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Regenerating soil fertility through cover crops and soil conservation approach: experiences in adoption and evaluation of these innovative techniques

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Sola res rustica, quae sine dubitatione proxima et quasi consaguinea sapiantae est, tam discentibus egeat quam magistris.

Così come ciascuno sceglie fra i sapienti chi possa dare all'animo suo una solida formazione alla virtù: e solo la scienza agricola, che senza dubbio è vicina, per nobiltà e importanza, alla sapienza, non ha né chi la insegni né chi la impari!

Columella, *De re rustica, Liber Primus, Praefatio*

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General aims of the research project

Global agriculture is at the centre of a new revolution which requires on one hand to produce more and more, and with good quality, as the International Year of Plant Health 2020 reminds us, for a growing population and on the other to use less and less inputs impacting the environment – fertilizers, plant protection products, fuel. However, agriculture as a productive activity must be economically sustainable - therefore obtaining satisfactory yields at low cost is an objective for each farmer – but it must also be environmentally sustainable: first of all for the survival of the agricultural activity itself, and secondly because can be a key player in the fight against climate change.

In fact, agriculture deals with the management of the territory of vast areas on the planet, and the soil is the main tool for agricultural production and the most important asset of every farmer. Fertile soil allows you to produce a lot and well with few inputs, allows you to make a profit that makes its cultivation sustainable and constitutes one of the most important carbon stocks globally.

An agronomic approach has existed for almost a century which aims to conserve fertile soil, and which in recent years has also learned to regenerate its fertility where necessary: it is conservative agriculture.

One of the fundamental principles of this technique is the continuous coverage of the soil, together with the minimization of tillage, which may be absent, and the maximization of biodiversity. Continuous coverage is achieved by maintaining crop residues on the surface but in particular by cultivating intercrops, or plant covers. Usually mixtures of species are used which are devitalized before the next sowing. A particular case is represented by "indefinite duration covers", i.e. multi-year plant covers (typically legumes) in which cash crops (typically cereals) are sown without devitalizing the cover crops.

These approaches open up innovative agronomic systems such as intercropping rapeseed with multiple cover crops (some annual and some perennial) that offer ecosystem services such as the regulation of phytophagous insects, nitrogen nutrition and weed management. Weed management and nitrogen nutrition are also the keys to direct sowing of cereals with straw under indefinite duration cover. The reduction of inputs with a stabilization of yields and an increase in the quality of cereals, which can have increases of 1-1.5% in protein, can bring an improvement in agricultural profitability with a decrease in costs and an increase in the economic margin. Italian and foreign quality supply chains are pushing farmers to adopt innovative techniques that have a positive impact on the agroecosystem such as the sowing of flowering buffer strips to encourage the presence of insects useful in the regulation of phytophagous pests, similarly to what occurs in associated rapeseed.

Soil management techniques without tillage and the constant presence of crops, both for cash and for cover or other ecosystem services, present at least two challenges. The first is that of increasing organic matter in the soil, with the notable benefits on soil fertility and the environment, summarized in the 4p1000 initiative launched at COP21 to relaunch carbon sequestration in soils to be removed from the atmosphere. This profound improvement can also respond to the need to improve the health of multi-year crops such as vines and fruit trees, which experience widespread phenomena of death and decay which could find a solution in more vital and fertile soil.

The second challenge is to understand the dynamics of fertility in a new context, with different needs from conventional agronomic management. Agricultural soils managed with these innovative practices are one of the best opportunities to validate new methods both to study and describe the biological, chemical and physical fertility of a soil, and to monitor it, giving farmers new tools to manage fertility and control nutrition. of crops in a more effective, sustainable and economical way.

Obiettivi generali del progetto di ricerca

L'agricoltura mondiale è al centro di una nuova rivoluzione che chiede da una parte di produrre sempre di più e di buona qualità, come ci ricorda l'International Year of Plant Health 2020, per una popolazione crescente e dall'altra di impiegare sempre meno input impattanti sull'ambiente – fertilizzanti, prodotti fitosanitari, carburante. L'agricoltura in quanto attività produttiva deve essere però sostenibile economicamente – quindi ottenere rese soddisfacenti a basso costo è un obiettivo per ciascun agricoltore – ma deve esserlo anche ambientalmente: prima di tutto per la sopravvivenza dell'attività agricola stessa, e in secondo luogo perché può essere un soggetto chiave nella lotta al cambiamento climatico.

