maze of small relict green zones (Ingegnoli, 2015; PIM Centro Studi, 2009) which host nearly four hundred springs. On the other hand, the context is traditionally devoted to agricultural activities, with several intensive farms nowadays converted to specialized grain production (Bechini and Castoldi, 2009; Fumagalli et al., 2011).

Fontanili represent important biotopes showing low variation in hydrological, hydrochemical and thermal conditions throughout the course of the year, maintaining pure, low temperature groundwater during the summer period. This allows the development of typical communities of plants and animals now endangered (Bischetti et al., 2012; Kløve et al., 2011). *Fontanili* are included in the European Union's Habitats Directive Annex I, as a biotope, and in Annex II due to the endangered taxa they host, mainly aquatic species. Their waters also maintain temperatures higher than the atmosphere in winter, so that winter-irrigated meadows (*prati marcitoi* or *marcite*) flourished in the past and permitted high yields of forage production since the middle age. Two main concerns appear in the area, the first being the risk of losing the integrity of *fontanili* fragile biotopes due to intensive agriculture activities and uncontrolled urbanization. The second concern is focused on the risk of canceling the traditional source of irrigation, the waters of *fontanili*, indeed, that made possible the prosperity of agriculture in the last centuries. The modern decrease in the areas devoted to extensive agriculture in favour of land consumption, intensification and urbanization is causing the genetic isolation and physical deterioration of *fontanili* and the best strategy advocated to slow down this threat would be the creation of a network of ecological corridors able to connect *fontanili* biotopes to each other.

Landscape ecology tools and methods are suitable to address such situations, starting from the assumption that environmental patterns have strong influence on ecological processes (Turner, 1989). In highly urbanized and fragmented contexts such as the one surrounding *fontanili*, the anthropogenic activities alter the ecosystems' integrity by fragmenting land, depressing key ecosystem services and, importantly, affecting human health (Forman, 1995; Ingegnoli, 2015; Pelorosso et al., 2016). These alterations interfere with critical ecological processes such as gene flow, species dispersal capacity and fitness of communities, as well as biodiversity conservation, ecosystem resilience and ecosystem services' efficiency (Pelorosso et al., 2016). In particular, land fragmentation is commonly considered among the major driving forces altering ecosystems and, consequently, the ecosystem services they provide. What makes landscape ecology interesting is the multitude of methodologies that have been implemented for these kind of analyses. For example, the modeling of ecological corridors between the remnants of naturalness in a landscape has been implemented through least-cost analyses (Adriaensen et al., 2003), circuit theory (McRae et al., 2008) or, conversely, other kinds of analyses based on the so-called geometric approach. The last aims at determining patterns of functionality through the landscape by using geometrical and mathematical functions that, in spite of being rather simplistic to understand, are often criticized as their linkage with ecological functions is not always straightforward (Groom and Schumaker, 1993; Kupfer, 2012; Schumaker, 1996). However, some useful applications as FRAGSTATS (McGarigal et al., 2012) are freely available to compute numerous indices describing the landscape structure at any spatial scale. What is important to highlight is that the simultaneous application of multiple methods to a certain context may improve the reliability of results by integrating their pro's and eliding their weaknesses. The exclusion of one method or the other might leave behind some useful information of ecological functionality, instead. Kupfer (2012) wrote about the need to rely on moving window analyses when calculating structural indices, along with making those calculations species-specific and not claiming results related to "ethereal" species of interest. Scolozzi and Geneletti (2012) indicated the need to rely on species' home ranges, along with considering computation times and, obviously, data retrieval costs and their availability when determining the scale of observation. Some of these methods behave similarly to an Occam's razor, as the availability of large computational resources (and existing indices) tend to accommodate ecological models of gigantic complexity rather than simple models with few species-specific parameters, which more likely return accurate results, because of the more straightforward meaning they carry. We found another concern in these kind of analyses is that of considering equally fragmented areas as identically functional to species movement or conservation, whereas in reality they act diversely. This may be the case of roads and hedges, often structurally similar but functionally opposed. *Vice-versa*, areas with similar ecological functionality in respect to a species could be diversely fragmented, such as forested areas and hedges. To this end, a novel methodology was tested that collimates a species-specific parameter, called permeability to species movement, and a measure of fragmentation expressed by the Aggregation Index. The first is an expert opinion estimated parameter indicating how easily a species could pass through a land use class; the second indicates how aggregated or fragmented the structure of an area is, through a numerical interval according to the formulation of He et al. (2000). A fuzzy analysis approach aimed at reducing uncertainty in the model (Zadeh, 1965) permitted to create a novel indicator of ecological functionality, based on the participative intervention of a multidisciplinary expert panel. Using circuit theory (McRae et al., 2008) and the novel indicator as a measure of ecological functionality, it was possible to model ecological corridors between *fontanili*. More information on the methodology can be found in Chapter 2, which reports the paper we published on Ecological Indicators in the year 2016.

The second context of interest was that of a high altitude rural district in Alta Valtellina (province of Sondrio), where pastures and meadows represent the primary resources for livestock farming. The context hosts several cattle farming sites which provide the production of milk and traditional dairy products, but it also represents one of the most important tourist locations of Northern Italy. The most critical concern about the area lies in the deterioration of meadows, which has become noticeable in the last years, both in terms of hay production and quality and also of their aesthetic appeal at landscape level. What makes alpine rural districts such this interesting for agro-ecological analyses is not only the massive presence of semi-natural agro-ecosystems, but also their duality in providing multifunctional services. Meadows, indeed, provide a multitude of goods and services, traditionally being the most important resources in terms of fodder production in marginal regions, along with providing recreational, aesthetic and touristic services (Junge et al., 2015).

Nevertheless, agricultural and territorial policies of the last decades have been disharmonious with respect to the management of mountain regions. At first, they pushed for an increase of production in the more fertile valley floors, while nowadays the leading action goes towards the rehabilitation of abandoned areas. Consequences have been noticeable: evident management changes met the alpine farms in the last years, with profound socio-economic and productive shifts (Monteiro et al., 2013, 2011) and important alterations of permanent hay-meadows at the basis of the whole alpine farming context (Fava et al., 2010). Alpine meadows are mined by two main kinds of alterations, among others: the land abandonment phenomena (Gellrich et al., 2007) and, secondly, the intensification of livestock farming processes (Guerci et al., 2014). Both the two concerns are dictated by a decrease of biodiversity and by the loss of traditional coenoses, which hosted taxa linked to traditional management (Marini et al., 2008; Prosser, 2001). In parallel, coenoses and species are rapidly renovating so that new typologies of meadows appear (Scotton et al., 2014) with different characters from traditional ones. The last represent, on the contrary, the outcome of management strategies lasted centuries (Ellenberg, 1988). Landscape-scaled alterations such as the homogenization of the landscape matrices, on one hand, and the fragmentation of grassland on the other cause the shift of biodiversity patterns (Monteiro et al., 2013; Tscharntke et al., 2005) while the most marginal and less productive areas have been discarded, as a consequence of modernization and rationalization of agriculture (Riedener et al., 2014). The most fertile areas followed an opposite pathway, being subjected to a trend of intensification regarding fertilization, stocking rates and number of cuts (Guerci et al., 2014; Gusmeroli et al., 2012; Scotton et al., 2014). This certainly yielded an incredible improvement with respect to forage production, but it also exerted strong negative effects on coenoses (Plantureux et al., 1987), among which eutrophication, a reduction of the floristic richness and a shift away from long-established meadows typologies. These trends are recognized as major multidimensional issues, as the loss of grassland threats centuries of traditional land use, also impacting the equilibrium of these agro-ecosystems (Monteiro et al., 2011). Issues regard the need of food supply (Ceballos et al., 2010), the lack of forage production (Liu et al., 2006) and the loss of biodiversity (Niedrist et al., 2009) among others. For these reasons, alpine species-rich grasslands have been placed among the most threatened ecosystems in Europe (Gusmeroli et al., 2012). After decades of marginal land abandonment and intensification of residual farming systems, one of the primary concerns appears to be the conservation of traditional farming practices, which could provide the preservation of semi-natural agro-ecosystems as meadows and pastures. In this research, we aimed at answering to two main research questions: (i) What are the conditions of permanent meadows in this context, in terms of their inherent biodiversity and productivity? (ii) How does the modern farm management affect plant composition, quality and biodiversity of these coenoses? The methods we used to investigate nearly 200 meadows, at the territorial scale of this rural district, are explained in Chapter 3, which reports the manuscript we submitted to the Agriculture, Ecosystem and Environment journal in the year 2016.

The third, and last, context we were occupied in was that of the fodder agro-ecosystems of the Po Valley in Northern Italy, with the analysis of an agronomic field experiment located in Lodi and lasted more than two decades. Data about five fodder cropping systems with measured biomass, crude proteins and milk feed units yields for a 21-years timespan were available thanks to the collaboration of CREA (*Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria*) at Lodi. The investigation was thus based on the field scale, with a view to temporal shifts in production and a deepened analysis of yields trends. The explored context appears of primary importance given the need of achieving more and more fodder of high quality and, contemporarily, avoiding the environmental burdens that affect the whole context of the Po Valley. In fact, the agricultural scenario of the plains of Northern Italy has been characterized by intensification processes and over-simplification of cropping systems, since the mid 1950's (Giardini and Ziliotto, 1988). Intensification trends in this sense have had an outstanding importance in the emersion of socio-economic benefits, also thanks to the leading actions of the Common Agricultural Policy: it has led to a specialization of farming systems, with improved mechanization processes and economic advantages. On the other hand, significant detrimental effects emerged over time, mining the multifunctional nature of plain agro-ecosystems, above all the simplification of cropping systems, environmental pollution and surpluses of production deriving from surpluses of agronomic inputs. In parallel, the majority of plain areas in Europe have been subjected to uncontrolled urbanization and land fragmentation, with the consequence of losing traditional extensive cropping systems as permanent meadows (Ingegnoli, 2015; Pierik et al., 2016). The increases in yield have been obtained at the expense of organic matter in soil, among others (Gilmanov et al., 2007). All these premises make clear the necessity to assess environmental impacts in agriculture (Lijo et al., 2014; Poeschl et al., 2012), searching for alternative cropping systems and shifting towards their extensification. A proper choice of cropping systems may alleviate the environmental burdens, keeping nutrients' balances at a tolerable level; nevertheless, the quality of production and, consequently, economic advantages for the farmers have to be jointly considered to fulfill the scope of sustainability.

One of the possibilities in this sense is that of cropping rotation systems, which, while prominent in the last centuries, have been discarded nowadays, altering the overall aesthetics of the traditional agricultural landscapes of Northern Italy (Ingegnoli, 2015). Among other benefits, cropping rotations augment the retention of organic matter in soils, with crop residues that influence physical, chemical and biological soil properties and help to maintain or even improve soil fertility over time (Brankatschk and Finkbeiner, 2015). Beside this advantage, subsequent crops could benefit from the previous ones with greater biomass production even reducing fertilizers doses, and, importantly, the cleansing effects in respect to weeds are known for meadows if inserted into rotations (Tomasoni et al., 2003). All these factors could alleviate the application of agrochemicals, guaranteeing at the same time a diversification of production, which would be greatly welcomed in a context such as the one here described. In fact, large areas of the Po plain are characterized by two simple cropping systems, according to Bacenetti et al. (2015). One of them is a single crop, mainly maize [*Zea mays*] or sorghum [*Sorghum vulgare* Pers., or *Sorghum bicolor* L. Moench], where the crop follows itself season after season. The other is an annual double crop: a winter crop, typically wheat [*Triticum sp.pl.*], barley [*Hordeum sp.pl.*] or triticale [*x Triticosecale* Wittmack] followed by maize. These cropping arrangements may satisfy the requirements of husbandry, but they certainly exert strong negative effects in terms of nitrate balances, N_2O atmospheric emissions and fossil energy required, among other burdens. It is clear the need to more deeply investigate all the cropping possibilities as to reinforce the weak agricultural system of the Po Valley, so the analysis of long-term agricultural experiments could represent a way of promoting viable solutions for the reduction of environmental burdens and for the strategic programming of farming systems. The aim of this study was to compare the performances of diversified cropping systems over a continuous long-term field trial. Five fodder cropping systems were compared: (i) a one-year double-crop rotation of autumn-sown Italian ryegrass + spring-sown silage maize; (ii) a three-year rotation of grain maize (first year), autumn-sown barley + silage maize (second year), and Italian ryegrass + silage maize (third year); (iii) a six-year rotation of Italian ryegrass + silage maize (years 1,2,3) + mixed meadow of white clover (*Trifolium repens* L.) and tall fescue (*Festuca arundinacea* Schreb.) for hay-making (years 4,5,6); (iv) a continuous grain maize monocropping; and (v) a permanent meadow. The five cropping systems were compared in terms of biomass, Milk Feed Units and Crude Proteins yields at different agronomic input levels, over a 21-years timespan. Additive Main Effects and Multiplicative Interaction (AMMI) analyses were used to investigate crop performances over time, among other statistical techniques. All the methods are presented in Chapter 4, which reports the manuscript we submitted to the European Journal of Agronomy in the year 2016.

The three chapters following describe the work carried out in detail, by presenting the papers published or already submitted to international scientific journals. A general conclusion after the presentation of the three studies concludes this thesis, by proposing a broader, semantic analysis of the research performed during my PhD.

2. Designing ecological corridors in a fragmented landscape: A fuzzy approach to circuit connectivity analysis

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Abstract

Landscape connectivity analysis is a major tool in supporting biodiversity conservation. Several methodologies have been developed to tackle it by following two main paths. The first path exploits graph approaches and models focal nodes' connections on a resistance/conductance matrix depending on focal species' movement potential. The second path considers geometrical pattern analyses based on the calculation of structural landscape metrics. These approaches separately investigate functional and structural features of the landscape, and may come short of a total definition if used separately. Here we propose a new scalable, modular, participative and open-source procedure based on Fuzzy logic to combine the functional and structural aspects of connectivity. We applied this method on the highly fragmented landscape of the Po Plain, focusing on its rare and endangered plain springs named *fontanili*. We identified an expert panel and involved it in the assignation of permeability values of land use classes with respect to the capacity of movement of animal species typical of *fontanili*. We concurrently performed a quantitative evaluation of the landscape fragmentation with a moving window. We found that the functional and structural evaluations were poorly correlated in the area under study (Pearson's $r = -0.35$, $P < 0.001$).

We thus integrated these two non-overlapping analyses of the landscape by Fuzzy logic using thresholds and combination weights obtained from questionnaires proposed to the expert panel. The resulting index, termed Fuzzy Functionality Index (FFI), improved the level of information associated with landscape classification. By merging functional and structural aspects of the landscape, the FFI allowed us to discriminate different functional values of equally permeable parcels and *vice-versa*. We demonstrate that FFI may act as a conductance measure in a circuit theory approach, highlighting ecological corridors between focal points of species' distribution. We present FFI as an effective predictive index to inspect complex and non-linear landscape dynamics.

Keywords: biodiversity conservation, landscape analysis, ecological corridors, graph analysis, circuit theory, fragmentation, Aggregation Index, fuzzy analysis, participative process

1. Introduction

Landscape ecology relies on the notion that environmental patterns have a strong influence on ecological processes (Turner, 1989). Anthropogenic activities such as urbanization and intensive agriculture are often harmful to natural and semi-natural environments because they alter landscapes' structural integrity (Forman, 1995; Turner et al., 2001; Ingegnoli, 2015; Pelorosso, 2015), depressing key ecosystem services, affecting human health (Hermann et al., 2011; Ingegnoli, 2015) and creating obstacles to ecological flows of autochthonous species, while promoting the spread of alien taxa (Gardner et al., 1993; Crooks and Sanjayan, 2006; Minor and Urban, 2008; Monteiro et al., 2013). These alterations interfere with critical ecological processes such as gene flow, dispersal capacity and fitness of plant and animal populations, as well as biodiversity conservation, ecosystem resilience and ecosystem services' efficiency (Pelorosso et al., 2015). At present, many of the inhabited world areas are characterized by a diffuse fabric of anthropogenic aggregates and by agricultural zones representing the remnants of extensive ancestral *green zon*es (Ingegnoli, 2015). Intensive farming activities increase the compactness of the landscape matrices, worsening their structure (Forman, 1995; Turner et al., 2001). In these areas the remaining hedgerows, woodlands, and wetlands become key indicators of the complexity/criticality of the landscape, as they represent the structural driving forces to support local/regional biodiversity (Walz, 2011).

The Po Plain (Northern Italy) is one of the most complex territories in Europe (Murphy et al., 2009; Ingegnoli, 2015), hosting several populated cities besides the regional capital: Milan. Here, the processes of land consumption have reached critical levels (PIM, 2009). With a population ranging from 1,000 to 7,100 inhabitants/km^2, the urbanized texture covers more than a third of the Milan district and the street density, which represents a driver of landscape fragmentation, is steadily increasing. The agricultural matrix in the Milan district is prevailingly intensive (Bechini and Castoldi, 2009) and sustains itself on a pervasive historical irrigation network (Gomarasca et al., 2005). In this context, one of the most fragile patches of “naturalness” is represented by the plain springs, locally known as *fontanili* (Bischetti et al., 2012; Kløve et al., 2011; Gomarasca et al., 2005). These important biotopes show low variation in hydrological, hydrochemical and thermal conditions throughout the year. The purity of the waters and their low temperature during summer (constantly around 12-14°C) allow the development and the maintenance of typical communities of plants and animals which are now endangered (Bischetti et al., 2012; Kløve et al., 2011; Gomarasca, 2001). *Fontanili* are included in the European Union’s Habitats Directive Annex I, as a biotope (code 32-60), and in Annex II because of the several endangered taxa they host: two species of amphibians, five species and two genera of fishes, one species of crustaceans, some aquatic insects and molluscs. The current decrease in the areas devoted to agriculture in favor of land consumption and urbanization is causing an increased isolation and therefore a remarkable deterioration of *fontanili*. Local administrations and research institutions have been studying how to stop or at least slow down the *fontanili* deterioration process, advocating that the best strategy in this sense would be the creation of a network of ecological corridors able to connect the biotopes to each other. It is increasingly clear that, especially in complex systems such as the Po plain, a tight network of ecological corridors may maintain an efficient flow of energy and species, favoring the ecological balance and resilience capacity of the landscape (Muradian, 2001; Pelorosso et al., 2015).

The first step to implement conservation through the design of ecological corridors in complex environments is the analysis of the landscape features under their many different aspects. With this aim, different landscape analysis approaches have been proposed. Among the most widely used are the *geometric approaches*, which aim at linking ecological functions to structural (geometrical) features of landscape parcels by surveying their uniformity/patchiness (Forman and Godron, 1986; O’Neill et al., 1997; Kupfer, 2011). While they are intuitive, geometric approaches may not provide straightforward relationships between ecological processes and landscape structure (Groom and Schumaker, 1993; Schumaker, 1996; Cardille et al., 2005; Kupfer, 2012; Pelorosso et al., 2015). Metric values are in fact heavily dependent on data resolution and on the extent of the study area (Turner, 1989; Moody and Woodcock, 1995; Schumaker, 1996; Huang et al., 2006), requiring careful manipulation, analysis and interpretation of data (Kupfer, 2012). Ecological patterns and processes vary within spatial and temporal ranges and with the variation of the organizational scales (Levin, 1992), highlighting the fact that scale-based approaches could have great interest and potentiality (Banks-Leite et al., 2011; Lindenmayer and Likens, 2011). Moreover, attempts to link landscape metrics with the wildlife responses have often been equivocal (Tischendorf, 2001): metrics may in fact capture landscape features irrelevant for the species' capacity to perceive the landscape (Lindenmayer et al., 2002).

A second landscape analysis method is that of *raster-based approaches.* This method is able to capture the gradients of environmental resources and habitat quality by representing continuous surfaces of any habitat variable (McIntyre and Hobbs, 1999; Kent, 2009; Murphy and Lovett-Doust, 2004). These approaches consider landscape patches in relation to their permeability/resistance to the movement of organisms of interest. A peculiar class of raster approaches involves a moving window analysis, where for each cell of landscape is assigned a new permeability value calculated and mediated on the neighboring cell values. This approach can be effective at capturing the neighborhood effects and at examining the effects of scale on forest patterns (Riitters and Coulston, 2005; Riitters et al., 2009).

A third approach in studying landscape complexity is that of *graph theory*, or network analysis, which models the functional response of a target species within a landscape pattern (patch size, shape, location) providing a spatial network representation (Urban et al., 2009). Graphs efficiently represent spatial relationships among habitat patches (Urban and Keitt, 2001), among focal species in landscapes (Fortuna et al., 2008), and habitat reserves (Fuller et al., 2006) even at the scale of a single raster cell (Adriaensen et al., 2003; Drielsma et al., 2007; Pinto and Keitt, 2009). The *electric approach* proposed by McRae et al. (2008) falls in this category. In it, a (group of) species represented as electrons moves through a resistance matrix between inlet and outlet nodes: results are interpretable in terms of isolation-by-resistance (McRae et al., 2006).