L'agricoltura, infatti, si occupa della gestione del territorio di vaste aree sul Pianeta, e il suolo è lo strumento principale per la produzione agricola e il patrimonio più importante di ogni agricoltore. Un suolo fertile permette di produrre molto e bene con pochi input, permette di realizzare un utile che renda la sua coltivazione sostenibile e costituisce uno degli stock di carbonio più importanti a livello globale.

Esiste da quasi un secolo un approccio agronomico che mira a conservare il suolo fertile, e che negli ultimi anni ha imparato anche a rigenerarne la fertilità ove necessario: è l'agricoltura conservativa.

Uno dei principi fondamentali di questa tecnica è la copertura continua del suolo, assieme alla minimizzazione delle lavorazioni, eventualmente assenti, e alla massimizzazione della biodiversità. La copertura continua si ottiene con il mantenimento dei residui colturali in superficie ma in particolare con la coltivazione di colture intercalari, o coperture vegetali. Solitamente si impiegano miscugli di specie che vengono devitalizzate prima della successiva semina. Un particolare caso è rappresentato dalle "coperture a durata indeterminata", ovvero coperture vegetali poliennali (tipicamente leguminose) nelle quali si seminano le colture da reddito (tipicamente cerealicole) senza devitalizzare quelle di copertura.

Questi approcci aprono a sistemi agronomici innovativi quali la consociazione del colza con più colture di copertura (alcune annuali e altre perenni) che offrono servizi ecosistemici quali la regolazione degli insetti fitofagi, la nutrizione azotata e la gestione delle infestanti. Gestione delle infestanti e nutrizione azotata sono le chiavi di lettura anche della semina diretta dei cereali a paglia sotto copertura a durata indeterminata. La riduzione degli input con una stabilizzazione delle rese e un aumento della qualità dei cereali, che possono avere aumenti dell'1-1,5% di proteina, possono apportare un miglioramento della redditività agraria con una diminuzione dei costi e un aumento del margine economico. Filiere di qualità italiane ed estere stanno spingendo gli agricoltori ad adottare tecniche innovative che abbiano un impatto positivo sull'agroecosistema come la semina di fasce tampone fiorite per favorire la presenza di insetti utili nella regolazione dei fitofagi, similarmente a quanto si verifica nel colza associato.

Le tecniche di gestione del suolo senza lavorazioni e la presenza costante di colture, sia da reddito che per copertura o altri servizi ecosistemici, mettono di fronte ad almeno due sfide. La prima è quella dell'aumento della sostanza organica nel suolo, con i notevoli benefici sulla fertilità del suolo e sull'ambiente, riassunti nell'iniziativa 4p1000 lanciata in seno alla COP21 per rilanciare il sequestro di carbonio nei suoli da sottrare dall'atmosfera. Questo miglioramento profondo può rispondere anche all'esigenze di migliorare la salute di colture poliennali quali vite e alberi da frutto, che vivono diffusi fenomeni di morie e deperimenti i quali potrebbero trovare soluzione in un suolo più vitale e fertile.

La seconda sfida è quella di conoscere le dinamiche della fertilità in un contesto nuovo, con esigenze diverse dalla gestione agronomica convenzionale. Proprio i suoli agrari gestiti con queste pratiche innovative sono una delle migliori opportunità per validare nuovi metodi tanto per studiare e descrivere la fertilità biologica, chimica e fisica di un suolo, che per monitorarla dando agli agricoltori strumenti nuovi per gestire la fertilità e pilotare la nutrizione delle colture in maniera più efficace, sostenibile ed economica.

Contribute 1

Biofuture. Biodiversità e valorizzazione dell'ortofrutta e degli ecosistemi nelle aree tipiche di produzione. Strategie per incrementare la biodiversità del suolo e dell'ambiente. Manuale operativo del progetto BIOFUTURE.

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Introduction

Soil is one of the last great scientific frontiers and the rhizosphere is the most active part of this frontier where biogeochemical processes influence a large series of processes at both landscape and global scales. A better understanding of these processes is critical to maintain the health of the planet and nourishing the organisms that live on it (Science, 2004; Morrissey et al., 2004 cited in David H. McNear Jr., 2013).

Soil represents the support for life and ecosystems, it is a reserve of genetic heritage and raw materials, guardian of historical memory, as well as an essential element of the landscape (European Commission, 2002). It follows that the soil is at the centre of balance, a reserve of water, nutritional elements and biodiversity, an element on which food chains depend, it is an integral part of the landscape and guardian of our cultural evolution. In summary, it is the support of all human activities (Vieri, 2012).

However, the soil seems to be increasingly fragile, poor in organic matter and vitality, in short less fertile: from 1944 to 2009 the damage from hydrogeological instability amounted to 52 billion euros, with huge annual expenses for reimbursements, reconstructions and safety measures, without counting the damage to people caused by extreme climate events (Vieri, 2012).