For an unbiased and realistic analysis, measures of connectivity should consider both structural and functional aspects of the landscape in a holistic approach. Opdam et al. (2008) highlighted how ecological quality and connectivity are the main determinants to model species' distribution in landscapes. More recently, other researchers underlined how an eco-profile should be designed for target species to allow for species-specific connectivity assessments (Kupfer, 2012; Pelorosso et al., 2015). Such designs must be based on generalized ecological traits of key species demanding an ecosystem network, at an appropriate scale. With this aim, some authors have designed green infrastructures (Benedict and McMahon, 2002) evaluating species movement as costs to move, using least cost distance approaches (Adriaensen et al., 2003). Functionally, such networks could behave as physical links between habitat patches (Freemark et al., 2002) defined on quality-determining factors like width and the connectivity capacities (functionality in terms of movement) (Forman, 1995). In these contexts, fragmentation undoubtedly represents a fundamental element acting as a quantitative descriptor of the landscape structure and organization (Kuttner et al., 2013). Fragmentation is recognized as a major cause of habitat loss, inter-patch dispersal and species' decline (Fahrig and Merriam, 1985; Fahrig and Paloheimo, 1988; Pulliam, 1988; Yahner, 1988; Thomas et al., 1990; Foster and Gaines, 1991; Saunders et al., 1991; Lamberson et al., 1992, 1994; Schumaker, 1996, Ingegnoli, 2015). However, it is clear that if fragmentation is used alone as purely geometrical element it might have a fairly limited information content, as, for example, it could paradoxically equalize areas with opposite ecological values because of an equivalent fragmentation pattern (*e.g.* hedges vs. roads). Conversely, equivalent spatial structures may conceal contrasting ecological values (*e.g.* forests vs. urban tissues). On the other hand, the sole use of graphs can only rely on landscapes' qualitative traits, therefore omitting the objective depiction of landscape patterns such as fragmentation rates. Given the complexity of urban landscapes both in terms of fragmentation and heterogeneity (Scolozzi and Geneletti, 2012; Tannier et al., 2012; Braaker et al., 2014), the need to complement structural information with species-specific parameters (biological and beyond) becomes very clear.

In this study, we aimed to develop a new synthetic index able to merge the species-specific permeability values, based on expert opinion, with structural fragmentation data, evaluating the potentiality of the new merged result through the circuit theory. We started from the assumption that the two measures alone are not sufficiently exhaustive to fully describe landscapes, and that they might lose the functional connectivity significance if not jointly considered. The deriving functional connectivity index, termed Fuzzy Functionality Index (FFI), was tested as a participative, non-deterministic base to a circuit theory application to provide ecological corridors. We applied our method in a pilot study considering the *fontanili*, fragile environments of the Po Valley, demonstrating the FFI's capacity to identify ecological connections between *fontanili*, as well as to highlight the most critical areas to be proposed as zoning and conservation priorities. We discuss the implications of this method in the broader perspective of ecological planning in fragmented areas.

2. Material and Methods

2.1. Study Area

The study area is located in the Po Plain (Northern Italy) and covers part of the Milan District (Fig. 1). The study area is about 1,350 km^2, its North-South borders delimiting the *fontanili* areal distribution. Mean altitude is 108 m a.s.l. with average slope 0.59%. The prevailing soils are loam, silty-loam and sandy-loam (Regional Geographical Database - RGDB, 2014). From North to South, soil texture becomes finer and coarse material content decreases (Fumagalli et al., 2011) allowing the emergence and the leakage of groundwater.

This geographically well-defined area is named the "springs zone" or "*fontanili* belt" (Bischetti et al., 2012; Gomarasca, 2001; Kløve et al., 2011). Until 1960s, *fontanili* biotopes were very common in the area, reaching 941 units. Since then, 448 *fontanili* have been completely lost, 122 dried out, and only 371 remain active. At present, many of the remaining *fontanili* appear to be in a bad functional state (Bischetti et al., 2012; Gomarasca, 2001). The network of ditches and canals spanning the study area, in part derived from *fontanili*, is complex and often associated with a mazy system of hedges that, for more than a thousand years, has characterized the local agricultural landscape (with 5.7 km/km^2 of channels and ditches, 21% of which derived from *fontanili*).

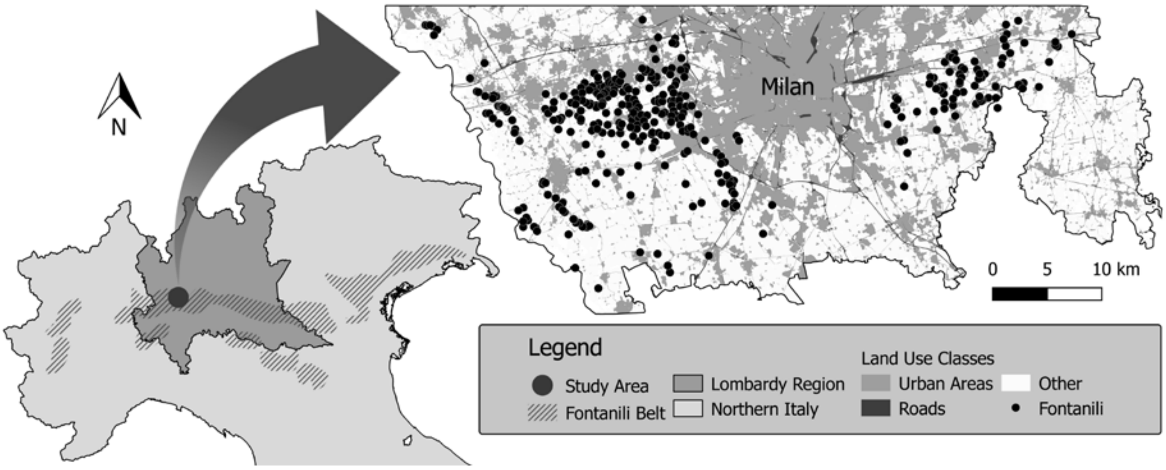

Fig. 1. Overview of the study area highlighting urbanized areas in relation to fontanili distribution

2.2. Methods

This study was conducted in a series of sequential steps, involving an expert group in a participative workflow and using open source software, according to the flowchart in Figure 2. The workflow underwent seven main points described through this section: i) definition of an expert panel, ii) definition of a target species group, iii) assembly of a permeability dataset, iv) mapping of *fontanili* distribution, v) fragmentation analysis, vi) implementation of fuzzy analysis to aggregate the main spatial variables, and vii) connectivity analysis between *fontanili*.

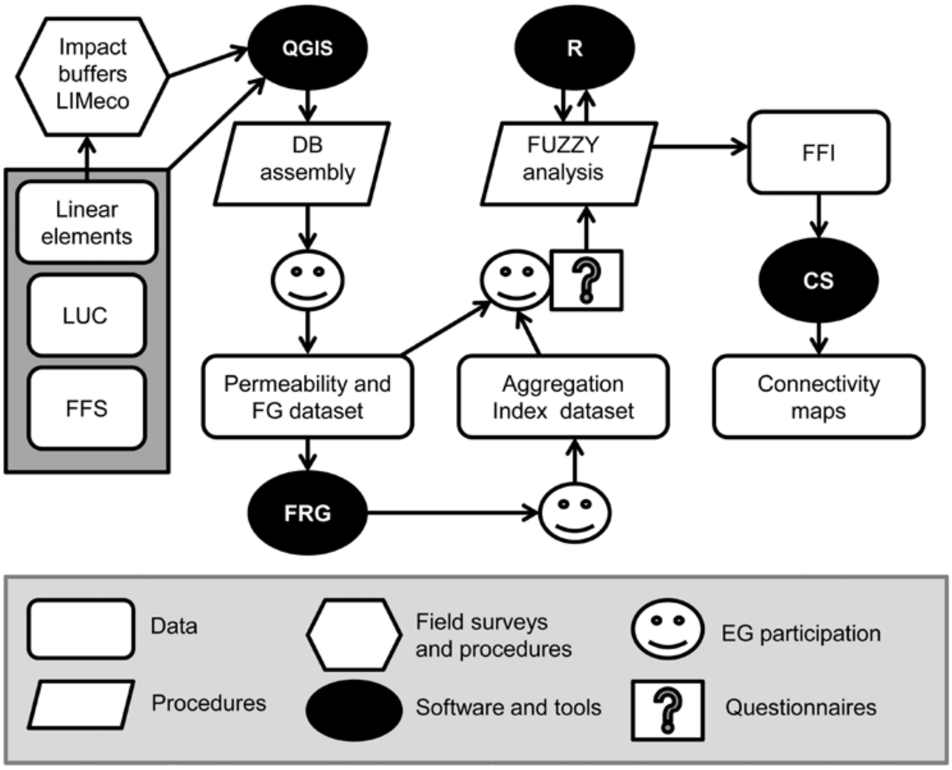

Fig. 2. Flowchart summarizing the main methodological steps. Software (open source) used is QGIS, FRAGSTATS (FRG), Circuitscape (CS) and R. Other abbreviations are LUC: Land Use Cover classes (derived from RGDB together with the Linear elements); FFS: Fontanili Focal Species; FG: Functional Groups; FFI: Fuzzy Functionality Index; EG: Expert Group

2.2.1. Expert panel

Species-specific parameters such as permeability to movement could be derived from several types of direct measures such as telemetry, trapping, behavioral data, measures of gene flow or it can be estimated through expert opinion (Epps et al., 2007; Rayfield et al., 2010; Sawyer et al., 2011). Among these possible approaches, the expert opinion was preferred as it is flexible and does not need complex and costly instruments or *ad hoc* field researches. A heterogeneous expert panel was selected to perform three critical tasks: i) critically evaluate permeability values given to landscape features, ii) revise parameters for each methodology adopted, and iii) participate in the compilation of fuzzy questionnaires (Supplementary Material).

The acquisition of knowledge is a hierarchical process starting with the collection of facts, proceeding with the application of learned content to solve problems, and finally arriving at the synthesis and evaluation of learned content (Bloom, 1956). Expert panel evaluations were carried out following the procedures described in the Ericsson handbook (Ericsson et al., 2006). A rigorous method should incorporate a measure of uncertainty for the proper elicited expert knowledge and should entail an assessment of the internal or external validity of findings. With this in mind, we followed four steps: i) identify, select, recruit and retain of experts; ii) elicit the expert knowledge; iii) confront and resolve bias, uncertainty and aggregation of expert knowledge; iv) validate the expert knowledge. Points iii) and iv) are addressed in the Discussion section.

The starting point for an expert knowledge study is to identify an appropriate pool of potential participants (Murray et al., 2009), which depends on a clear understanding of project scope and objectives. Experts were recruited according to their professional background and knowledge of the study area (Elbroch et al., 2011). To favor the best elicitation of expert knowledge, all meetings with experts were done in person, as computer mediated interaction may fail to meet equality in communication between individuals of differing statuses (Christopherson, 2007). Initially, we organized groups of experts to formulate "skeletons" of the questionnaires that were subjected to the experts from time to time. Questionnaires were presented to the individual experts. Later, as anonymous documents, they were submitted to the subgroups assessment. In this phase, we were careful to avoid the emergence of dominant personalities which could influence the assessment of the group (MacDougall and Baum, 1997). To do this, the authors acted as dominant elements of the group, coordinating and guiding the discussion at all times.

The panel comprised 28 persons encompassing varied ages, study and work backgrounds, to create a favorable dialogue between the parts in a multidisciplinary team. The principal requirement for participants was their previous involvement in environmental and/or urban planning projects focusing on the study area. Twelve are MSc experts in agro-ecology or in ecology (two biologists, two naturalists, four agronomists, two biotechnologists, one has a degree in forest sciences, and one in agricultural biotechnology), four are ecological modeling experts (two biologist, two agronomists and one physicist), and six are employed in the strategic management of agriculture and forests of the Milan municipality (two agronomists, three architects and one lawyer). The remaining six work in a national NGO (*Legambiente*: http://international.legambiente.it/) focusing on environment and biodiversity protection.

2.2.2.Target species group

A group of key species was defined to inspect the functionality of landscape features in terms of permeability to movement (*Fontanili* Focal Species, FFS). FFS considers the typical communities of *fontanili* and of their associated hedges (Bischetti et al., 2012; Gomarasca, 2001). The taxa considered are: amphibians and reptiles such as newts, frogs and toads, water snakes (*Natrix spp.*) and Aesculapian snake (*Zamenis longissimus*) (Ildos and Ancona, 1994; Kolozsvary and Swihart, 1999; Swihart et al., 2002; Sillero et al., 2014; Ficetola et al., 2015); insects: *Carabidae spp.* and *Dytiscidae spp.* (Cole et al., 2002; Buse et al., 2007); micromammalians (Bosè and Guidali, 2000). The sensitivity of FFS to fragmentation, urbanization, landscape degradation and pollution is widely reported (Laurence et al., 1996; Blaunstein et al. 1997; Flax and Borkin, 1997; Waldick 1997; Strijbosch & Van Gelder, 1997; Zuiderwijk et al., 1998; Daszak et al., 2001; Gardner, 2001; Kjoss and Litvaitis, 2001; Taylor and Fox, 2001; Gentilli, 2004). When known, the presence of northern pike (*Esox lucius*), chub (*Squalius cephalus*), common minnow (*Phoxinus phoxinus*), bullhead (*Cottus gobio*) and crayfish (*Austropotamobius pallipes*) (Smith et al., 1996; Hanfling and Weetman, 2006; Blanchet et al., 2010) is also taken in account to indicate the elevated quality levels of major streams. The FFS group have a home range between 10 and 100 m^2 and can move daily from 10 to 200 meters. These data influenced the choice of working resolution.

2.2.3. GIS dataset assembly

The localization and the development of ecological corridors is closely linked with the typologies of land use and with the needs of the target species (Adriaensen et al., 2003; Watts et al., 2010; Zeller et al., 2012; Kuttner et al., 2013). First, a permeability value was assigned to each land use class, considering the several aspects of each class that may have an effect on FFS movement. When classes of permeability had different influences on surrounding parcels (e.g. roads), diversified impact values were adopted to discriminate the possible subtype classes (e.g. for roads: local roads, state roads, highways and so on). Similarly, rivers and major canals were considered permeable migration routes for water FFS, but with different values of permeability depending on water pollution levels.

The GIS database for permeability was created with QGIS 2.2.0 (QGIS Development Team, 2013) starting from the Regional LUC vector map provided by the Regional Geographical Database (RGDB, 2014), based on CORINE classification. Incorrect or obsolete land use data were updated by comparing the land use data with Google Earth® orthoimages (Google Earth®, 2012-2014). Polyline elements such as hedges, tree rows, roads, railways and main water courses available in the RGDB were also updated and converted to polygons. The physical width of streets was calculated on orthoimages by averaging several measurements for each of the four street classes: highways (four lanes - 22 m wide), national (four lanes - 16 m wide), provincial and municipal roads (two lanes – 8 m wide) and secondary roads (5 m wide). Widths of 30 m and 15 m were assigned to hedges and to tree rows, respectively, according to the General Forestry Plans Directories of Lombardy Region. The width dimensions of the main water courses were derived through orthoimage observations and evaluations. Canals and ditches of the minor irrigation network were excluded from the dataset, as information was lacking about their hydrological conditions throughout the year.

Landscape permeability values in regard to FFS were attributed to the GIS database. Permeability values ranged from 1 to 10, where 1 correspond to the minimum of permeability and 10 to the maximum. These values were at first identified by the authors and then validated through the participative consultation of the expert group. All cover class data were also synthesized into six functional groups, as proposed by Kuttner et al. (2013): *artificial matrix, disturbed matrix, valuable matrix, connecting corridors, dissecting corridors* and *stepping stones*. Functional groups were not used for any analytical mean. This grouping combines everything having similar spatial, geometric and ecological characteristics, synthesizing dispersed data in semantic groups. Despite being apparently simplistic, this synthetic approach is useful to aggregate results in a way favorable to an integrative discussion approach.

The linear elements in the dataset were integrated with impact buffer values to add information about their impacts (positive or negative) on the surrounding landscape elements. Impact buffers consider two aspects: i) extension of the effect (width dimensions) and ii) magnitude of the effect, in terms of decreased or increased permeability of the underlying land use (impact value). Buffer effect values on permeability were negative for roads and railways, positive for hedges, and negative, neutral or positive for main water courses (depending to the physical-chemical water quality – see below).

The buffer dimensions of roads and railways were estimated through phytosociological surveys focused on changes of the roadsides plant communities, assuming that vegetation structure and composition change with pollutants, local temperature variations and traffic volumes (Lee et al., 2012; Neher et al., 2013). We used the presence of therophytes and allochthonous species, and their coverage alterations, as indicators of detrimental effects of roads (comparing these observations with data of local plant communities not impacted by roads). Impact extension was recurrent for road categories, hence a mean width dimension was proposed for each of them. The impact value of roads and railways' permeability buffer was calculated by averaging (mathematically) the underlying land use permeability value with the minimum permeability value proper of those linear features. This operation permitted us to render discrete the impact gradient that goes from the road or railway (permeability value 1) to the surrounding areas (variable permeability value, depending on land use).

The buffer dimension of hedges was evaluated by observing their mean shadow extension (through observations made in the field and on orthoimages). Their positive buffer impact on the surroundings was obtained by summing a + 2 value to the underlying land use permeability value, according to the expert group: this was used to emphasize the importance of semi-natural linear elements that serve as viable habitats (Ferrier, 2002) and break, in a positive way, the spatial contiguity of the agricultural matrix. The tree rows' impacts were not considered due to their poor vegetation structure and biodiversity.

The buffer dimensions of main water courses were estimated by a phytosociological approach, observing the extension of plant communities linked to wet habitats. The buffer extension varied from 2,000 m for broader natural rivers to 40 m for large channels with concrete bottoms and for drainage ditches of lesser importance. Water courses showed positive, null or negative impacts depending on their water quality, calculated through a reclassification of the LIMeco Index (a synthetic indicator of water quality calculated on the concentrations of dissolved Oxygen, Ammonia, Nitrates and total Phosphorus). Water quality data were obtained from the Regional Environmental Agency (ARPA, 2014) and enriched by our own field chemical analyses (data not shown). The five LIMeco values, *high, good, sufficient, poor* and *bad* were reclassified in numerical values +2, +1, 0, -1 and -2 to be summed to the permeability value of underlying areas. Every step was revised and validated by the expert group.

A stepwise geoprocessing-synthesis through QGIS provided the final permeability dataset in vector format, integrated with all impact buffers. The obtained values ranged from the minimum of 1 (complete resistance) to the maximum of 12 (best land use plus the best positive impact). The minimum permeability, equal to 1, could not be worsened by any negative impact. For subsequent analyses, the vector dataset was rasterized to obtain a permeability raster dataset. Rasterization processes were computed in R 3.1.0 (R Core Team, 2014) using the packages R/raster (Hijmans et al., 2014) and R/rgdal (Bivand et al., 2014) with 100 m and 25 m resolutions to obtain two raster datasets at different detail, according to the ecological characters of the key species FFS (home range, movement skills and so on).

2.2.4. *Fontanili* mapping

The study targets, *fontanili*, were not included in the working dataset to avoid assigning them an *a priori* permeability value. Neither buffers nor quality values were assigned to *fontanili*. *Fontanili* were mapped in QGIS based on expert field recognition during the years 1999-2014, improving and completing the FonTe Regional database (Bischetti et al., 2012). *Fontanili* point features were rasterized to perform further connectivity analysis, converting each to a pixel; rasterization was achieved in R as above.

2.2.5. Fragmentation analysis

The permeability raster datasets at the two resolutions (100 and 25m) were used to compute several landscape fragmentation indexes through FRAGSTATS 4.2 (McGarigal and

Ene, 2012); at first, a general descriptive analysis of landscape and class levels was computed through the “no sampling” option which returned numerical values showing the real patch subdivision. Later, the whole area was sampled by a square moving window to avoid patch subdivision, therefore considering landscape as a continuous surface delineated by a gradient of ecological characters (McIntyre and Hobbs, 1999; Murphy and Lovett-Doust, 2004; Kent, 2009; Kupfer, 2012). Assuming that the landscape is not only hierarchically structured (Forman and Godron, 1986; Kotliar and Wiens, 1990), but also composed of overlapping units (Ingegnoli and Pignatti, 2007; Ingegnoli, 2015) we studied landscape complexity (fractionation) through different structural indices combinable, in FRAGSTATS, with the moving window: Aggregation, Cohesion, Contagion, Shannon Diversity, Simpson Diversity, and Patch Richness indices. The 100 m resolution raster was examined with a 500 m sided moving window, whereas the 25 m raster was examined with a 100 m sided one. The maps obtained with each index were thereafter analyzed by the expert group, which decided that Aggregation Index (AI; He et al., 2000; McGarigal and Ene, 2012) was the most informative and easily readable in terms of landscape complexity. AI (Eq. 1) indicates, in a 0-100 range, how much the environmental classes within the moving window area are fragmented (AI = 0) rather than aggregated (AI = 100):

$$AI = \left(\frac{g_{ii}}{max\_g_{ii}}\right) \cdot 100 \qquad \text{(Eq. 1)}$$

where the number of like adjacencies between pixels of class type *i* within the moving window area (g_{ii}) is compared to the maximum number of like adjacencies (max_g_{ii}) available, then the ratio is reported as a percentage (AI).

2.2.6. Fuzzy analysis

Fragmentation indices are able to highlight the structural pattern of a landscape, but they are not interpretable in terms of functionality if not in conjunction with species-specific variables. In other words, structural connectivity measures need to incorporate specific information about functionality to avoid misinformation (*e.g.*, how could an equal fragmentation value either for a hedge or for a street differentiate their functionality?). The combination of diverse variables such as AI and P appears difficult though, typically because their relationship is not linear and it often deals with some degree of uncertainty. Fuzzy logic (Zadeh, 1965; Klir and Yuan, 1995) allowed us to manage the membership of variables to sets as not determined in absolute terms (0 or 1) but rather in a blurred manner, represented by the real interval between 0 and 1. This makes fuzzy logic relevant when dealing with uncertainty, in particular for modeling (Foody, 2006), impact analysis (Silvert, 1997; Carozzi et al., 2013), landscape planning (Allmendinger and Haughton, 2009), and clustering purposes (Equihua, 1990; Chevene et al., 1994). A fuzzy algorithm was thus developed to combine AI and P in a participative process, thanks to the guidance of a panel of experts answering a dedicated questionnaire (supplementary material).

The aggregation procedure of AI and P followed Sugeno's method of fuzzy inference (Sugeno, 1985) where three membership classes were identified for each variable: favourable (F), unfavourable (U) and partial. This means that F indicates whether a value assumed by the variable is completely favourable, U whether completely unfavourable, whereas partial indicates the transitional interval between F and U. A membership function is used for each variable to classify values belonging to the partial set. Thresholds need to be identified to discriminate the membership of values to the three sets, as they represent the limits of the transitional interval. In practice, a threshold represents the minimum/maximum value below/above which the variable (either AI or P) assumes a full membership to F, U or partial set. Questionnaires were proposed to the expert panel to identify four thresholds (two for each variable), finally obtained by averaging and rounding the panel responses: AI_{max} (completely U), AI_{min} (completely F), P_{max} (completely F), P_{min} (completely U) (supplementary material). Two *S*–shaped membership functions (*e.g.* Bellocchi et al., 2002) with values ranging from 0 to 1 and from 1 to 0 were defined for P (S_1, Eq. 2) and AI (S_2, Eq. 3) respectively, based on the four thresholds as above:

$$S_1(P, P_{max}, P_{min}) = \begin{cases} 1 & P \geq P_{max} \\ 1 - 2[(P - P_{max})/(P_{max} - P_{min})]^2 & mean1 < P < P_{max} \\ 2[(P - P_{min})/(P_{max} - P_{min})]^2 & P_{min} < P \leq mean1 \\ 0 & P \leq P_{min} \end{cases} \quad \text{(Eq. 2)}$$

$$S_2(AI, AI_{max}, AI_{min}) = \begin{cases} 0 & AI \geq AI_{max} \\ 2[(AI - AI_{max})/(AI_{max} - AI_{min})]^2 & mean2 < AI < AI_{max} \\ 1 - 2[(AI - AI_{min})/(AI_{max} - AI_{min})]^2 & AI_{min} < AI \leq mean2 \\ 1 & AI \leq AI_{min} \end{cases} \quad \text{(Eq. 3)}$$

where *mean1* = (P_{min} + P_{max})/2 and *mean2* = (AI_{min} + AI_{max})/2.

In the same questionnaires, experts were asked to propose weights for the four full membership combinations of the two variables. Weights (W) ranged from 0 to 1, with 1 indicating the best ecological functionality expected: i) P_{max} and AI_{min} (W1- which indicates F for P and F for AI), ii) P_{max} and AI_{max} (W2, which indicates F for P and U for AI), iii) P_{min} and AI_{min} (W3, which indicates U for P and F for AI), iv) P_{min} and AI_{max} (W4, which indicates U for P and U for AI). These final combination weights resulted from averaging and rounding the expert panel responses.

In accordance with Sugeno (1985), a set of decision rules was defined to obtain a final 0 – 1 interval for both AI and P, according to their membership to the three subsets, F, U or partial. The combination weights were used to compute a weighted average of fuzzy sets to derive crisp values (defuzzification). A complete and detailed description of the fuzzy method used for the aggregation is available in Bellocchi et al. (2002) with the complete sets of equations and numerical examples. The algorithm was carried out in R in a matrix layout, importing AI and P raster datasets as dataframe. The resulting synthetic indicator was named Fuzzy Functionality Index (FFI), ranging from 0 (worst ecological functionality) to 1 (best ecological functionality). FFI was translated into ASCII tables using R/SDMTools (VanDerWal et al., 2014), then mapped. Custom scripts are available upon request.

As an explanation of the algorithm, a graphical overview of all possible combinations of AI (0–100) and P (1–12) to derive FFI (0-1) is provided in Figure 3 based on thresholds and combination weights derived from questionnaires (see Results and Discussion). Different thresholds and weights would change the graph's appearance, but in all cases the two S-shaped membership functions of AI and P will intersect at four steps where FFI assumes the value of the respective combination weight.

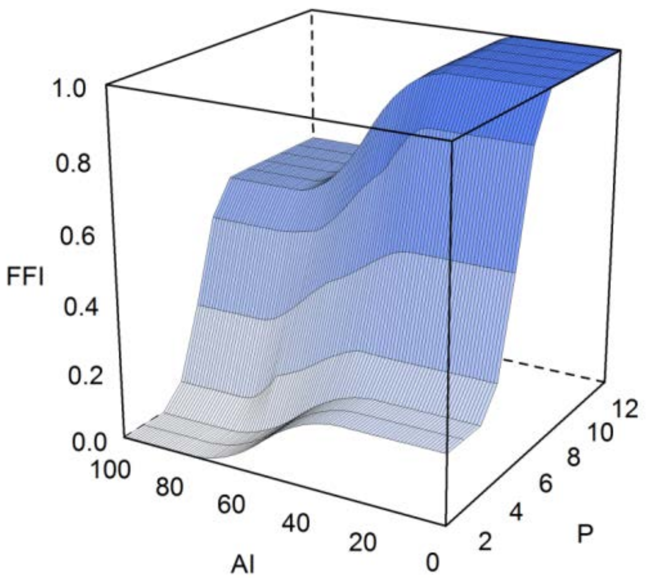

Fig. 3. Graphical overview of FFI (Z-axis), obtained by all possible combinations between AI (X-axis) and P (Y-axis). FFI increase is highlighted by the white/blue scale (white: minimum value; blue: maximum value)

2.2.7. Connectivity analysis

FFI raster maps of the area under study were employed as conductance matrices to obtain connectivity maps using Circuitscape 4.0.5 (McRae et al., 2008; McRae et al., 2009). 371 active *fontanili* were used as focal nodes. The *one-to-all* algorithm based on eight cells neighborhood was chosen to obtain cumulated current maps. In this method, current is assigned to one focal node at a time dissipating within a narrower spatial range and better mimicking FFS movements. Rather than forcing nodes to connect indefinitely through the landscape, this method highlights the isolation grade between them. The calculation was carried out for the whole area on the 100m raster dataset. The detailed raster dataset at 25m resolution was used in specific areas only so as to reduce the number of focal nodes per computation. The resulting maps reporting current distribution across the landscape were examined in R using R/raster package and the results displayed by QGIS.

3. Results and Discussion

3.1. Permeability values, functional groups and buffer widths

Table 1 presents LUC permeability values, ranging from 1 to 10 (minimum and maximum value), and the six functional groups (those proposed by Kuttner et al., 2013), elaborated by authors, discussed and validated by the expert group. For completeness, class denominations and codes, according to Functional CORINE Land Cover classification, have been added. These values, joined with all parcels of the landscape, resulted in thematic maps of permeability and functional groups, which will be discussed below.

Table 1. Correspondence between land use/cover classes (LUC), CORINE Land Cover (CLC) classification code, Functional Groups, and Permeability values. All values were proposed by the authors and evaluated through the Expert Group consultation

LUC classes	CLC code	Functional Group	Permeability
Continuous urban fabric	1.1.1.	Artificial matrix	1
Discontinuous urban fabric	1.1.2.	Artificial matrix	1
Single building marginal to urban fabric	1.1.2.3.1.	Artificial matrix	4
Industrial and commercial units	1.2.1.1.	Artificial matrix	1
Agricultural units	1.2.1.1.2.	Artificial matrix	4
Areas of special installations	1.2.1.2.	Artificial matrix	1
Road network and associated land	1.2.2.1.	Dissecting corridor	1
Railways	1.2.2.3.	Dissecting corridor	1
Airports	1.2.4.	Artificial matrix	1
Mineral extraction sites	1.3.1.	Valuable matrix	5
Dump sites	1.3.2.	Artificial matrix	1
Construction sites	1.3.3.	Artificial matrix	1
Land without current use	1.3.4.	Disturbed matrix	3
Parks	1.4.1.1.	Valuable matrix	6
Sport facilities	1.4.2.1.	Artificial matrix	1
Leisure areas	1.4.2.2.	Disturbed matrix	3
Recreation settlements	1.4.2.3.	Artificial matrix	1
Arable land prevailingly without dispersed vegetation	2.1.1.1.	Disturbed matrix	5
Arable land with scattered vegetation	2.1.1.2.	Disturbed matrix	5
Horticultural crops	2.1.1.3.1.	Disturbed matrix	4
Foil tunnel and greenhouse horticultural crops	2.1.1.3.2.	Artificial matrix	2
Gardening and non-food crops	2.1.1.4.1.	Disturbed matrix	3

Foil tunnel and greenhouse gardening and non-food crops	2.1.1.4.2.	Artificial matrix	2
Domestic horticultural crops	2.1.1.5.	Valuable matrix	5
Rice fields	2.1.3.	Disturbed matrix	4
Vineyards	2.2.1.	Disturbed matrix	5
Orchards	2.2.2.	Disturbed matrix	5
Other cultivated ligneous species	2.2.4.2.	Valuable matrix	7
Grassland prevailingly without trees and shrubs	2.3.1.1.	Valuable matrix	8
Grassland with trees and shrubs	2.3.1.2.	Valuable matrix	9
Permanently irrigated meadows	2.3.1.3.	Valuable matrix	8
Tree rows	2.4.3.2.	Connecting corridor	7
Hedges	2.4.3.4.	Connecting corridor	10
Broad-leaved forests	3.1.1.	Stepping stone	10
Broad-leaved forests along rivers	3.1.1.3.	Stepping stone	10
Poplar plantations	3.1.1.5.	Stepping stone	7
Recently planted broad-leaved forests	3.1.4.	Stepping stone	8
Gravel bed vegetation	3.2.2.2.	Valuable matrix	9
River bank vegetation	3.2.2.3.	Valuable matrix	8
Shrub areas with significant presence of trees	3.2.4.1.	Valuable matrix	8
Shrubs within abandoned agricultural areas	3.2.4.2.	Valuable matrix	7
Beaches, dunes and sand plains	3.3.1.	Valuable matrix	8
Sparsely vegetated areas	3.3.3.	Valuable matrix	7
Inland marshes	4.1.1.	Valuable matrix	10
Water courses	5.1.1.	Connecting corridor	LIMeco Values
Natural water bodies	5.1.2.1.	Valuable matrix	5
Artificial reservoirs	5.1.2.2.	Valuable matrix	5

Buffer widths and relative impact values assigned to linear elements of the landscape are presented in form of buffer areas improving (*e.g.* hedges and clean rivers) or worsening (*e.g.* roads and polluted rivers) the permeability values of the nearby territory (Table 2). The final processing of all presented values is the result of the close cooperation between the authors and the members of the expert group. One of the prominent issues in the dialogue between experts and authors is that of maintaining a satisfying level of impartiality and of correctness in judgments. Previous literature evidence that a key asset is the number of experts necessary to provide sufficient breadth of knowledge to capture the parameters of interest and associated uncertainty. For group-based methods, the available guidance suggests that effective group dynamics and profitable discussions can occur with up to 12 (Cooke and Probst, 2006) or even 15 participants (Aspinall, 2010), half of the experts employed here. Some authors identify three types of uncertainty involved in similar approaches: aleatory, epistemic, and linguistic (Morgan and Henrion, 1992; Hora, 1996; Elith et al., 2002). The aleatory uncertainty is included in the system due to its inherent stochasticity, and cannot be totally eliminated. The epistemic uncertainty is caused by lack of empirical knowledge, and was minimized collecting differing and synergic information through continuous and purposeful discussion between the 28 experts. Linguistic uncertainty was reduced by the continuous interaction with expert panel representatives, minimizing ambiguity of terms used both in the explanations and in the questionnaire elaborations.

Table 2. Impacting features with their corresponding permeability value, impact extension (buffer width) and mean of impact calculation. Roads and railways impacts are obtained by averaging land use values with the proper road permeability value equal to 1. Other impacts are obtained by summing or subtracting the proposed coefficient. Watercourses' buffer width varies with the extension of related plant communities. All values were proposed by the authors and validated through the expert group consultation

Impacting features	Permeability	Buffer width (m)	Impact calculation
Highways and ring roads	1	300	average
National roads	1	200	average
Provincial roads	1	150	average
Secondary roads	1	50	average
Railways	1	50	average
Hedges	10	20	2
"High" LIMeco water courses	10	Variable	2
"Good" LIMeco water courses	9	Variable	1
"Sufficient" LIMeco water courses	8	Variable	0
"Poor" LIMeco water courses	6	Variable	-1
"Bad" LIMeco water courses	5	Variable	-2

3.2. Permeability to FFS movement in the landscape

Permeability (P) values, analyzed through the resulting maps (e.g. Fig 5a) and synthetically read through the functional groups, outline a high heterogeneity (Fig. 4a). Stepping stones (P = 9.66 ± 2.02) and connecting corridors (8.59 ± 2.1) are the most permeable groups, followed by the valuable matrix (7.21 ± 1.67). The disturbed matrix, mainly represented by intensive agricultural areas (Bechini and Castoldi, 2009; Fumagalli et al., 2011), has a wide extension and includes maize crops and rice paddy fields. Here, P values show a lower interquartile range (4.48 ± 0.98); this is due to the more uniform spatial pattern, characterized by larger parcel size, that causes the dilution of buffer effects. An even tighter range characterizes artificial matrices (1.15 ± 0.65) and dissecting corridors (1.01 ± 0.14), arguably the less permeable groupings, as they represent strongly deteriorated land uses, such as asphalted roads, which it is impossible to improve unless a full restoration and cessation of disturbance are achieved.

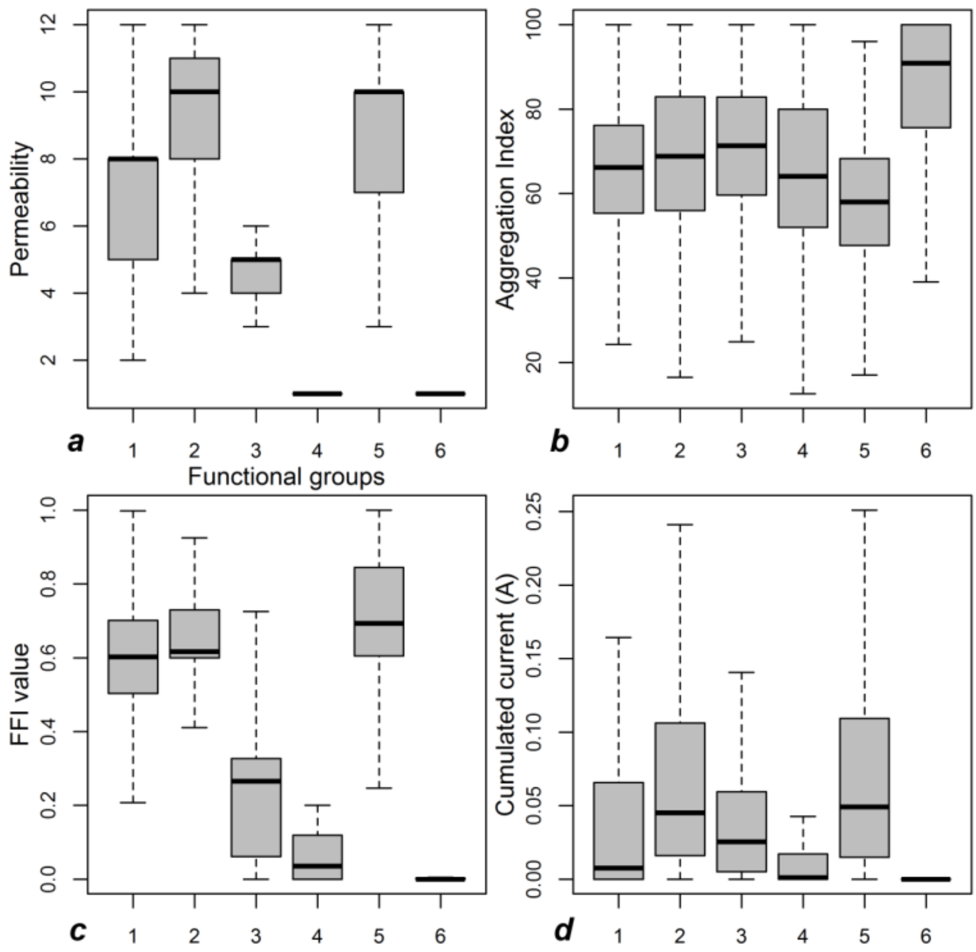

Fig. 4. Boxplots showing the main results derived from the analysis of maps: a) Permeability; b) Aggregation Index, calculated on the basis of 500m square moving windows; c) Fuzzy Functionality Index (FFI) value; d) cumulative current between fontanili, expressed in amperes. Map data are aggregated through six functional groups, in order: 1-valuable matrix; 2-stepping stones; 3-disturbed matrix; 4-dissecting corridors; 5-connecting corridors; 6-artificial matrix

The wide variation observed within functional groups in Figure 4a results from the approximation in functional groups assignation: various land cover classes refer to the same group and the complex spatial pattern increases P variability. Impact buffers of parcels, either negative or positive, contribute to P variation within classes. For example, a dense network of roads and an even thicker, sprayed, hedges distribution characterizes the landscape under study, correspondingly decrementing and incrementing the intrinsic P of adjacent parcels. The greater portion of variation extends below the median (Fig. 4a), indicating negative buffer effects within valuable and disturbed matrices and connecting corridors. This is the case with connecting corridors that, while generally highly permeable, are often interrupted by roads and crop fields. Stepping stones alone show a relevant variation between the median and the third quartile, indicating that water courses have positive impacts. Otherwise, some positive impacts seem to be masked. The distribution of P within functional groups is expectedly varied, reflecting the high variability of the landscape under study. Neither does the synthesis of functional groups precisely indicate specific functionality of parcels, nor does P fully describe the complexity of the study area. Parcels' functionality is barely described by the P values alone (Fig. 4a), and may benefit by considering the addition of a landscape structure component.

3.3. Aggregation Index and landscape structure

The Aggregation Index (AI) indicated how much the chosen classes are fragmented (AI = 0) or aggregated (AI = 100) within the landscape, based on the spatial distribution and contiguity of patches. The "no sampling" AI computation at class level, which clusters the whole area into hard - lined defined patches (Fig. 5b and 5c), resulted in a high level of patch heterogeneity.

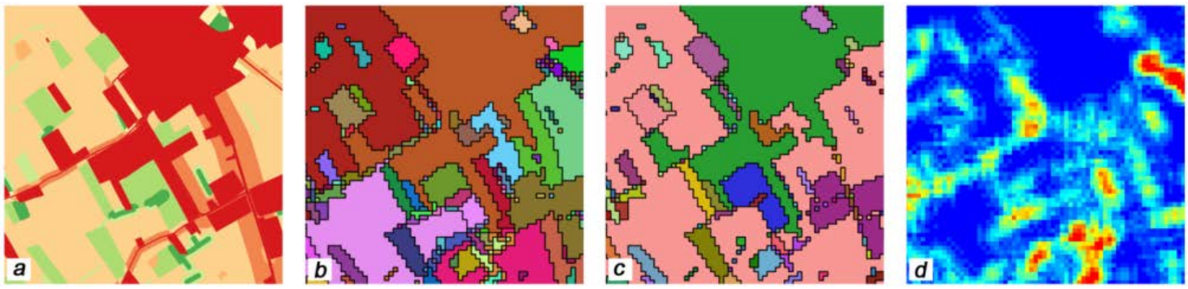

Fig. 5. Example maps showing different interpretations for the landscape: a) Permeability map, in red – green ascending scale, as it derives from the Permeability dataset at 25m resolution. b) "No sampling" derived patches, computed through FRAGSTATS and starting from the previous permeability map; note that each color represents a unique patch. c) "No sampling" derived patches but this time computed with functional groups instead of permeability. This method achieves a lower number of patches (also here, different colors for each patch). d) AI is calculated through the use of a 100 m square moving window at 25 m resolution. AI is represented through a continuous gradient of colors, from blue (aggregated areas) to red (fragmented areas). Here, any defined patch subdivision is avoided

The "no sampling" AI analysis divides the area under study (135,013 ha) into 15,116 patches with an average area of 8.9 ± 223.5 ha, indicating numerous small patches and a highly heterogeneous distribution of their extents. However, mean AI in the landscape equals 64.53, pointing to a general medium-high level of aggregation due to the predominance of aggregated areas attributable to farmlands, but in particular to the urban areas (PIM, 2009). In fact, urbanized classes occupy 32.14% of the total area and have an AI of 82.36, confirming the high aggregation of the urban fabric. Agricultural classes occupy 39.01% of the total area, with variable AI from 64.2 to 71.5, highlighting a high cohesion within subareas. Fragmentation of patches rises with the increasing of permeability classes: the three most permeable classes (P = 10, 11 and 12) together represent only 6.33% of the landscape with the lowest AI values, ranging from 27.63 to 73.99. In fact, hedges and tree rows are interspersed within the agricultural matrix, so they are not continuous. Broad-leaved forests and poplar plantations are rare and heterogeneous, mainly related to the principal water courses. This "no-sampling" description is a function of the chosen point of view (*e.g.* target species or planner), as the number and organization of patches depends on the chosen criterion of classification (Fig. 5b and Fig. 5c). In addition, the physical impact buffers mapped along the linear patches (not existing in reality, but only in the form of graphical elements), alter computations, as highlighted by Gustafson (1998).

The moving window analysis clearly overcomes these limitations, providing a continuous description of the landscape (Fig. 5d). In particular, the AI moving window analysis allowed us to define the landscape as a continuum rather than as a rigid mosaic of adjacent patches, highlighting the nuances and the gradients existing in the landscape (a more realistic interpretation of the landscape in an ecological sense). This analysis shows a certain blurriness due to window overlapping (Fig. 4b). In particular, the connecting corridors (AI = 57.96 ± 14.59) and the dissecting corridors (66.2 ± 19.15) remain the most fragmented functional groups; the artificial (85.63 ± 15.76) and disturbed (70.89 ± 16.25) matrices the most aggregated ones. Moreover, the high interquartile range for any box in Fig. 4b corresponds, in the map, to a continuous AI value gradient between any parcel (or pixel) and its margins (neighboring pixels) within that group.

3.4. Assembling functional and structural aspects of the landscape

P and AI values share only a limited correlation (Pearson's r = -0.35, P < 0.001), likely due to their different geometries and meaning. P refers to physically mapped parcels, whereas AI highlights dynamic transitions between pixels through a moving window, differentiating homogeneous areas from fragmented ones. As shown, P values do not carry information about landscape structure. On the other hand, AI values do not differentiate highly functional parcels (*e.g.* hedges) from less functional ones (*e.g.* roads): thus, differently permeable parcels may share the same AI index and *vice-versa*. The Fuzzy logic approach is capable of combining the two approaches, mitigating their relative limitations. Being a non-linear synthesis of P and AI, FFI emphasizes the character of blurriness, typical of living entities and thresholds and weights of environment interactions of AI and P contribute to explain such blurred dynamics. Thresholds (AI_{max}, AI_{min}, P_{min}, P_{max}) and combination of weights (W1, W2, W3, W4), resulted from questionnaires, show a rather unbalanced subdivision of AI and P ranges: AI_{max} = 78.33 (out of 100), AI_{min} = 30.41, P_{max} = 7.16 (out of 12), P_{min} = 3.08. In other words, AI values above 78.33 and to 100 are plateauing to AI_{max}, and are considered completely aggregated. The same goes for AI values below 30.41, all flattened to null aggregation (AI=0). Similarly, P values above 7.16 and to 12 (maximum P possible) are evenly considered maximum permeability, P values below 3.08 are flattened to the minimum of P=1. The resulting combination weights (W1 = 1, W2 = 0.6, W3 = 0.2, W4 = 0, in a scale from 1, best, to 0, worse) indicate fragmented and highly permeable parcels (W1 - *e.g.* hedges) as the best choice, while aggregated and impermeable land uses (W4 - typically, the urban fabric) as the worst. In between, highly permeable and aggregated parcels (*e.g.* forests and permanent grasslands) are not as good in quality as the best choices. Fragmented and impermeable land uses (typically, the road network) are not as poor as the worst choice.

Mapped FFI values, in an ascending functionality scale from 0 to 1, are numerically shown in Fig. 4c. The connecting corridors show the highest values (FFI = 0.71 ± 0.17), followed by the stepping stones (0.67 ± 0.13) and the valuable matrix (0.59 ± 0.2). The highly permeable, fragmented features (*e.g.*, hedges) are slightly differentiated from the highly permeable but homogeneous areas (*e.g.*, broad-leaved forests). The disturbed matrix shows a lower FFI (0.24 ± 0.19), being rather homogeneous and much less permeable than the previous described situations. Minimum FFI is reached by the artificial matrix, with minimum variation (0.018 ± 0.05), due to high AI and minimum P characterizing contiguous urban areas. Notably, the dissecting corridors (0.062 ± 0.07) are recovered by the null value assigned in Fig. 4a, indicating that functionality should not be assumed *a priori* using functional groups, but that it depends on either structure and species-specific parameters translatable as AI and P: although P is minimum for dissecting corridors, lower AI makes FFI increase, correctly indicating that not all roads in the area act as barriers. It should be noted that boxes in Fig. 4c generally show reduced variance compared with Fig. 4a, due to fuzzy thresholds and the simultaneous consideration of P and AI. Again, a higher variation above the median is noticeable for FFI (Fig. 4c), as opposed to the general tendency of P (Fig. 4a). All groupings except for the artificial matrix show a balanced distribution of FFI values around the median, in that every previous P value now assumes a different ecological meaning if parcels are either aggregated or fragmented. In other words, FFI blends the notion of fragmentation with the evaluation of species movement capabilities. The good ability of FFI to combine AI and P values also becomes evident when considering some elements of agricultural areas (previously underrated by P). Here, fragmented hedges are read and interpreted by FFI as a gradient of increasing functionality from the center of the element to its borders. On the contrary, a gradual and continuous decrease in functionality towards the border is evident when the adjacent landscape coverage is closed by a road.

3.5. Circuit modeling using FFI

Further considerations about FFI values are possible when they are used as a conductance measure in circuit analysis. Cumulated current values obtained (expressed in amperes) are shown in Fig.4d. Current values are maximum for connecting corridors (mean = 0.104 ± 0.23) and stepping stones (0.117 ± 0.28) with high variation. Valuable and disturbed matrices allow slightly less current accumulation (0.06 ± 0.15 and 0.05 ± 0.11, respectively). Dissecting corridors allow minimum current passage (0.02 ± 0.08). Artificial matrix is expectedly inadequate to FFS movement (median = 0), given its minimum functionality. Resulting maps are shown in Fig. 6 for both the whole landscape (at a resolution of 100 m) and a sample area in detail (at a resolution of 25 m); FFI map and reclassified Circuitscape currents are overlaid.

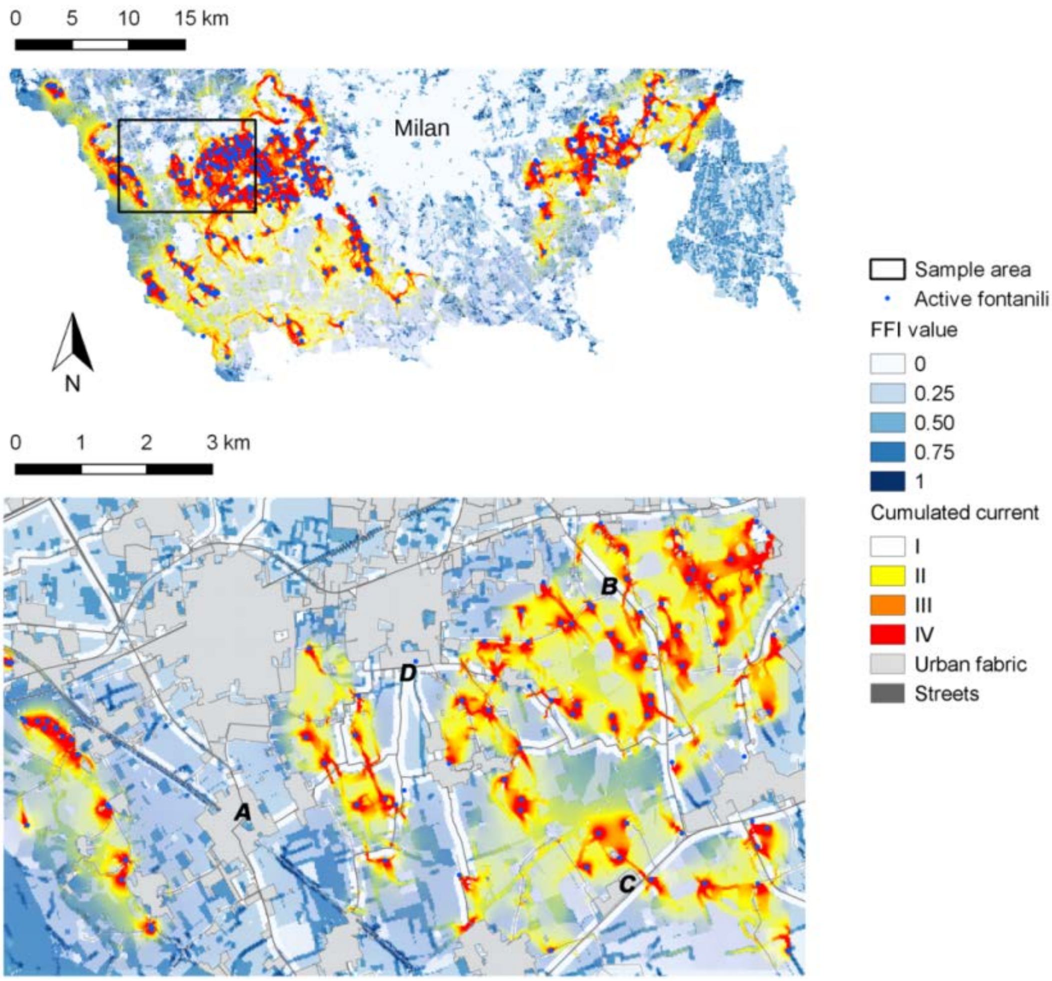

Fig. 6. Result maps of FFI value and cumulative current. Cumulative current has been reclassified in four ascending classes (I – IV, null to high current). The whole study area (upper frame) is presented with 100 m resolution. A sample area (framed in the black rectangle) is expanded in the lower frame, with 25 m resolution. Defined and sharp corridors are highlighted between fontanili. Letters A and D identify barriers to the movement of FFS, whereas letters B and C indicate passages for FFS through two local roads

Diffused urbanization and spatial heterogeneity induce clear isolation between *fontanili* clusters (Fig. 6). Isolation of focal points can be best observed in the upper panel of Fig. 6, where at least three clusters are visible: one lies within forests towards the extreme West; one is located West of Milan and coincides with the arable maize belt, although it expands towards the Southern paddy fields. A third cluster expands from Milan to the East. The Southern part of the area does not provide any significant connection.

A detailed view of connections within clusters is exemplified in the lower panel of Fig. 6. Historically, *fontanili* have been related to agricultural areas as irrigation sources (Bischetti et al., 2012; Kløve et al., 2011) and their "heads" and "ditches" have been surrounded by hedges and wood communities (Minelli et al., 2002 in Kløve et al., 2011). Pinch point corridors highlight those hedges and woods close to the minor irrigation network (hedges act as proxy variables for canals and ditches). In agricultural areas, current dispersal mainly relies on parcels previously grouped as connecting corridors and/or disturbed matrix, where arable land is interspersed with hedges. In the Western area, large connections rely on woods, linked to a big river (Ticino river) previously grouped as stepping stones. In the Eastern portion, connections mostly rely on permanent meadows and hedges, previously grouped as valuable matrix and connecting corridors. It is clear that functional connectivity relies upon parcels' permeability, structure and spatial contiguity, so that mapped results cannot be predicted by land cover classes or functional groups only. In fact, more insightful and realistic results were obtained with the FFI method, if compared to simpler land use quality assessments. As support, roads and contiguous urban areas mainly represent barriers (Fig. 6, letters A, D), but in few cases minor roads are crossed by current (Fig. 6, letters B, C). This is the case with ditches and road crossings, where hydraulic contiguity is ensured by siphons and hedges present at the roadsides. FFI is here able to detect a functionality gradient that is possible to use to highlight candidate corridors. By neglecting the structural gradient, Circuitscape would have considered all roads as barriers due to the null conductance attributed to them through LUC permeability. The same applies to watercourses, that could act as connecting corridors (West and East of Milan) or even dissecting corridors (South of Milan) depending on water quality, permeability and fragmentation gradients of the neighbouring landscape units. This "double characteristic of some barriers", using a definition by Pelorosso et al. (2015), seems to be well considered, evaluated and resolved by the methodology presented. Taken together, these data provide a good estimate of the accuracy and consistency of the values produced by the expert group. The validation of expert knowledge may come by the comparison with empirical data (Bart, 2006) and with the predictions derived from models produced by processing real data (Pullinger and Johnson, 2010; Drescher and Perera, 2010a & 2010b; Iglecia et al., 2012). In the case of *fontanili*, the goodness of expert evaluations is backed by the faithful description

FFI makes of ecological corridors (Fig. 6). Such data, consistent with reality, sustain the accuracy and the quality of the information derived by the expert Group, which provide directions and values that underlie the construction of the entire path leading the FFI application.

3.6. Concerning FFI, corridors and planning scales

The mapped results in Figure 6 allow one to discuss the behavior of FFI at different scales. The FFI map for the entire area (at 100m) was compared to a smaller sample area (at 25m). Firstly, the visual comparison of maps ended with the expert panel agreement about the two layers' similarity. However the spatial Pearson's correlation between the two FFI maps ($r = 0.774$, $P < 0.001$) indicated that the unexplained variance was equal to 40%, which may be interpreted as a loss of detail caused by the coarser resolution. High variation still clearly depends on the input layers' resolution (for the two P raster layers, $r = 0.845$, $P < 0.001$, 28.6% of unexplained variance). As FFI depends on P resolution and on dimensions/shape of the moving window through which AI is calculated (which also depends on P, in turn), a large amount of inconsistencies between outputs seems ascribable to input detail, rather than caused by the formulation of FFI itself.

Two important aspects of corridors produced through FFI should be highlighted. On the one hand, corridors at the two scales are visually comparable, as also shown by McRae et al. (2008). On the other hand, a complete Circuitscape run with 371 focal nodes and more than 2 million FFI cells at 25 m resolution was not possible even using a high-performance laptop, making this step a computational bottleneck. With the FFI cells at 100 m resolution, instead, the process is computed in a timeframe of minutes. Therefore, Circuitscape had been applied to the whole map at 100 m resolution, and on limited sample areas of relevant interest at 25 m resolution (as in Fig. 6, lower panel). Since the output routes were limited to such areas with fewer cells and focal nodes, we cannot deepen a critical comparison of scales. However, we found the simultaneous interpretation of FFI and current maps could support planning processes both at regional level (wide area) and municipality level (detail). In agreement with Scolozzi and Geneletti (2012) and Pelorosso et al. (2015), planning scale and area extension should be (and were) decided respecting both planning demands and specific ecological characteristics of the landscape, also considering pertinence to target species in question, data availability, data retrieval costs and computation times.

4. Conclusions

We have produced a tool blending information about qualitative traits of the landscape (permeability) with its structural features (the Aggregation Index), naming it the Fuzzy Functionality Index (FFI). FFI aims to fill the gap between structural connectivity, exclusively based on landscape structure (geometry), and functional connectivity as an ecological issue, so as to better describe the potentiality of movement of focus species. In FFI computation, fragmentation (or, inversely, aggregation) is not meant as a structural counterpart of connectivity, but acts itself as a constituent of an assessment process of functionality in a non-linear, participative, focal-species oriented fuzzy approach.

The use of a moving window let us investigate landscape structure by means of a continuous surface, exempt from a rigid patch-tiling process. The combination of such gradients with specific permeability was parameterized and validated through simple questionnaires, involving an expert panel. Our results show that FFI is able to go beyond an *a priori* functional classification of land cover classes, often adopted by land use managers. FFI potential is underlined by its application on *fontanili,* particular and endangered biotopes which occur in a highly urbanized and fragmented area. The results obtained were explored and validated by a multidisciplinary expert panel and by land use managers, closing the loop started from landscape evaluation at the onset of the study. In the complex process, the proposed method followed an efficient, high-throughput and open-source pipeline to support landscape management in a typical degraded and fragmented landscape. This is of particular relevance when considering the fast pace at which land degradation is affecting wider and wider areas of the world.

Overall, we have shown that the whole process is very flexible. It permits one to assemble available geographic data, participants' knowledge and open source software to obtain a simple-to-read, easy-to-interpret mapped index at virtually no cost. This is a strategic and overriding concern, since it can promote the application of planning strategies to preserve the integrity of the landscape even in developing countries, which often lack relevant budgets but are more and more affected by uncontrolled land consumption.

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Supplementary Material: questionnaire for the Fuzzy Functionality Index (FFI) analysis

First step: identification of permeability thresholds

A permeability (P) map of the area, with values ranging from 1 (minimum permeability) to 12 (maximum permeability) was presented to the panel. At the same time, a table comprising LUC classes and their proposed permeability values (similar to Table 1) was shown to the panel. In this way, the panel was allowed to recognize mapped parcels, then verify and validate the permeability value proposed for each land use type in the table, or suggest a new value based on their expertise and on the observation of maps. For this kind of questionnaire, we suggest the use of simple and effective coloring schemes in maps, such as yellow-to-green, to indicate to the ascending scale of permeability of the index. A straightforward interpretation of the variable can be thus achieved. An example map, cropped from the real one shown to the panel, is shown in Figure S1.

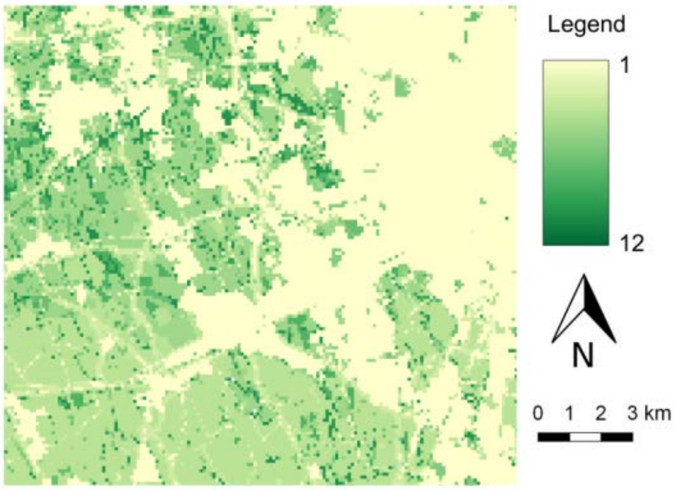

Figure S1. Sample box of the permeability map proposed to the expert panel

Then, we invited the expert panel to reclassify the whole permeability scale in three groups: i) slightly permeable, ii) averagely permeable, and iii) highly permeable land use classes. Finally, we asked them to propose the two threshold values that delimited such classification identifying, at the same time, the two P thresholds needed: P_{min}, under which all values represent the minimum permeability; P_{max}, above which all values indicate equally high permeability of parcels. To support the answerers, a detailed legend with 12 classes was provided. The threshold values were needed to reclassify P into an S-shaped curve as described in the Material and Methods section.

Second step: identification of Aggregation Index thresholds

A map derived from the *no sampling* analysis by FRAGSTATS software was created based on the permeability value as the key variable. Such a map indicated each patch of equal permeability in the study area, highlighting them with different colors. It was proposed to the expert panel to show them the overall fragmentation of the territory. Since each mapped patch had different colors, the degree of heterogeneity (high or low) and the structural aspect of the landscape were sufficiently easy to understand by the experts.

Then, several moving window analysis maps were presented to the panel, depicting numerous fragmentation indices, computed on the landscape. After a brief inspection with the guidance of the authors, the panel confirmed that the Aggregation Index was the simplest and most effective one to describe the landscape structure. This map highlighted, through an easily readable color gradient, the dynamic transitions between former patches, in terms of the Aggregation Index (AI). It was also explained that the moving window, being 500 m sided, was able to intersect more than one patch at a time (since the minimum patch was a 100 m sided square), favoring a more accurate reading of gradients between patches. In the case of AI, a new color scheme was necessary to avoid possible biasing. This decision was taken because AI values are not easily interpretable. In general, fragmentation measures cannot be interpreted as “more is better” or, *vice-versa*, “less is better”, in terms of ecological functionality, because they do not carry any species specific information. The formerly presented color scheme (yellow-to-green) was thus avoided and the chosen one (green-white-purple) is depicted in Figure S2, applied to a real cropped AI map.

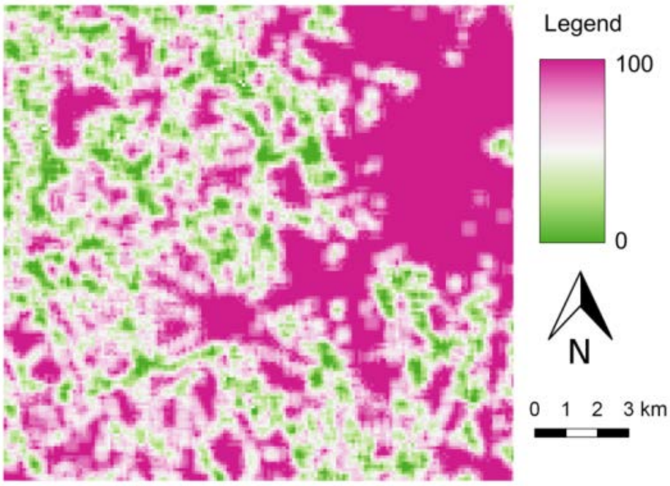

Figure S2. Sample box of the Aggregation Index map proposed to the expert panel

AI and P maps were presented together to the expert panel. We specified that a certain AI value (represented by color in the map) could have been related to areas characterized by high or low P without linearity: AI was indeed able to put in evidence only the structure of the landscape, but not necessarily its P and *vice-versa*. To be clearer, we indicated some examples in maps: both aggregated forests or urban zones could have the same AI value if their aggregation measure was equal; on the other hand, both roads or hedges could have the same AI value if equally fragmented. In both cases, it was evident that the ecological meanings of those areas were however totally different.

Then, as happened for the permeability map, we invited the experts to carefully suggest two thresholds also for AI, the first (AI_{max}) over which everything was similarly aggregated, the second (AI_{min}) under which everything was similarly fragmented. To aid the answerers, a detailed legend with 12 classes was provided. The threshold values were needed to reclassify AI into an S-shaped curve as described in the Material and Methods section.

<u>Third step: identification of combination weights</u>

Four possible combinations between the formerly proposed thresholds were required by the fuzzy procedure, to make possible a linkage between AI and P. Each combination needed the attribution of a numerical weight (W1 to W4) to become a parameter for the fuzzy procedure. Such weights merely represent the “scores” of combinations of variables, in order to achieve the final reclassification of the two in FFI. We presented and explained the four possible combinations to the expert panel (all the intermediate possibilities are not excluded, but computed by the fuzzy procedure):

W1) P max and AI min (that means, highly permeable and fragmented areas);

W2) P max and AI max (that means, highly permeable and aggregated areas);

W3) P min and AI min (that means, less permeable, fragmented areas);

W4) P min and AI max (that means, less permeable, aggregated areas).

We invited the expert panel to attribute a score to each of the four combinations, indicating their efficacy in improving and protecting ecological connections between *fontanili*, the study target. The proposed evaluative scale, divided into seven scores, was therefore compiled: worst (weight = 1), insufficient (weight = 0.8), poor (weight = 0.6), sufficient (weight = 0.4), good (weight = 0.2), very good (weight = 0.1), best (weight = 0). Only the seven judgment scores, without the numerical weights, were shown to the panel during the response process to avoid biases.

<u>Fourth step: averaging thresholds and weights</u>

All thresholds (P_{min}, P_{max}, AI_{min}, AI_{max}) and weights (W1, W2, W3, W4) derived from the questionnaires were averaged on the expert panel size (in our case, n = 28) and the mean values rounded. The resulting values were used as parameters for the Fuzzy Functionality Index computation. Our results can be found in the Results and Discussion section.

<u>Questions we proposed to the expert panel</u>

The questions were not presented at the same time and we carried out several consequent meetings with the panel of experts.

1) You can see here a permeability map of the area, beside a table indicating LUC classes and their relative permeability. Given the target species we focus on and your knowledge about the area, do you agree with our proposed values? If not, suggest new values for certain land use classes and explain your reasons.

2) Thresholds are needed for our approach and you are requested to participate in their assignation. Looking at the map and the LUC table thoroughly, please divide the permeability range into three sections (slightly, averagely and highly permeable) and indicate which values of permeability (in the range 1 to 12) would discriminate those sections.

3) You can see here a map that shows you the amount of equally permeable patches in the area. Every color indicates a different patch, so the more colors, the more heterogeneous is the landscape. Do you recognize the landscape pattern based on your expertise on the area?

4) Which one of the following moving windows indices best describes the landscape pattern in your opinion?

5) You can see here a map depicting the Aggregation Index for the area, calculated through a moving window. Please divide the AI into three sections (mostly fragmented, average, mostly aggregated) as you did for permeability and indicate the values that would discriminate those sections.

6) [After the explanation of combinations between thresholds] You are requested to assign a score to each combination, so please indicate for each of them (W1, W2, W3, W4) how much it is ecologically functional in respect to the target species. We propose the following scores: worst, insufficient, poor, sufficient, good, very good, best. Please explain your choices.

3. Meadows species composition, biodiversity and forage value in an Alpine district: relationships with environmental and dairy farm management variables

Submitted to Agriculture, Ecosystems and Environment. Under review

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Abstract

Alpine meadows have been exposed to profound management shifts in modern times: changes in the plant species composition and biodiversity losses are known issues within Alpine contexts, often occurring in favor of augmented foraging capabilities. In this study, we analyzed the relationships between the plant species composition, biodiversity and forage value of meadows and two sets of variables, environmental and management ones, in an Alpine dairy district in Northern Italy. The management variables explained marginal variance: only the number of cuts per year remarkably explained the plant species composition and biodiversity of coenoses. The number of cattle and the field applied nitrogen marginally described the most intensively managed communities. Overall, the environmental and topographic variables better described the variability of responses. In particular, an increase of the Landolt Nutrient Index was associated with an increase of the forage value opposite to a decrease of the Shannon Index. The negative correlation between the two responses highlights a known dilemma which especially refers to high altitude communities as the ones under study, which are subjected to important environmental constraints. Some *taxa* as *Anthriscus sylvestris*, *Heracleum sphondylium* at the lower altitudes, *Rumex acetosa* and *Polygonum bistorta* in the humid meadows at higher altitudes, were found to critically unbalance the species composition thus the overall biodiversity. This is certainly the most critical finding, explainable by the late first cuttings commonly adopted by all farmers, other than being a result of long-term intensive management strategies. Overall, homologated

management choices could not explain the wide ecological variability investigated, but indeed they made possible to understand how the system should be deeply revised, in respect to limiting environmental constraints and community productive capabilities.

Keywords

Alpine meadows; functional agro-biodiversity; dairy farms; sustainability; variance partitioning

1. Introduction

Meadows and pastures have always been the forage basis for livestock breeding in the Alps and largely characterize the Alpine landscape (Ellenberg, 1988; Fava et al., 2010; Monteiro et al., 2013). In particular, meadows ensure feed supplies for the critical periods of vegetative rest, whereas pastures support cattle feeding in the summer. In the last decades, these two semi-natural agro-ecosystems have gone through widespread degradation processes, as a result of profound socio-economic shifts (Bätzing, 2003) and equally deep changes in the farming strategies. In particular, two main kinds of alteration have involved meadows: the land abandonment (Gellrich et al., 2007; Hopkins and Holz, 2006) and the intensification of livestock systems (Andrighetto et al., 2003; Strijker, 2005; Guerci et al., 2014).

The land abandonment phenomenon concerns the most marginal parcels, mainly the steeper ones, less-accessible and more difficult to manage. Often these have been converted to grazing (Rudmann-Maurer et al., 2008) while, in parallel, labor diminished in parcels yielding only small returns (Tasser and Tappeiner, 2002). In addition, in several arid mountain regions, the abandonment of grasslands has been due to changes in the irrigation regimes, as modernization and rationalization of agriculture led to the exclusion of marginal areas with poor accessibility, with the consequence of decreased productivity and land use conversion (Werner, 1995; Riedener et al., 2014). Another general issue, the high rates of urbanization in the lower parts of Alpine valleys, was investigated by Monteiro et al. (2011), due both to conversion of grasslands towards other crops or human settlements besides the total land abandonment. These trends are recognized as major multidimensional issues; in fact, the loss of grassland threatens centuries of traditional land use and it impacts its relevant ecological and economical values at the same time (Monteiro et al., 2011; Poschlod and WallisDeVries, 2002), especially regarding the need of food supply (Ceballos et al., 2010), the lack of forage production (Liu et al., 2006) and the loss of intrinsic biodiversity (Niedrist et al., 2009) among others. In fact, such dynamics have been remarkable, therefore Alpine species-rich grasslands have been placed among the most threatened ecosystems in Europe (FAO, 2008; Gusmeroli et al., 2012).The intensification of livestock systems, instead, has had consequences for the most accessible and productive meadows. Farms in the

Alpine context have become larger and more modern (Streifender et al., 2007) and often specialized breeds such as Holstein Friesian and Brown Swiss substituted traditional cattle, the latter more prone to tolerate constrained environments as the Alpine one (Scotton et al., 2014). Cattle have reached high standards of milk production and feeding rations earned higher energy and protein contents, often by purchasing concentrates from plain areas (Sturaro et al., 2009), with the drawbacks of opening nutrient cycles (Penati et al., 2011). Increasing stocking rates have characterized most of the enlarging farms (Penati et al., 2013), therefore nitrogen loadings on the field have significantly increased (Gusmeroli et al., 2012; van der Hoek et al., 2004) as sometimes happened for the number of cuts (Scotton et al., 2014). These factors have provided an undeniable increase in hay biomass yield, though they have exerted strong negative effects on the ecosystem (Plantureux et al., 1987), especially in the case of unbalanced cutting frequencies in respect to fertilization rates (Dietl and Lehmann, 2004). The eutrophication of coenoses, the appearance of nitrophilous species and the reduction of biodiversity are among the major negative effects (Marini et al., 2008), mostly penalizing the species linked to traditional management (Prosser, 2001). In utmost cases, new typologies of meadows appeared with untypical plant species compositions (Scotton et al., 2014), which seem far from traditional ones that connoted Alpine landscapes for centuries (Ellenberg, 1988). Besides, landscape-scaled alterations such as the homogenization of the landscape matrices and the fragmentation of grassland occurred (Tscharntke et al., 2005), thus shifting biodiversity patterns and altering management strategies such as hay-making processes and livestock grazing scheduling.

The restoration of degraded grasslands, together with the preservation of their extension, is the primary condition for the maintenance of viable farming systems in the Alps. This requires accurate knowledge of vegetation and its determinants, which are partly natural (environmental constraints) and partly anthropogenic (management choices). The knowledge of vegetation and its determinants is also the prerequisite to guide management strategies towards the sustainability of farming processes and to ensure the production of good quality forage.

In this research we evaluated the relative importance of environmental and management factors on plant species composition, biodiversity and forage value of meadows in an area of the European Central Alps. Until now, few studies have been devoted to the topic, probably because of the burden of field investigations and the difficult interpretation of the data. Naturalistic studies have mainly focused on plant species composition and biodiversity, while agronomic studies have mostly investigated the productive aspects. In this work, we tried to combine the two approaches, with the aim to respect multi-functionality, which is one of the peculiar characteristics of grasslands.

2. Materials and methods

2.1. Study area

The study area is located in the upper Valtellina Valley, district of Sondrio, Northern Italy (46°28'06.3" N; 10°22'12.4" E) namely within the territory of the Alta Valtellina Upland Authority (Figure 1).

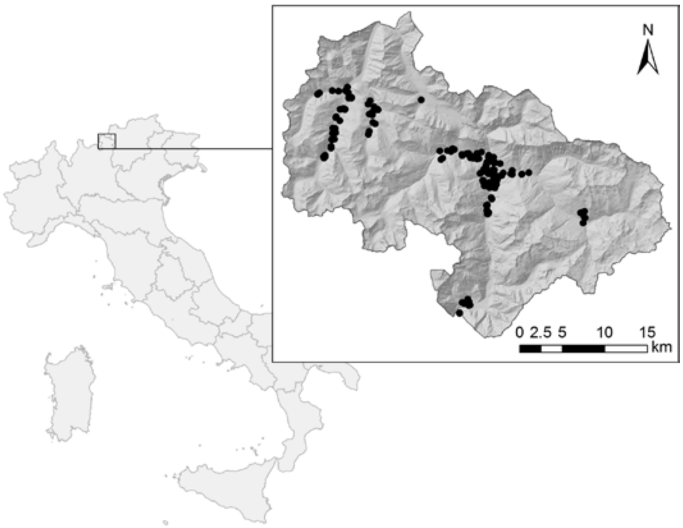

Figure 1. Overview of the surveyed meadows (dots) within the boundaries of the Alta Valtellina Upland Authority

The alluvial plain is relatively small, compared to the numerous alluvial cones, due to the high altitudes ranging from 628 to 3850 m a.s.l. and to the mean slope of 56%, according to the Regional Geographical Database (RGDB, 2015). In detail, the surveyed portion of meadows in the area of interest stands within a wide range of altitudes, from 823 to 2221 m a.s.l. with a mean of 1466 m a.s.l. and 14.6% slope on the average. Data from the Worldclim database (Hijmans et al., 2005) indicate a mean annual rainfall of 769 mm and a mean annual temperature of 6.5°C for the location of Bormio. At the higher altitudes of Livigno municipality, total rainfall equals 886 mm and mean temperature 2.9°C. Agricultural activities include a relatively large number of dairy cattle farms, thus meadows represent the prevalent land use class in the lower portions, while pastures and spruce forests dominate the highlands (Gusmeroli et al., 2004). Meadows of the area are phytosociologically attributable to the associations of *Arrhenatheretum elatioris* and *Trisetetum flavescentis*. *Arrhenatheretum elatioris* occupies the lowest and flat areas, whereas the higher plots are dominated by *Trisetetum flavescentis*. The higher the anthropic pressure, the lower becomes biodiversity, with few species becoming dominant (*Anthriscus sylvestris*, *Polygonum bistorta* and *Heracleum sphondylium* among others). Otherwise, where management becomes more extensive, coenoses tend to preserve their characters of naturalness.

This research concerned the meadows of dairy farms of the area with more than 20 LU (livestock units). Smaller farms were ignored, given the impossibility to recover reliable management information. The surveyed farms were 23, with little more than 30 LU on average, that represent almost half of the milking cows raised in the context. Cows are mainly Italian Brown breed and their individual daily milk production is less than 20 kg, on the average. Livestock density is high on the average, being of 2.50 LU ha^{-1}. Consequently, feed self-sufficiency is low, less than fifty percent on the average, similarly to other Alpine areas (Cozzi et al., 2006; Giustini et al., 2007). However, large differences are noticeable between farms.

2.2. Management data

Management strategies were surveyed by proposing *ad hoc* questionnaires to farmers. Questionnaires regarded livestock, cattle breeds, their age and number, stocking rates, feed rations, their costs and composition, dairy production, fertilization, hay-making processes, number of cuts and overall structure of the buildings. All declared variables were mainly referring to the past year, except for steady data as building dimensions or meadows extension. The declared hay production was verified by expert opinion and based on experience gained by the authors in previous surveys (data not shown). A nitrogen farm gate balance was performed (Thomassen and de Boer, 2005; Penati et al., 2011) to calculate the field applied nitrogen, collecting data about the inputs of concentrates and forages and the dairy production leaving the farm.

2.3. Floristic surveys and environmental data

Meadows were analyzed in 191 randomly selected plots (Figure 1), by layering on farms, altitude and slope, in order to capture all the variability. In each plot, a 10 m $\times$ 10 m square was randomly chosen, avoiding field borders to reduce edge effects. The vegetation included into the chosen plots was investigated through phytosociological relevés in the years 2014 and 2015, according to the Braun-Blanquet methodology (Braun-Blanquet, 1964), and visually estimating species coverage on a percentage scale. Aspect, slope, geographical coordinates and altitude were collected with a DGPS. Geographical data were integrated and corrected with the creation of a Digital Elevation Model based on RGDB. The distance occurring from the surveyed plots to farm centers was computed in straight line, as the fragmentation of parcels and the lack of precise data about unpaved tracks did not permit detailed route analyses. All geographical analyses involved in the creation of a geographical database were performed with ESRI ArcGIS 10.2.2 (ESRI, 2014).

To analyze the biodiversity of meadows, the Species richness, the Shannon Index (Shannon, 1948) and the Species Evenness (Legendre and Legendre, 1979) were obtained for each plot. The forage value was estimated according to Klapp–Stählin (Werner and Paulissen, 1991-1992) and weighted on the percentage cover of species. This index evaluates the preference of a plant species for cattle, when it is growing in its natural state within the plant community. This index ranges from - 1 (refused or poisonous species) to 8 (highest preference), with 0 referring to species without any grazing interest. In principle, an increase of the index corresponds to an increase in the nutritional value and digestibility of the species (Gusmeroli et al., 2007). Ecological indicators proposed by Landolt (1977) were calculated for each plot according to Ter Braak and Barendregt (1986) as they provided information about: soil moisture, soil nutrient availability, humus, soil acidity, soil texture, light, temperature and continentality.

2.4. Data analysis

The effects of the two sets of management [M] and environmental [E] variables (explanatory variables) (Table 1) on plant species composition, Shannon Index and forage value were analyzed by applying the variation partitioning approach (Borcard et al., 1992). The Shannon Index was considered as biodiversity measure, being highly correlated both with the Floristic Richness ($r = 0.78$, $P \leq 0.001$) and with the Species Evenness ($r = 0.76$, $P \leq 0.001$), of which it represents a synthesis. In each set, the most correlated variables (Pearson's $r > 0.7$ or Point-biserial's $r > 0.7$ when dealing with dichotomous variables, as the number of cuts) and the ones with Variance Inflation Factors (VIFs) greater than 10 (Belsley et al., 1980) were excluded to deal with inter-set collinearity.

Table 1. Depiction of management and environmental variables with their descriptive statistics (The management variables are derived from 23 units, environmental ones from 191)

Variables	Description	Units	Mean	SD	CV(%)	Min	Max
Management							
TAL	Total Agricultural land	ha	325.8	422.5	129.7	6.33	1438
TMA	Total meadow area	ha	22.42	25.58	114.1	3.71	123.65
THP	Total hay production	Mg D.M.	94.02	85.66	91.1	16.8	395.7
n.cat	Number of milking cows	n	32.04	23.28	72.6	9	95
Brown	Italian Brown cows over total	%	76.38	35.24	46.1	0	100
HC	Heifers and calves over total cattle	%	58.52	21.87	37.4	16	117
LD	Livestock Density	LU/ha	2.50	2.87	114.7	0.29	12.4
FPCM	Fat Protein Corrected Milk	kg/d	18.24	5.68	31.1	7.59	28.97
IOFC	Income Over Feed Cost	€/d	2.47	2.98	120.7	-1.02	11.63
N.milk	N in milk	%	0.102	0.034	33.0	0.04	0.16
N.field	Farm N balance	kg N/ha	124.1	90.45	72.9	32.95	341.87
N.eff	N efficiency	%	29.61	13.22	44.7	14	76
Con	Concentrates in cow ration	%	37.57	14.72	39.2	5	55
NMKE	N milk efficiency	%	25.22	6.86	27.2	15	41
OHT	Own hay over total hay	%	44.04	17.89	40.6	7.7	80.4
PF	Purchased feed in cow ration	%	49.03	27.26	55.6	-6.7	92.3
Environmental							
Alt	Altitude	m	1466	356	24.27	823	2221
Slo	Slope	%	14.58	13.57	93.04	0	65
Dist	Distance from farm center	m	1752	2084	118.9	23.8	9400
M	Landolt Moisture Index	-	2.89	0.25	8.6	2.15	3.79
L	Landolt Light Index	-	3.46	0.27	7.84	2.41	3.98
T	Landolt Temperature Index	-	2.99	0.37	12.27	1.63	3.74
pH	Landolt acidity Index	-	2.98	0.2	6.65	2.2	3.66
N	Landolt Nutrient Index	-	3.39	0.29	8.55	2.35	3.94
H	Landolt Humus Index	-	3.17	0.24	7.72	2.31	4.19
Tx	Landolt soil Texture Index	-	3.95	0.23	5.72	2.92	4.64

The species × relevés matrix was simplified excluding the species occurring in less than 5% of the relevés (Ter Braak and Smilauer, 2002). A preliminary Detrended Correspondence Analysis with by-segments detrending (DCA) excluded any linear response of species along the environmental gradient, thus the Constrained Correspondence Analysis (CCA) was chosen as the preferable ordination method. A set of partial CCAs was performed and the total inertia was partitioned between the two pure effects [E] and [M] and their shared portion [E ∩ M], after distinct forward selection procedures for the two sets. Statistical significance was tested for pure effects with a Monte Carlo permutation test (n = 999), whereas the shared portion of the effects, being derived from subtraction, was not testable.

The Shannon Index and the forage value were adopted as response variables in multiple linear regression models, where the descriptors were chosen by distinct forward selection procedures, one for each of the two sets of variables. The overall explained variation was partitioned into pure [E] and [M] effects and shared [E ∩ M] ones based on partial Adjusted R^2, and significance was tested with 999 permutations, as above. Also here, the shared portion [E ∩ M] was not testable. Univariate linear regressions were adopted *a posteriori* to describe the relationships between responses and factors, and only the significant ones ($P \leq 0.05$) were retained. Thereafter, the Shannon Index was subjected to one-way ANOVA to test differences between the number of cuts. All statistical procedures were computed through R 3.1.2 (R Core Team, 2014) and the R/vegan package (Oksanen et al., 2015).

3. Results

The forward selection procedures retained the variables shown in Table 2, where the R^2 values indicate the amount of explained variation. Regarding the plant species composition, 13 variables were retained, with nine from the environmental set and four from the management one. The Shannon Index depended on nine variables, seven environmental and two management ones. Its average value was 3.53 ± 0.49, with an overall richness of 204 species. The forage value was explained by nine variables, whereof eight environmental and only a management one. Its average value was 5.25 ± 0.76 on the -1 to 8 ascending scale.

Table 2. Results of the forward selection of the explanatory variables for the CCA procedure and for the multiple linear regressions. F and P values are presented for each variable, together with the overall explained variation (R^2). Variable codes: Alt – Altitude; N – soil Nutrient index; pH – soil acidity index; M – soil Moisture index; T – Temperature index; Tx – soil Texture index; L – Light index; Dist – distance from the farm center; n.cut – number of Cuts; N.field – N to field, derived from the farm's N balance; n.cat – number of cattle; Con – concentrates in diet (%)

Environmental set [E]				Management set [M]			
Variable	F	P	R^2 (%)	Variable	F	P	R^2 (%)
Plant species composition							
Alt	4.91	0.001		n.cut	13.87	0.001	
pH	7.43	0.001		Con	1.88	0.043	
N	7.92	0.001		n.cat	1.28	0.121	
U	8.75	0.001		N.field	1.27	0.122	
T	8.13	0.001					
G	7.10	0.001					
L	6.48	0.001					
Slo	2.55	0.001					
Dist	2.08	0.004					
			32.23				11.17
Shannon Index							
N	84.28	0.001		n.cut	25.31	0.001	
Alt	23,12	0.001		n.cat	5.05	0.026	
L	39.93	0.001					
T	30.34	0.001					
M	17.59	0.001					
Slo	4.37	0.038					
Tx	4.39	0.04					
			53.98				12.36
Forage value							
N	136.09	0.001		N.field	3.34	0.068	
M	95.99	0.001					
L	37.29	0.001					
pH	45.33	0.001					
Alt	6.68	0.010					
T	15.46	0.001					
Dist	6.22	0.013					
Tx	0.44	0.500					
			63.84				1.2

Table 3 indicates the partition of variance, expressed by R^2, between the two explanatory sets [E] and [M], and their shared portion [E∩M]. The most relevant amount of variance is significantly explained by the environmental variables alone: 24.0% for the plant species composition, 43.0% for the Shannon Index and 62.8% for the forage value The management set of variables does not account for any significant variation for any of the responses and the amounts of explained variances are minimum. Also the shared effects between the two sets do account for a small part of the total variability and, in addition, their significance is not provable.

Table 3. Variance partitioning table for the plant species composition, the Shannon Index and the forage value. P values of the testable fractions [E] and [M] and the overall model significance (Monte Carlo Permutation test, n = 999 permutations) are reported in round brackets. The overall R^2 for the plant species composition expresses the total constrained inertia, which is the amount of floristic variability summarized by the explanatory variables in the CCA model

Pure effects		Shared effects	
Environmental [E]	Management [M]	E ∩ M	Overall R^2 (%)
Plant species composition			
24.0 (0.001)	2.98 (0.091)	8.18	35.2 (0.001)
Shannon Index			
43.0 (0.001)	1.42 (0.071)	10.94	55.4 (0.001)
Forage value			
62.8 (0.001)	0.15 (0.45)	1.06	63.9 (0.001)

Figure 2 shows the ordination biplot along the first two axes of Constrained Correspondence Analysis, where the relationships between species and the explanatory variables retained by the forward selection procedure are highlighted. The biplot captures 48.41% of the total constrained inertia, the latter being the amount of floristic variability explainable by the variables retained (28.57% is captured by the first axis and 19.84% by the second axis). To correctly interpret the biplot, the arrows have to be closely considered. Their lengths are proportional to the importance of the explanatory variables they represent, namely to their variance, while the angles between the arrows indicate the degree of correlation between the variables (the more positively correlated they are, the more acute is the angle; on the opposite, the more negatively correlated they are, the more obtuse is the angle; right angles indicate no correlation). Species whose projection on the direction of an arrow falls in the quadrant where the arrow lays are plus variant for that variable; if falling in the opposite quadrant, species show below–average values. The closer to the origin, the closer species are to average values. The closer species are among them in the biplot space, the more likely they behave at the same manner.

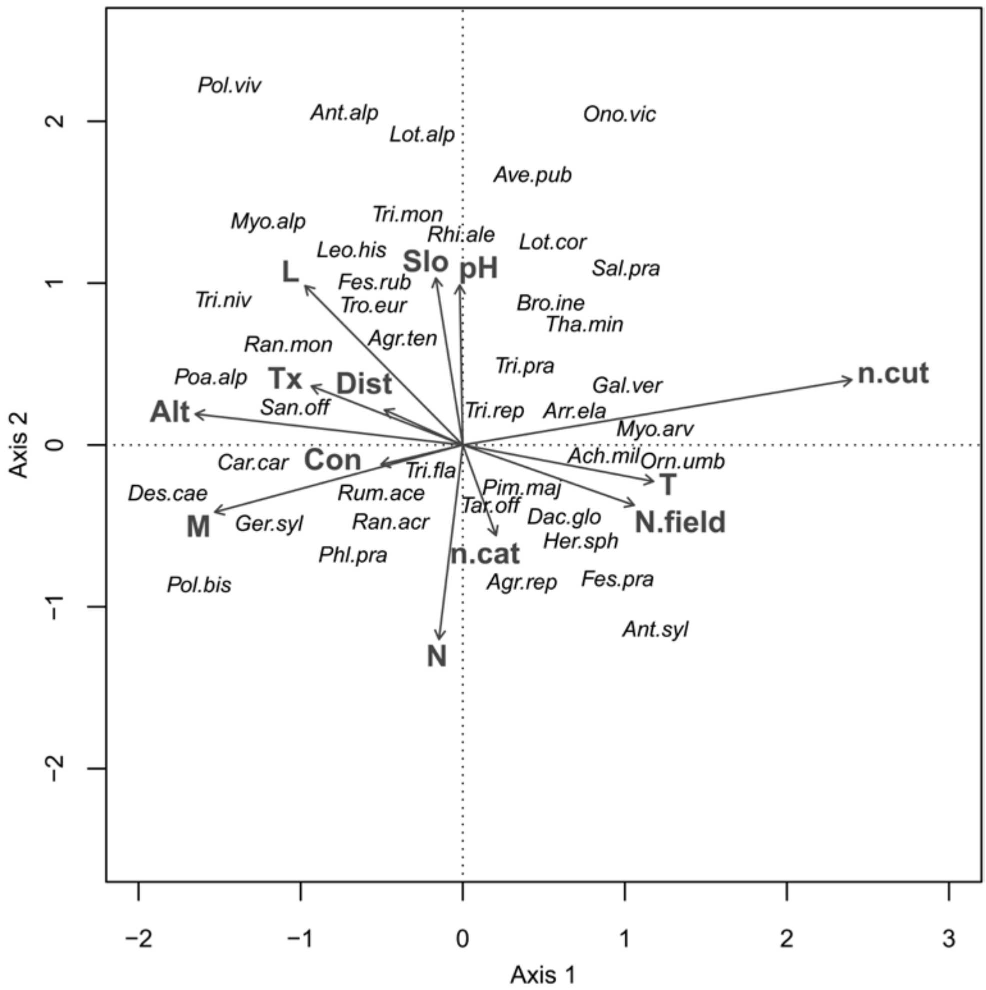

Figure 2. Ordination biplot along the first two axes of the CCA, which summarize 41.48% of the total explainable inertia. Vectors indicate the environmental and management variables, with codes referring to Table 2. Only the most significant species are shown. Species codes: Ach.mil – Achillea millefolium; Agr.rep – Agropyron repens; Agr.ten – Agrostis tenuis; Ant.alp – Anthoxanthum alpinum; Ant.syl – Anthriscus sylvestris; Arr.ela – Arrhenatherum elatius; Ave.pub – Avenula pubescens; Bro.ine – Bromus inermis; Car.car – Carum carvi; Dac.glo – Dactylis glomerata; Des.cae – Deschampsia caespitosa; Fes.pra – Festuca pratensis; Fes.rub – Festuca rubra; Gal.ver – Galium verum; Ger.syl – Geranium sylvaticum; Her.sph – Heracleum sphondylium; Leo.his – Leontodon hispidus; Lot.alp – Lotus alpinus; Lot.cor – Lotus cornicolatus; Myo.alp – Myosotis alpestris; Myo.arv – Myosotis arvensis; Ono.vic – Onobrychis viciifolia; Orn.umb – Ornithogalum umbellatum; Phl.pra – Phleum pratense; Pim.maj – Pimpinella major; Poa.alp – Poa alpina; Pol.bis – Polygonum bistorta; Pol.viv – Polygonum viviparum; Ran.acr – Ranunculus acris; Ran.mon – Ranunculus montanus; Rhi.ale – Rhinanthus alectorolophus; Rum.ace – Rumex acetosa; Sal.pra – Salvia pratensis; San.off – Sanguisorba officinalis; Tha.min – Thalictrum minus; Tar.off – Taraxacum officinale; Tri.fla – Trisetum flavescens; Tri.niv –Trifolium nivale; Tri.pra – Trifolium pratensis; Tri.rep – Trifolium repens; Tri.mon – Trifolium montanum; Tro.eur – Trollius europaeus

Finally, Figures 3 and 4 report the most significant simple regressions on the variables retained by the forward selection procedure for Shannon Index and forage value, respectively.

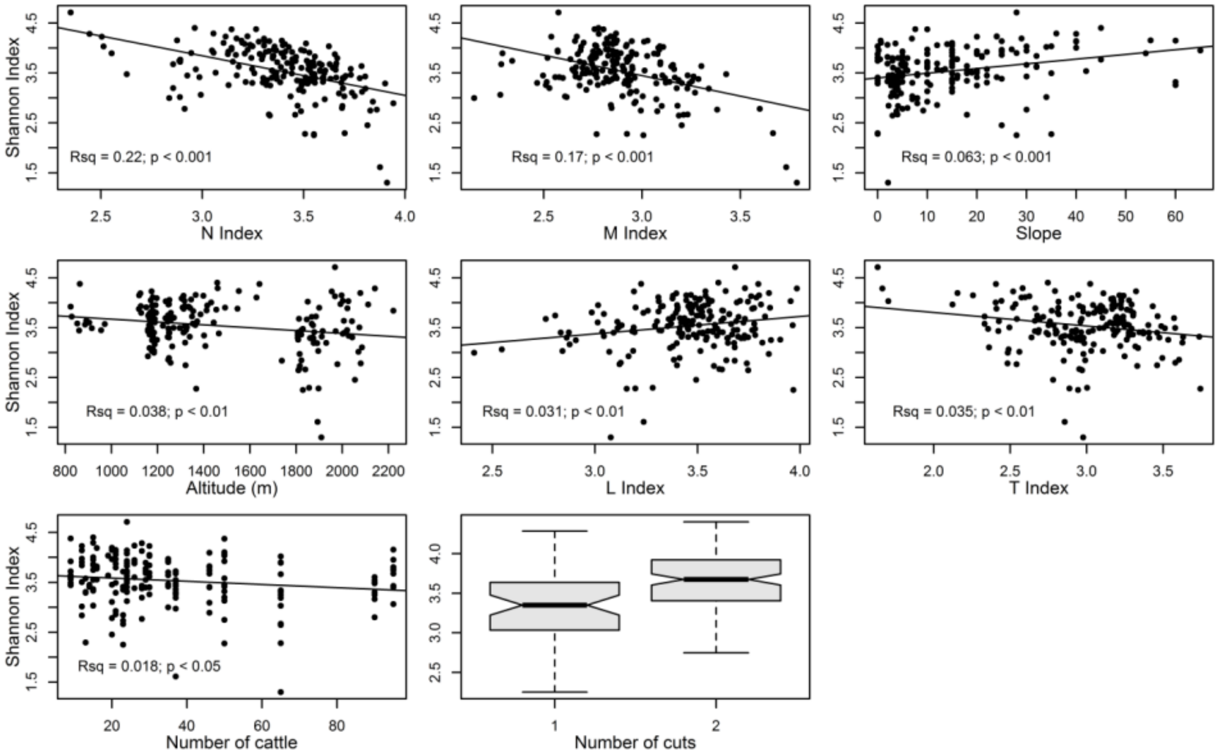

Figure 3. Linear regressions of the Shannon Index on the most important descriptors. The boxplot highlights significant differences between the number of cuts, inspected with one-way ANOVA (P < 0.001). Notches indicate 95% C.I. for the median

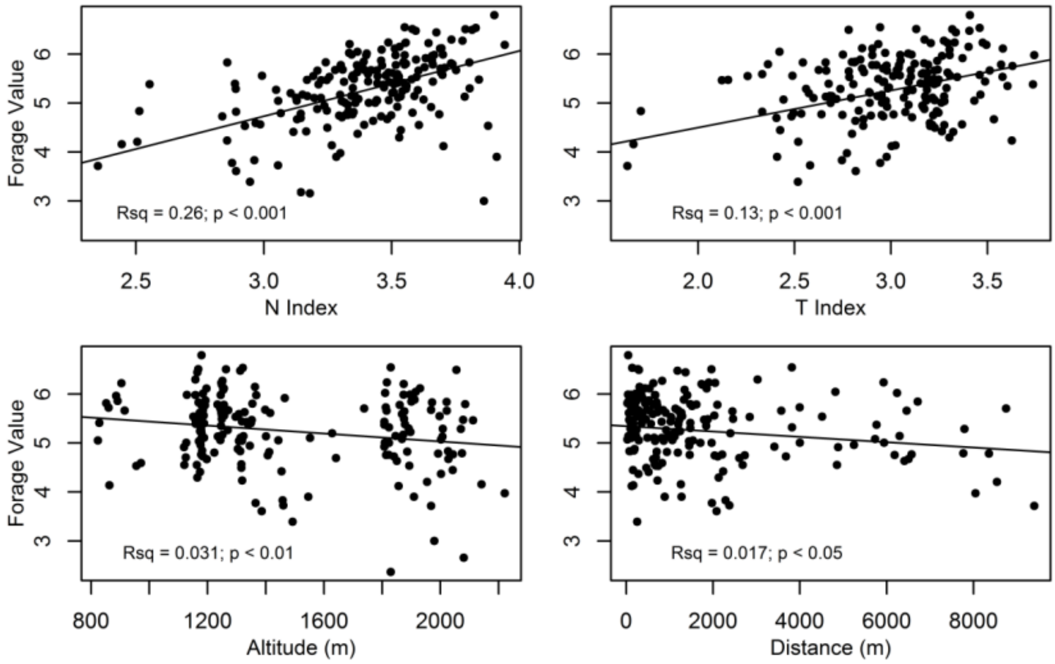

Figure 4. Linear regressions of the Forage Value on the most important descriptors

4. Discussion

Regarding the plant species composition (Figure 2), we observe a general contrast between environmental factors, with the only exception of Landolt Temperature index (T), and the most important management factors, namely the number of cuts and the nitrogen at field (n.cut and N.field). Meadows located at higher altitudes are more well-lighted and hygrophilous and they are less intensively managed (with less cuttings and smaller amounts of fertilizers). The floristic composition of steeper sites, with less acidic soil matrices, is characterized by microthermic and oligotrophic species, such as *Anthoxanthum alpinum, Leontodon hispidus, Lotus alpinus, Myosotis alpestris, Polygonum viviparum, Trifolium nivale* and others. In less steep sites, the increased moisture and fertility favors more hygrophilous and mesotrophic species, in particular *Carum carvi, Deschampsia caespitosa, Geranium sylvaticum, Phleum pratense, Poa alpina, Polygonum bistorta and Ranunculus montanus.*

Meadows at lower altitudes are more intensively managed in general. In the flatter parcels, where soils become more acidic, their coenoses include nitrophilous forbs like *Anthriscus sylvestris* and *Heracleum sphondylium*, together with mesophilous species like *Agropyron repens, Achillea millefolium, Dactylis glomerata, Festuca pratensis, Pimpinella major* and *Taraxacum officinale*. With decreasing slopes and soil acidity, these species are replaced by *Avenula pubescens, Bromus inermis, Onobrychis viciifolia, Lotus corniculatus, Salvia pratensis, Thalictrum minus* and other species.

Arrhenatherum elatius and *Trisetum flavescens* are the most diffused grass species, retrieved in almost all of the relevés and largely specialized in providing good forage. This also happens for some legumes, among which *Trifolium pratense and Trifolium repens*. All of these species are typical of the phytosociological associations of *Arrhenatheretum elatioris* and *Trisetetum flavescentis*, the most common communities of Alpine meadows.

Regarding biodiversity, the Shannon Index (Figure 3) increases by slopes and decreases by altitude. It is also negatively correlated to N, M, and T indices, positively to L one. R^2 values are often small for variables when isolated, although these results are consistent with other studies (*e.g.* Gusmeroli et al., 2012), and this is expected as a consequence of the ecological complexity of coenoses, where the effects of environmental variables have to be conjunctively considered and cannot be disentangled.

The management variables retained in the multiple regression model (Table 2), namely the number of dairy cows and the number of cuts, only explain 12% of the Shannon Index variability, 11% of which is shared with the environmental set. With increasing herd size the index tends to reduce, as highlighted from the regression reported in Figure 3. Concerning the number of cuts, the Shannon Index shows a mean of 3.28 ± 0.60 for parcels cut once, significantly lower ($P \leq 0.001$) than those cut twice (mean = 3.65 ± 0.39). This could be explained by the different altitudes and temperatures of lowland meadows *versus* high elevation ones. The single cut is not a choice but a constraint due to the shorter growing period, within environments at high altitude. In a synergic way, these extreme conditions drastically reduce the number of species able to grow, so the Shannon Index diminishes, especially if exposed to intensive management strategies. Unlike biodiversity, the forage value tends to raise with decreasing elevation and enhancing N and T indices (Figure 4). Indeed, the correlation between the Shannon Index and the forage value was significantly negative, although the coefficient value was rather weak ($r = - 0.16$, $P \leq 0.025$). The forage value also reduces with increasing the distance from the farm center. The only management factor retained by the multiple regression model was the field applied nitrogen, but its explanatory power was negligible.

Overall, these results are similar to those reported by Gusmeroli et al. (2012). They found that a set of management variables and nearby landscape ones did not significantly explain any of the responses, as the major part of the variability was due to environmental factors, referring to a neighboring area at lower altitudes. On the opposite, the Shannon Index was found declining with the number of cuts, in contrast to this work, where an enhancement was found passing from 1 to 2 cuts. This could be explained since higher altitudes are characterized by a large amount of constraints, typically the shorter growing period during summer, the more rigid temperatures and, generally, higher soil moisture content, at least referring to the substrates we studied. In fact, all of these variables were found significant in the explanation of the responses *per se*, although the enormous variability found in this relatively small context did not permit any reasonable explanation of variances at times. Meadows management, including the number of cuts, is therefore dictated by those constraints. In fact, field practices and their scheduling are rather homogeneous for the area, so that the effects of management variables could be masked within the whole context. Another reason for the low explanatory power of management variables is that the huge climatic variability in time corresponds to changes in the scheduled field interventions, mostly referring to the first cut and to fertilization, both variable between the years and certainly depending also on the altitudes and slopes in the area. The N balance, computed for all the involved farms, represents such an example: it has been calculated as the mean nitrogen used to fertilize the total meadow area, because any reliable information could not be derived from interviews about doses, neither typologies (slurry, manure, etc.) and every year variable amounts of organic fertilizer are spread in certain parcels depending on weather conditions. In spite of these premises, data confirm a general tendency to mostly fertilize parcels near to the farm center (field applied nitrogen diminishes with increasing distances, $r = -0.19$, $P \leq 0.01$) also due to the negligible slopes where farm centers rest on.

As found in Scotton et al. (2014) and Gusmeroli et al. (2012), biodiversity and forage production capabilities do not always collimate: the more meadows are fertilized, the higher hay production they get (Gough et al., 2000) and often the forage value increases, but the lower the Shannon Index gets in parallel. That means, a balance between these two ecosystem services of meadows (productive and conservative services) should be advocated especially where environmental constraints weaken the resilience of plant communities.

5. Conclusions

The poor capacity of management factors to explain the species composition, specific biodiversity and forage value of meadows in the explored area is attributable to a substantial homogeneity of practices. Their variability is best explained by environmental variables, due to the fact that meadows are subjected to relevant constraints in this particular area at high altitudes. Nevertheless, the critical presence of nitrophilous forbs puts in evidence an excessive fertilization *versus* the possibilities meadows can support at these elevations, with typical eutrophication consequences. These include unbalanced plant composition, thus decreased biodiversity, and also the forage value is impacted in general, although the latter often augments within intensively managed communities in the lower sections of the area. In synthesis, earlier first cuts and less conspicuous nitrogen application rates are to be considered among the answers to these problems, at least to guarantee a recovery of biodiversity, especially at higher altitudes, even if it would mean a reduction in forage yields.

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4. Comparative analysis of North Italian cropping systems performances in a long-term field experiment

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Abstract

This study compares biomass, Milk Feed Units (MFU) and Crude Proteins (CP) yields in different cropping systems in Northern Italy, over a period of 21 years (1986-2006). Five fodder cropping systems were compared: (i) a one-year double-crop rotation (R1) of autumn-sown Italian ryegrass + spring-sown silage maize; (ii) a three-year rotation (R3) of grain maize (first year), autumn-sown barley + silage maize (second year), and Italian ryegrass + silage maize (third year); (iii) a six-year rotation (R6) of Italian ryegrass + silage maize (years 1,2,3) + mixed meadow of white clover (*Trifolium repens* L.) and tall fescue (*Festuca arundinacea* Schreb.) for hay-making (years 4,5,6); (iv) a continuous grain maize (CM); and (v) a permanent meadow (PM). All cropping systems were subjected to two levels of agronomic inputs: high (A), indicating the amounts of synthetic fertilizers N-P-K, manure and herbicides normally applied by farmers in the region at the start of the survey, and low (B) consisting in a reduction of the amount of fertilizers, both synthetic and manure (-30%) and of the herbicide rates (-25%) compared to treatment A. With regards to cropping systems we found R1 > R3 > R6 > PM > CM in terms of biomass yield, with a slightly different trend for the MFU yield, where CM > PM, whereas R6 > R1 > PM > R3 > CM regarding the CP yield. The two agronomic input treatments always resulted A > B for each parameter, with effects less than proportional to the decrease of inputs and independent from the years. Only CP yields significantly depended on the interaction of the two factors. The five cropping systems also significantly varied between the 21 years of experiment: all of them decreased their yield performances except for PM which improved in biomass, MFU and CP over time. The higher yield variability over the years was that of CM and PM, whereas the three rotations (R1, R3, R6) appeared more stable over time. Overall, the double-crop R1 permitted the highest yields in terms of biomass and MFU, whereas the more complex R6 came out on top in terms of CP, remarkably followed by PM, both due to the presence of white clover, and even though the abundance of forbs increased in time. These findings suggest the importance of complex cropping systems which, despite not always being the most productive in terms of biomass, could provide major quality of fodder besides guaranteeing a remarkable agricultural diversification.

Keywords: Agronomic inputs; Grain maize monoculture; Cropping systems; Permanent meadow

1. Introduction

The agricultural scenario of the Po Valley in Northern Italy has been characterized by intensification processes and over-simplification of cropping systems since the mid 1950's (Giardini and Ziliotto, 1988). Particularly, the intensification of agricultural practices, in accordance to the design of the Common Agricultural Policy (CAP), has had an outstanding importance in the emersion of socio-economic benefits (Tomasoni et al., 2003). In addition to a remarkable increase of crop yields and the reach of higher efficiencies in mechanization, also a specialization of farming systems occurred. On the contrary, significant detrimental effects emerged over time and became of interest in the CAP scenario, above all the simplification of cropping systems, environmental pollution and surpluses of production both due to surpluses of inputted agronomic factors (Parente, 1996). The same increases in yield have been obtained at the expense of organic matter in soil, and more and more increasing energy inputs (fertilizers, irrigation, herbicides, machining).

In the area, one of the most important European agricultural system with a strong trend of urbanization (Pierik et al., 2016), the intensification processes determined a tremendous decrease of the number of farms and cultivated soil surface.

Nowadays it is becoming necessary to assess environmental impacts in agriculture (Gonzalez-García et al., 2012; Poeschl et al., 2012; Lijo et al., 2014), besides searching for cropping alternatives, undeniably shifting towards the extensification of cropping systems. Extensification processes should, however, guarantee the economic sustainability of farming systems (Robertson and Swinton, 2005) also if the reliance on fertilizers and external inputs comes short. A proper choice of crop rotation may alleviate the environmental problems increasing nutrient uptake and keeping unavoidable nutrients losses at tolerable level. Therefore, crop rotations can be regarded as sustainability enhancing mechanisms developed by the farmers (Fresco and Kroonemberg, 1992).

Brankatschk et al. (2015) exemplify the positive effects of cropping rotation systems, starting from the importance of crop residues that influence physical, chemical and biological soil properties and help to maintain or even improve soil fertility over time. In crop rotations, species have the potential to increase the biomass production of the following crops, besides contributing to the sequestration of organic matter in soils (Peoples and Baldock, 2001). According to Cowell et al. (1995) and Zegada-Lizarazu and Monti (2011), crop rotations provide improved soil texture, structure and fertility thus making possible the increase of crop yields even reducing agrochemicals and synthetic fertilizers doses. In addition, weed seed banks could be controlled by crop rotations, particularly concerning the “cleansing” effect of meadows if inserted in rotations (Tomasoni et al., 2003) and suggesting the potential reduction of herbicide doses. It is also undeniable that complex cropping schemes with numerous species and cultivated varieties could improve biodiversity and diversified production, with a view to market requirements and resistance to plant diseases. Similar positive occurrences could be described for permanent meadows, as these agro-ecosystems provide a large variety of public services besides their hay-making function (Gusmeroli et al., 2012).

Large areas of the Po Valley are mainly characterized by two cropping systems (Bacenetti et al., 2015), one of which is a single crop (mostly maize or sorghum), where the crop is cultivated season after season, and a double crop, where the winter crop, usually wheat, barley or triticale, is followed by maize. In a forage-production optic, this arrangement may satisfy the requirements of husbandry, but on the other hand it exerts a strong negative effect on the environment, in terms of nitrate losses (Randall and Goss, 2001), fossil energy required (Pimentel and Patzek, 2005) and N_2O atmosphere emissions (Crutzen et al., 2008) among others. In addition, the cited cropping arrangement appears completely different from the traditional one of Northern Italy that, in the last centuries, mostly relied on permanent meadows and maize, or crop rotations based on multiple consequent crops, rotational and irrigated meadows.

It is clear the need to more deeply investigate all the cropping possibilities as to reinforce the weak agricultural system of the Po Valley, for example, analyzing long-term experiments of differentiated cropping systems and their performances, thus promoting viable solutions for the reduction of environmental burdens and for the strategic programming of farming systems. For this sake, a long-term cropping system experiment was carried on since 1985.

The aim of this study was to compare the performances of five cropping systems over a continuous long-term field trial. The five cropping systems were compared in terms of biomass yield, Milk Feed Units (MFU) yield and Crude Proteins (CP) yield at different agronomic input levels, over time. This could represent a viable approach to tackle agricultural systems programming in Northern Italy (or in similar agronomical environments) in a context of sustainability, where the productive role of agriculture should be fulfilled, both augmenting biodiversity of production and diminishing environmental burdens.

2. Materials and methods

2.1. Study Area and experimental design

Details of the methodology were formerly presented by Onofrii et al. (1993), Tomasoni et al. (2003) and Borrelli et al. (2014). In brief, the experiment, established in 1985 and still ongoing, is located at Lodi, Po Valley, Northern Italy (45°19’ N, 9°30’ E, 81 m a.s.l.). Soils are sandy-loam and classified as mollic Hapludalf, with subacid pH, low in nitrogen, organic matter, and exchangeable potassium, and with good provision of assimilable phosphorus. The major characteristics of the soil in the arable layer (0–0.3 m) are: clay (<0.002 mm) 9.9%; silt (0.05–0.002 mm) 24.7%; sand (2–0.05 mm) 65.4%; organic matter 0.15 (g kg^{-1}); available soil water 9.76 (m m^{-3}); bulk density 1.58 Mg m^{-3}; pH (H_2O) 6.5; C.E.C. 11.3 ($cmol_{(+)}$ kg^{-1}).

The climate, typical of the lowlands of North-Western Italy, is sub-continental (Figure 1) with an average annual rainfall of 797 mm and an average annual mean daily temperature of 12.9 °C, January being the coldest month (1.1 °C) and July the hottest one (22.9 °C) (Borrelli and Tomasoni, 2005).

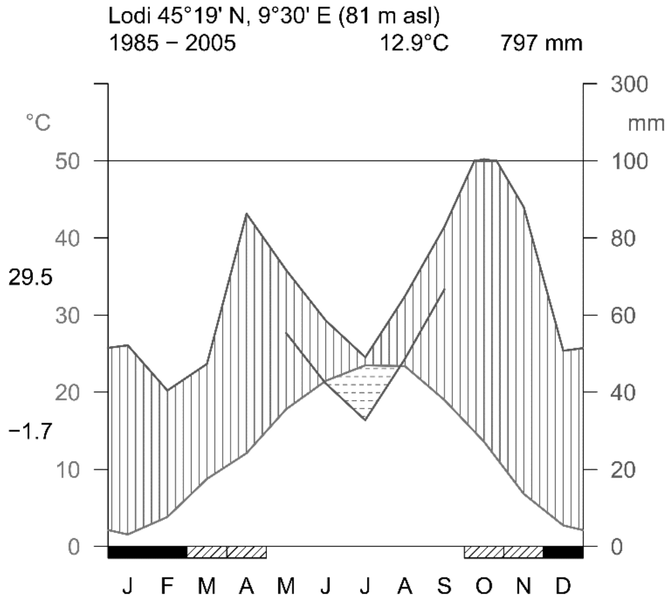

Figure 1. Walter and Lieth climate diagram for the period: 1985-2005

Overall, the experiment compares five cropping systems and two agronomic input levels. The five cropping systems (Table 1) are: (i) an annually-repeated double crop (coded as R1) of autumn-sown Italian ryegrass (*Lolium multiflorum* L.) + spring-sown maize (*Zea mays* L.) both used for silage; (ii) a three-year rotation (R3): autumn-sown barley (*Hordeum vulgare* L.) + spring-sown maize, both for silage / Italian ryegrass + maize (both for silage) / grain maize; (iii) a six-year rotation (R6): 3 years of Italian ryegrass + maize (both for silage) / 3 years of meadow (Ladino white clover, *Trifolium repens* L., + tall fescue, *Festuca arundinacea* Schreb.) for hay making; (iv) a continuous grain maize cropping (CM), and (v) a permanent meadow (PM).

Table 1. The cropping systems under comparison and single crops in detail. IR: Italian ryegrass; SM: silage maize; RM: rotational meadow; SB: barley; GM: grain maize; PM: permanent meadow

Six-year Rotation (R6)	Three-year Rotation (R3)	Annual Rotation (continuous double copping) (R1)	Continuous grain maize cropping (CM)	Permanent meadow (PM)
IR - SM_1	SB - SM	IR - SM	GM	PM
IR - SM_2	IR - SM			
IR - SM_3	GM			
RM_1				
RM_2				
RM_3				

Each cropping systems has received two levels of agronomic inputs, corresponding to high (treatment A) and low (treatment B) conventional agro-techniques conditions for the region, respectively. The treatment A represents the usual agronomic input applied by the farmers of the area at the outset of the experiment, in the year 1985. Treatment B (low input) receives about 70% of the amount of organic and synthetic fertilization and 75% of herbicide amount compared to A. The purpose of such input reduction was to assess whether the yield response was proportional to the reduction of agronomic inputs. The amount of organic and synthetic fertilization applied to individual crops and cropping systems are reported in Table 2.

In treatment B, the herbicide rate has been 4 kg ha^{-1} of (metolachlor + terbuthylazine)-based product applied at the pre-emergence stage (spring) on maize crop, either for silage or grain, in whatever rotation it was contemplated, and 3 kg ha^{-1} of methabenthiazuron-based product applied at the pre-emergence stage (autumn) in the R3 rotation. No other crops have received herbicide applications. A further difference between A and B treatments concerns soil tillage before autumn-sown crops. In the former treatment, the soil has been ploughed to a depth of 30 cm and then rotary-cultivated, while in the latter it has only been rotary-cultivated to a depth of 15 cm. All maize crops, either for silage or grain, in both treatments have been ploughed prior to sowing, and rotary-cultivated along the rows after the plant emergence, also to favor the burial of nitrogen fertilizer (half of total amount) applied at the post-emergence stage. Each year, four border gravitational irrigations, each of ca. 1000 m^3 ha^{-1} volume, have been provided to the whole trial (both treatments). Time of sowing and all other cultural practices have been those typical for each crop in the region. A detailed description of manure and synthetic fertilization and herbicide application rate, along with irrigation and soil tillage schedules can be found in Onofrii et al. (1993) and Borrelli et al. (2014).

Table 2. Amount of organic and synthetic fertilization applied to individual crops and cropping systems

	Synthetic (kg ha^{-1} y^{-1})						Manure (Mg ha^{-1} y^{-1})	
Input	A			B			A	B
Crop	N	P_2O_5	K_2O	N	P_2O_5	K_2O		
Italian ryegrass	150	100	120	105	70	84		
Silage barley	120	100	120	84	70	84		
Silage maize	250	100	100	175	70	70	40	28
Grain maize	250	100	100	175	70	70		
Rotated meadow	125	150	120	87	105	84	40*	28*
Permanent meadow	125	150	120	87	105	84	40	28
				Cropping systems				
R1	400	200	220	280	140	154	40	28
R3	340	167	180	238	117	126		
R6	263	158	170	184	123	119		
CM	250	100	100	175	70	70		
PM	125	150	120	87	105	84		

In the case of CM the maize stover has been returned to the soil, under the assumption that the farm adopting such cropping system does not use crop residues for farmyard manure preparation; in case of grain maize in R3, the stover has not been returned to the soil under the assumption that it is used for farmyard manure preparation.

The experimental design was a strip-plot with three randomized blocks, the main plots being represented by the input levels and the sub-plots by the compared cropping systems. All the phases of crop rotations have been present every year, in each combination of block and input level, to avoid possible confounding effects of the factor year when comparing rotations made up of different phases in different years. This implies that in each block and input level there have been one plot for each annual cropping system (CM, R1 and PM), three crops for the triennial rotation (R3) and six plots for the six-year rotation (R6). Altogether the trial includes 72 plots (12 crop-phases $\times$ 3 blocks $\times$ 2 input levels), each measuring 60 m^2 (6 $\times$ 10 m).

2.2. Data Analysis

Data from 1986 to 2006 were analyzed, excluding further observations due to a slight change in the agronomic inputs adopted. Three parameters were analyzed to compare

* rotational meadows received manure application only in the first year

cropping systems and agronomic input: biomass yield (Yield), Milk Feed Units (MFU) yield and Crude Proteins (CP) yield. The fresh biomass was harvested and weighted on the whole plot surface and a sub-sample from each plot was oven-dried at 60°C, until the dry weight was stable, to determine the dry matter content (Borrelli et al., 2014). Further analyses on the dry biomass aimed to determine the concentration in Milk Feed Units and Crude Proteins. MFU were calculated according to Jarrige (1978) and crude protein were calculated according to FAO (2003).

A factorial ANOVA was performed on the three formerly depicted parameters considering cropping systems (CS) and input treatments (Input) as fixed factors, whilst the Years Of Experiment (YOE) and blocks were considered as random factors. The model was a four-way mixed ANOVA, without replications. For this reason, a Cochran approximate test (Cochran, 1951) was performed for the two experimental factors and their interaction, where the model was not additive. Multiple comparisons were performed through Student-Newman-Keuls test. Interactions with YOE were analyzed through parallelism test of regression lines and Additive Main Effect Multiplicative Interaction (AMMI analysis). The AMMI analysis is generally useful to better visualize the dataset and to explore its pattern and structure in the case of significant interactions between factors (Gollob, 1968; Zobel et al., 1988). The significance of PC axes was obtained through Gollob's Test (Gollob, 1968). All analyses were performed through MSTAT software (MSTAT, 1989) and a spreadsheet. The Principal Component Analysis for the AMMI model was performed using R 3.1.2 (R Core Team, 2015) and the R/agricolae package (de Mendiburu, 2015).

3. Results

The analysis of variance indicates that the factors cropping systems (CS), input levels (Input) and years (YOE) significantly affected biomass yield, Milk Feed Units yield and Crude Proteins yield (Table 3).

Table 3. Means and significance of ANOVA for yield, MFU yield and CP yield in respect to the CS and Input factors. Different letters indicate significant differences at Student-Newman-Keuls test for P = 0.05

	Biomass (Mg DM ha^{-1} y^{-1})		MFU (units ha^{-1} y^{-1})		CP (kg ha^{-1} y^{-1})	
Cropping systems						
CM	8.97	e	10927	d	735	e
PM	11.17	d	7685	e	1714	c
R1	25.6	a	21408	a	1744	b
R3	19.75	b	17621	b	1364	d
R6	18.81	c	15027	c	1884	a
Inputs						
A	17.9	a	15496	a	1635	a
B	15.82	b	13572	b	1341	b
Significance						
CS	0.001		0.001		0.001	
Input	0.001		0.001		0.001	
YOE	0.001		0.001		0.001	
CS × Input	ns		ns		0.05	
CS × YOE	0.001		0.001		0.001	
Input × YOE	ns		ns		ns	
CS × Input × YOE	0.001		0.001		0.01	

Biomass yields for the three rotations R1, R3, R6 are significantly higher than PM and CM, the latter being the less productive crop system, with a mean of 8.97 Mg DM ha^{-1} y^{-1}. The 1-year rotation R1 permits the highest harvested biomass, with 25.6 Mg DM ha^{-1} y^{-1} on the average, significantly greater than the other rotation systems (+5.85 Mg DM ha^{-1} y^{-1} compared to R3 and +6.79 Mg DM ha^{-1} y^{-1} compared to R6, respectively +29.6% and +36,1%). Even among R3 and R6 there is a meaningful difference, in favor of the former (+0.91 Mg DM ha^{-1} y^{-1}, *i.e.* +5.0%), as well as different are the yields of CM and PM, with a margin for PM of 2.2 Mg DM ha^{-1} y^{-1} (+24.5%).

Also in terms of Milk Feed Units yield, the three cropping systems are superior to PM and CM. R1 still obtains the highest value: 21408 units ha^{-1} y^{-1}, roughly three times the lowest value provided by the permanent meadow (7685 units ha^{-1} y^{-1}). As for the biomass, the three rotation systems significantly differ between them, with gaps of 3787 units ha^{-1} y^{-1} (+21.5%) between R1 and R3, of 6381 units ha^{-1} y^{-1} (+29.8%) between R1 and R6 and of 2594 units ha^{-1} y^{-1} (+17.3%) between R3 and R6. Unlike biomass, CM recorded better performance than PM (+3242 units, *i.e.* +42.2%).

The situation changes in terms of Crude Proteins yield. The most productive cropping system becomes R6, with a yield of 1884 kg ha^{-1} y^{-1} statistically superior to all other treatments. R1 follows, then, in order, PM (with a remarkable value of 1714 kg ha^{-1} y^{-1}), R3 and CM, all separated by significant differences. CM provides only 735 kg ha^{-1} y^{-1}, about 2.5 times less than R6, due to the absence of white clover or other *Leguminosae*.

The agronomic inputs change all the three production parameters. The upper level involves increments of 2.08 Mg DM ha^{-1} y^{-1} (+13.1%) for the biomass, 1924 units ha^{-1} y^{-1} (+14,0%) for energy and 294 kg ha^{-1} y^{-1} (+21.9%) for crude proteins.

The CS × Input interaction is not significant for both biomass and MFU, whereas it is significant (P = 0.05) for Crude Proteins. As shown in Figure 2, the two input levels maintain the same general trend (level A > level B) for all five cropping systems but in case of R1, R6 and PM the reduction of agronomical inputs substantially affects CP whilst in case of CM and R3 the reduction of input quantities exercises little effect. In particular, R6 reaches the highest CP yield (2024 kg ha^{-1} y^{-1}) if subjected to input level A, not significantly higher than R1 with the same agronomic input but much higher than the same rotation with reduced inputs. The permanent meadow, if subjected to input B achieves a CP yield of 1535 kg ha^{-1} y^{-1}, which is not significantly different from R1 with the same input level (1553 kg ha^{-1} y^{-1}). In addition, R3, even if subjected to the input level A does not statistically differentiate from the latter, with 1506 kg ha^{-1} y^{-1}.

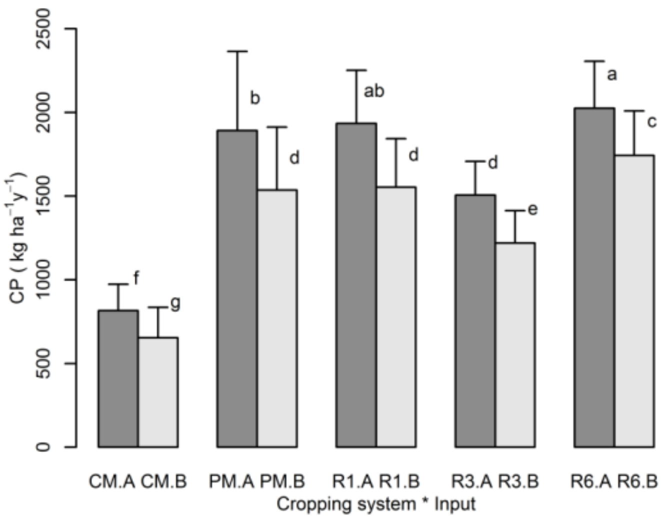

Figure 2. Mean Crude Proteins yields and standard deviation for the five cropping systems, split by the two levels of the agronomic Input (levels A and B, respectively high and low input). Different letters indicate significant differences at Student-Newman-Keuls test for P = 0.05

The effect of the factor Input does not change even between the years (interaction Input × YOE never significant) while the factor CS modifies its behavior over time (significant interaction for all three parameters).

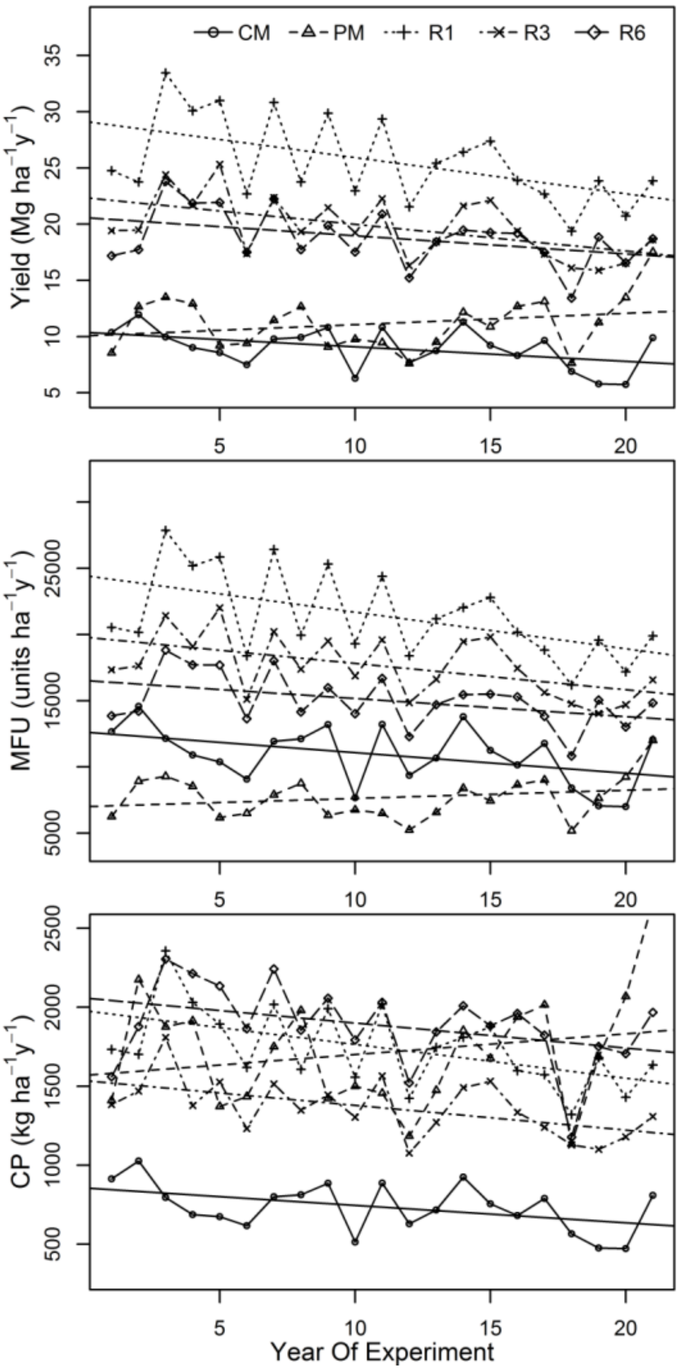

Figure 3. Time course of Biomass yield (Yield), Milk Feed units (MFU) and Crude Proteins (CP) for the five cropping systems over time (YOE from 1 to 21) and linear regression lines

As it results from the regression analysis (Figure 3), the permanent meadow slightly increases Biomass, MFU and CP yields over time, in contrast to the other crop systems, all of them decreasing their performances. The parallelism tests confirmed the different trend of PM with respect to other cropping systems, even if the regression lines for both PM and R6 did not result statistically significant (data not shown). Further information is given by AMMI analysis. The deviance decomposition table indicates the statistical significance of ordination axes through Gollob's Test and the percentage of variance explained by each of them (Table

4). As Gollob's Test has a tendency to be poorly conservative (Cornelius, 1993) and standing the small quantity of variance explained by the third axis, in the cases of Biomass and CP yields only the first two ordination axes were therefore considered.

Table 4. Deviance decomposition table for the AMMI analysis procedure. Significance values of the Gollob's Test are shown for the first principal components

Variance source	DF	SSE	MSE	F	P	Explained MSE (%)
Yield						
Interaction	80	1849544834	23119310			
PC1	23	1338000000	58173913	23.85	0.001	72
PC2	21	376680000	17937143	7.35	0.001	20
PC3	19	99960000	5261053	2.16	0.05	5
Residuals	17	34904833	872621	0.36	ns	2
Milk Feed Units						
Interaction	80	7885658160	98570727			
PC1	23	874800000	38034783	19.48	0.001	67
PC2	21	343740000	16368571	6.71	0.001	26
Residuals	36	20316359	507909	0.21	ns	7
Crude Proteins						
Interaction	80	3174077	39676			
PC1	23	13656000	593739	26.96	0.001	72
PC2	21	4124400	196400	8.92	0.001	22
PC3	19	772200	40642	1.85	0.05	4
Residuals	17	491867	12297	0.01	ns	3

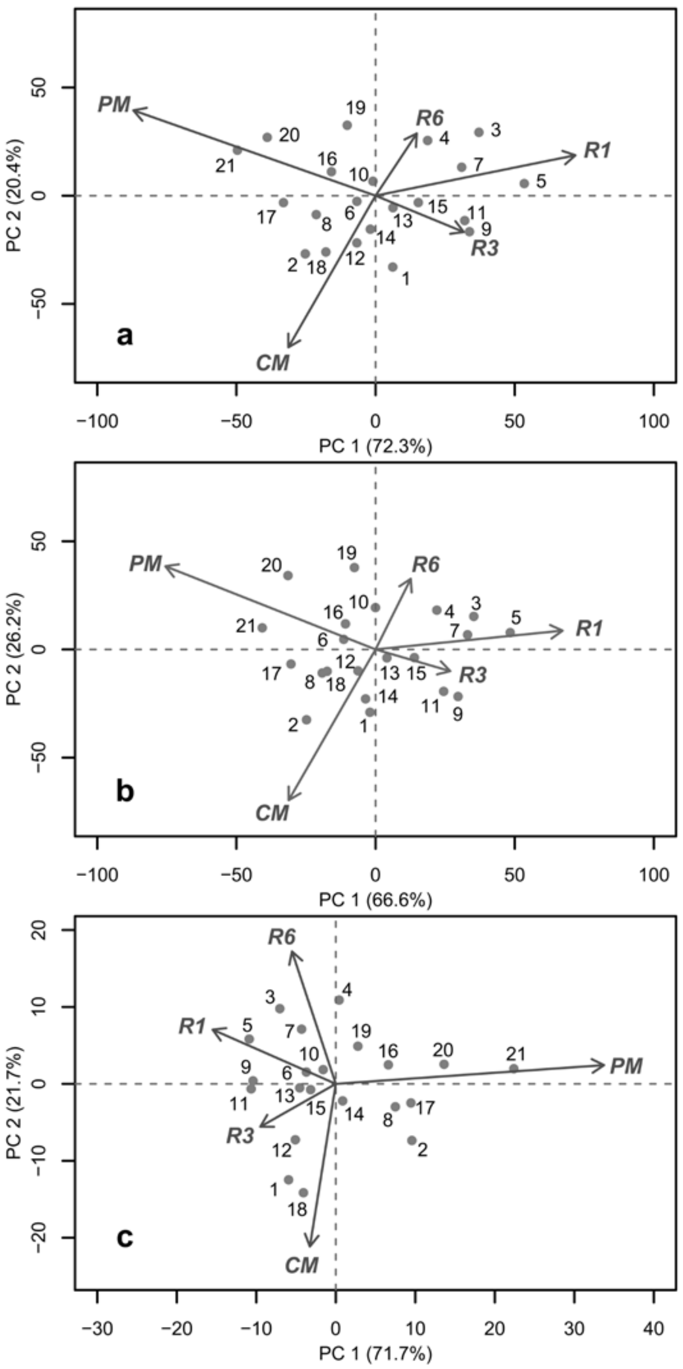

Figure 4. AMMI analysis biplot graphs of the CS × YOE interaction for: a) biomass yields; b) Milk Feed units yield, and c) Crude proteins yield

Type 2 AMMI biplot graphs are shown for the three response variables considering the first two PC axes *sensu* Gabriel (1971), with cropping systems expressed by vectors starting from the origin and ending at their scores (Gauch and Zobel, 1996; Vargas and Crossa, 2000). For biomass yield (Figure 4a) the biplot summarizes 92.7% of the variability. A considerable part of it is explained by CM and PM, according to the length of their vectors. Biomass for CM is higher in the first two years, decreasing towards the end of the examined period (years 19, 20, 21). PM, instead, shows augmented yield performances in the last three years, whereas a lower peak occurs in the 18^{th} year. R1, R3 and R6 rotations provide an optimum yield between the 3^{rd} and the 5^{th} year, then their performances decrease with a minimum in the last years observed (opposite quadrant of the PCs space). In particular, R6 and R3 explain little interaction variability, therefore they are the most stable cropping systems over time.

The biplot for MFU (Figure 4b) explains 92.8% of the variance and shows results similar to the previous, with PM and CM responsible for large variability. Also in this case, the CS $\times$ YOE interaction is more important in the first years for CM, less in the last three years. PM reaches higher performances in Milk Feed Units yield in the last three years, with a lower peak in the 18^{th}. R3 and R6 both decrease their performances over time, highlighting little interaction of factors, whereas R1 indicates higher variation in the first decade in respect to the second one. Both R6 and R3 appear to be the most stable cropping systems regarding Milk Feed Units yield over time.

Regarding Crude Proteins yield (Figure 4c), the first two components summarize 93.4% of the interaction variation. CM and PM still account for the larger variability, with CM decreasing performances with time and PM increasing in the last years. On the contrary, R1, R3 and R6 decrease their performances in terms of CP over time, but with R1 and R3 being the most stable cropping systems for this variable. R6 indicates larger interaction in the first decade than in the second one.

Overall, the 18^{th} year of experiment (that is 2002) indicates a consistent decrease in productions, probably due to an exceptional rainfall peak occurred in November. The total rainfall for that year was 1230.4 mm contrary to the mean of 797 mm y^{-1} in the observed period (Figure 1). The increase of productivity of PM over time is accompanied by a progressive increase of forbs (*Rumex* spp., *Plantago* spp., etc.) at the expenses of grasses. The amount of forbs has gradually increased during the years, as visible in Figure 5.

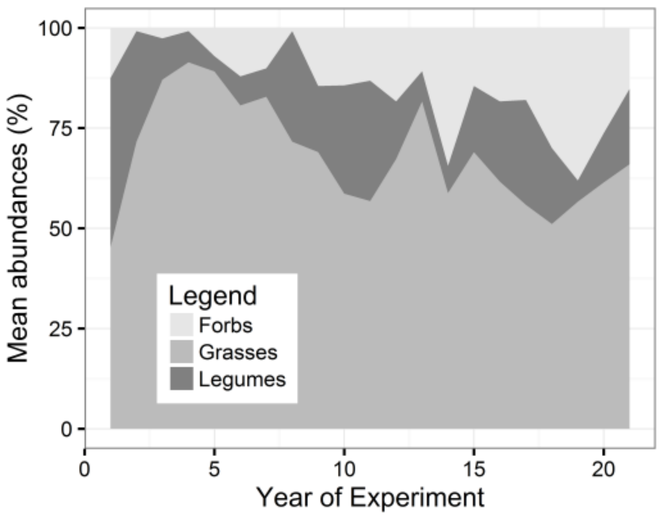

Figure 5. Mean species abundances (%) for PM, grouped by grasses, legumes and forbs over the experiment timespan

It is important to note that, within cropping systems, the values of CP yield are mostly determined by the presence of meadows, because the deriving forage is typically rich in white clover. Yet, the five cuts of the meadows have variable CP contents that can be explained by the variation of the grasses/legumes ratio along the season (data not shown). While the first cutting is typically abundant in grasses, and therefore low in CP, the subsequent cuts have higher white clover fractions thus higher CP contents are yielded.

4. Discussion

The annual double-crop Italian ryegrass + Silage maize (R1) is indisputably the most productive cropping system in terms of both biomass yield and Milk Feed Units yield. The common choice of the farmers is to maximize the Milk Feed Units locally produced, and to supplement the diet of dairy cows with protein concentrates, mostly soybean flour, which are purchased in the international market. In this way, the farming systems are over-loaded by external nitrogen, which in form of sludge/slurries can be source of water pollution. Nevertheless, mainly based on economic analysis, such intensive cropping systems are widely adopted by the livestock farmers of the area. The continuous grain maize cropping (CM), usually adopted by farms that dismissed livestock activities, showed the lowest production among the compared cropping systems, accompanied by the lowest crude protein yield. This is due to the fact that the harvested biomass for grain maize consists of about 50% of the total crop biomass. In parallel, maize stover is usually returned to the soil so that portion of biomass does not account for yield. Such low yield is accompanied by a low protein content of grain maize. Hence, compared to grain maize, silage maize allows the exploitation of nearly the total produced aboveground biomass, and it can also be easily rotated, particularly in light soil conditions, with Italian ryegrass, which represents an additional source of biomass, Milk Feed Units and Crude Protein on annual basis. Interestingly, the permanent meadow (PM), the less intensive cropping system in this comparison and the only one which excludes any possibility of crop rotation, produced merely 44% of biomass and 36% of Milk Feed Units compared to R1, but the amount of Crude Protein was very close to the one of R1. This is justified by the fact that white clover, a leguminous species providing a protein rich forage, not only was originally seeded in the permanent meadow, but it also aggressively expands in the meadow community over time, as generally known for the study area, reaching high abundances. Recently seeded white clover in the rotated three-years meadow of R6 also assures the highest Crude Protein productivity of this cropping system. Certainly, permanent meadows and rotations including rotated meadows prove to be effective in terms of Crude Protein yields. Overall, the arable cropping systems here depicted show decreasing trends of productivity over time. Such trends are mostly influenced by the performances of grain and silage maize, which seem to be mainly affected by the levels of agronomic inputs as highlighted by Stanger et al. (2008): the quantity of applied nitrogen plays an important role in maintaining and improving biomass yields in absence of rotations. Also Borrelli et al. (2014) indicated that the aboveground biomass productivity of grain and silage maize decreased over the course of the experiment with high inter-year variability, as here demonstrated through the AMMI analysis, and hypothesized the need for higher input regimes to insure monoculture yield stability. On the contrary, the permanent meadow is the only cropping system increasing its yields over time, also demonstrating the capability to reach high Crude Protein contents in the long term. This is consistent with Tilman (2000) and Tilman et al. (2006) who indicated that the raise of diversity leads to greater productivity, greater nutrient retention and improved ecosystem stability of the plant community. The advent of other plant Families, the selection of most competitive species and the functional complementarity of plants with different above- and below-ground structure is the plausible reason for the steady increase of productivity of permanent meadows over the years (Fornara and Tilman, 2008). Another benefit of permanent meadows surely regards the amount of input energy required, much less than the other four cropping systems, as demonstrated by Tomasoni et al. (2011), although their energy efficiency is lowered by small biomass yields. Also, the organic matter content in soil, as known, raises within unploughed grasslands whilst reducing in the case of arable crops (Battaglini et al., 2014; Haas et al., 2001) so that grasslands become essential to sequestrate Carbon (Gilmanov et al., 2007). All these reasons make permanent meadows one of the best candidates to be considered for agricultural extensification (Postma-Blaauw et al., 2010) and they should be encouraged as low energy-demanding cropping systems, which often are financially supported according to their advantages (Castoldi and Bechini, 2010). On the other hand, the permanent meadow demonstrates a substantial inter-year variability and this could be explained by two reasons. Firstly, the advent of other species temporarily changes the balance of the plant community and this happens especially in the case of novel communities, in search for their equilibrium (Ziliotto et al, 1988). Secondly, more self-sufficient cropping systems as meadows generally exhibit higher variability in time than those subjected to much higher agronomic inputs, as concluded by Tomasoni et al. (2011).

Overall, the reduction of agronomic inputs of 30% determines a less than proportional reduction of biomass, MFU, and CP yields. This confirms in the long period the observation that Onofrii et al. (1993) reported after the first six years of the experiment. However, as the agronomic inputs were combined into input packages, the individual effects of fertilization, soil tillage and herbicide cannot be disentangled. The reduction of input has a more pronounced effect in most intensive cropping systems (*e.g.*, R1 and CM), so that the other rotations and meadows should become the preferable choice when pointing to a reduction of crops' environmental burdens.

5. Conclusions

The six cropping systems under analysis have revealed different production potential in the 21-year trial. The annual double-crop Italian ryegrass + Silage maize was the most productive cropping system in terms of both biomass and Milk Feed Units yields, while more complex systems came out on top in terms of Crude Protein yield. The continuous grain maize cropping and the permanent meadow showed lower performance, although the permanent grassland provided good yields in terms of Crude Proteins. Higher agronomic inputs did constantly result into higher production in all cropping systems.

Over time, the gaps between cropping systems tended to reduce. In fact, yields generally decreased in the analyzed timespan, except for permanent grasslands whose yields showed an increasing trend, probably attributable to their capability to conserve organic matter in soils. This seems to be indirectly confirmed by a greater resilience of production in the more complex cropping systems with respect to the others, where the relative weight of the artificial meadow becomes higher.

Acknowledgements

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Summary and final conclusions

This research was aimed to integrate multiple techniques and scientific competences, needed to inspect the multifunctionality of agro-ecosystems and to assess biological conservation strategies, with a view to agricultural production. The most innovative aspect of the work relies on the multi-tool analysis of data at different scales in space and time, as a multi-scale approach is able to delineate a complete picture of the ecosystem services provided by agro-ecosystems. The scientific skills that were involved in this research and perfected during the PhD triennium are the following:

- ❖ Agronomy: to delineate the productive capability of agro-ecosystems, mainly referring to fodder crop systems at the scales of field and district;
- ❖ Ecology: to inspect the dynamics of meadows, their plant species compositions and biodiversity with regards to anthropogenic and environmental constraints;
- ❖ Landscape Ecology: to delineate a conservational strategy at the scale of landscape, with the usage of tools intended to thoroughly analyze every ecological process in large territories;
- ❖ Phytosociology: to collect data about phytocoenoses in various semi-natural agro-ecosystems;
- ❖ Multivariate Statistics: useful tools to elaborate large quantities of ecological data, both deriving from extensive field campaigns and long-term experiments;
- ❖ Geographic Information Systems: among the most useful tools in these domains, GIS software have been selected to aid the deep territorial analyses of entire landscapes and rural districts, and to support the creation of extensive databases needed for the evaluation of the ecological status of regions of interest.

With regards to the first project shown in this work (Chapter 2), we were asked to delineate a picture of the ecological status of *fontanili* within the district of Milan, and to analyze their ecological connections, where their biodiversity was not fully compromised. We knew this opportunity was tempting, as the context of interest both insists on an intensive agricultural matrix, both it is mined by anthropogenic burdens such as a strong urbanization trend. A broad literature review and some initial analytical choices were required at the start of the work, and we finally concluded that a large scale, landscape-scale approach was needed. Landscape ecology is a relatively young discipline founded on the analysis of ecological processes at the broad scale of landscapes, intended both as territories and as the entirety of the dynamics occurring within human, animal and plant communities. The creation of an extensive territorial database was needed to apply some existing techniques, with the help of open source software, but with time some drawbacks were found: we became aware of some limitations due to approximations of the methodologies applied and decided to create a new one from scratch, permitting better and more straightforward interpretation of results. This ended up with the integration of two main landscape ecology methods deriving from historically different thoughts: the analysis of landscape fragmentation was integrated with a species-specific assessment of permeability of movement with a fuzzy set of equations. This permitted a more correct interpretation of the ecological functionality of landscape units, because of its participatory nature and its meaningful formulation which achieves “the best of both worlds”, amplifying the accuracy of results and eliding the past limitations. Among other benefits, this approach is fully open source so it can be free of charge, meaning that it is applicable in poor countries, which are increasingly affected by land consumption but can only rely on little resources for research activities and territorial planning. This could emphasize the relationship between a reasoned landscape programming for enhancing agricultural activities, more and more demanded at the global scale, and the conservation of resources upon which production is, obviously, based.

The integration of biological conservation and agricultural production becomes important also with regards to marginal rural contexts founded on the maintenance of pastures and meadows as sources of biodiversity and high quality forage (Chapter 3). We were asked to inspect the quality of meadows in a vast rural district, both in terms of biological diversity and feeding capability, and decided to apply complex multivariate approaches to a wide database we collected. In this case, the importance of the work hinges on the analyses of meadow management strategies at the scale of district, which thereafter influences the whole alpine landscape. Livestock rural districts in marginal areas have been subjected to intensive management on one hand, and land abandonment on the other, with disharmonious consequences depending on altitudes, geography and social/economic pressures. In the context we analyzed, a great variability has been found with regards to meadow typologies and their agro-biodiversity, intended both as their biological variability and feeding capability in terms of quantity and quality. Statistically speaking, none of the management factors inspected has appeared incredibly useful to predict the agro-biodiversity of meadows; environmental constraints, due to the critical altitudes of the area, were important parameters, instead. Nonetheless, we were struggling with some decaying meadows where the amount of nitrophilous forbs puts in evidence the application of intensive strategies in the long run, accompanied by important biodiversity losses and reduction of fodder quality. To conclude, we think this study has highlighted a great variability of semi-natural resources at the level of marginal district, which, if not numerically explainable, is certainly worth improving and protecting. This variability directly derives from centuries of traditional farming, and it is at the basis of the typicality of products beside guaranteeing the preservation of particular landscapes.

With regards to the last paragraph, it is clear that observational studies are among the best strategies to assess agro-biodiversity when dealing with semi-natural resources. There, the dynamics of vegetation last incredibly long times, because of environmental constraints and perpetuated management activities. But, how is it possible to inspect agro-biodiversity in other agricultural contexts, where anthropogenic actions have changed the territory (and its resources) utterly? Chapter 4 wanted to answer to this question, with the analyses of a long term agronomic experiment. The only way to quantify the levels of agro-biodiversity in agricultural field contexts, strictly speaking, is to set up field experiments and analyze crop yields in the long run. Here, we had the opportunity to analyze a serious database which also includes rotations and permanent meadows: surely, it was a great chance to prove how agricultural diversity and the quality of production can collimate. In particular, the temporal scale of 21 years let us derive some interesting results, obtained with advanced multivariate techniques. First of all, rotations and meadows clearly overmatch continuous maize monocropping in terms of yields, fodder quality and, surely, biodiversity of production. Meadows appear to be the only cropping systems improving their performances over time, whereas the other ones show, some more than others, the signs of resources depletion and consequent losses of production and crop stability. This is certainly an important conclusion that matches modern agricultural policies and trends, going towards the rehabilitation of multi-crop systems and traditional fodder production.

In conclusion, agro-biodiversity has been assessed with a variety of tools and methodologies applied at different spatial and temporal scales, integrating diverse disciplines and contemplating both ecological aspects of conservation and agronomical aspects of production. Such an approach assures a wide comprehension of ecological processes that occur within agricultural contexts over space and time and it is able to highlight the complex connections between resources and outcomes. On the one hand, agro-practices are strongly influencing resources by depletion, with intensive choices contrary to the conservation of any biological diversity. On the other hand, agriculture is one of the primary activities able to rehabilitate those resources, by enhancing traditional cropping systems and turning them into modern, sustainable, sources of agro-biodiversity. This would assure the preservation and enhancement of such ecosystem services, which are at the basis of rural societies. Secondly, this would also ensure an important aspect more related to sociology, rather than agronomy: the rural livelihood, often forgotten in modern times, has progressively decreased in quality and only a rehabilitation of sustainable agriculture would be able to furnish viable solutions to increase the standards of living in rural areas. This is, by far, the most critical topic related to the social aspects of agro-biodiversity, which has emerged starting from mass exodus towards city centers, it continued with a widespread alteration of natural resources and intensification processes, and it has, by now, arrived at a turning point. This work has revealed some novelties by adopting multidisciplinary approaches, relating semi-natural elements each other, integrating conservation and production and combining strategies to break the ongoing process, making even clearer the need to "change gear" and to accelerate a deep rethink of the modern agricultural world. Future advancements for this study would be, indeed, (i) the application of these methodologies to other contexts, being them pliable, to prove their effectiveness in other areas of the planet, and (ii) the formulation of new and likewise modern tools to more and more expand the knowledge of ecosystem services.

References for Chapter 1 (Introduction)

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