2015 was the International Year of Soil, at the end of which the COP21 conference was held: what legacy remained from all this? Soil supports almost all agricultural and food production – it is estimated that 95% of our food is directly or indirectly produced from the soil (FAO, 2015) – it allows the production of wood and fibre, filters water and it allows you to drink it or grow fish or irrigate plants, but it also ensures the liveability of a territory, with its waterways, slopes, roads. FAO, promoter of the IYS2015 initiative, has summarised the importance of soil and its virtuous management in 6 key points.

1. Soils are the foundation for vegetation, whether cultivated or managed for feed, fibre, fuel, or medicinal products
2. Healthy soil is living soil. Soils are home to a quarter of our planet's biodiversity
3. Healthy soils are the basis of healthy food production
4. Soils filter and conserve water, increasing food security
5. Soils contribute to combating and adapting to climate change by playing a primary role in the carbon cycle
6. Soil is a non-renewable resource, and its preservation is essential for food security

Sustainability must be the founding character of the agriculture that we intend to practice in the near future. Economic sustainability, with sufficient and satisfactory production, but also environmental sustainability, to limit the negative effects of agriculture on the health of the agro-ecosystem (of which the soil is an essential pillar) and on that of man himself, farmer, and consumer. In fact, the growing demand for food by the growing world population should not be forgotten, but neither should the quality of the food we eat which must not only provide calories but also important nutrients necessary for health and well-being, and which must be at at the same time safe thanks to the absence of biological and chemical contamination: mycotoxins, pathogens, residues of plant protection products or other substances used in food processing. In this context we see how as the demand for production grows, the cultivated land decreases, due to the lost capacity to support agricultural production or its destination to other uses (energy or sealed under public and private infrastructures).

It is, therefore, a priority to maintain or restore the fertility of the soil to guarantee its productivity. Fertility can be defined as «the ability of a soil to be functional in the ecosystem to support biological productivity, maintain environmental quality and promote the well-being of animals and plants. The state of quality of the soil must be monitored to:

test agronomic productivity, verify the state of fertility at a local level for the development of specific projects, control the impact of agricultural activity and management systems at company, territorial and regional level or national» (Pisante, 2012).

When the Soil Science Society of America was created, USA was experiencing violent sandstorms that were causing the erosion of enormous quantities of soil, threatening the population of entire territories, and remembering this many years later serves to underline the centrality of soil in the well-being of a society. At the birth of that institution, farmers were encouraged to adopt practices that limited erosion, but even today many typical soil processes remain little known. Sandstorms, so-called dust bowls, still occur today alongside large-scale land degradation events across large regions.

Perhaps and even more than in the past, soil has a great potential influence on the territory and its population, and although our knowledge of processes within the soil has increased since the 1930s, many questions remain unresolved: does transition to conservation agriculture promote carbon sequestration in the soil? And how to explain the heterogeneity at the microscale level in the transformation of organic matter?

Given the scale of the challenges posed by our (ab)used soils, one might consider that if we do not intervene quickly, humanity may not have the opportunity to explore new frontiers in the future. This perspective, which considers how soil is crucial for life on earth, could stimulate new community interest in soil, supporting research and attracting new attention (Baveye et al., 2011).

Soil contains ¼ of the world's biodiversity, with an estimated 33% of world's soils as moderately or highly degraded: there is no time to waste. It can take up to 1000 years to produce a few centimetres of soil, and in the meantime 50 thousand km^2 are lost every year due to deforestation, unsustainable land use and management practices, overgrazing and climate change.

Soil is «a vital resource under increasing pressure, which must be protected to ensure sustainable development» (European Commission, 2002). Therefore, knowing the soil and its fertility in depth, and how man with his agricultural presence acts on these elements is of primary importance, in particular with a view to climate change and the need to preserve the fertility and productivity of our soils.

«Conservation agriculture (CA) aims to obtain sustainable and profitable agricultural systems and tends to improve living conditions of farmers through the simultaneous implementation of three principles at field level: minimum soil tillage; crop associations and rotations, permanent soil cover. CA has great potential for all types of farms and agroecological environments. It is of great interest to small companies; but perhaps it is urgently adopted in those where the limited means of production do not allow the strong shortage of time and qualified manpower to be overcome. It is a tool for reconciling agricultural production, improving living conditions and protecting the environment. CA is successfully applied in different types of production systems and in a diversity of agroecological zones. It is perceived by farmers as a valid tool for sustainable land management» (FAO, 2015).

In Italy it is also known as "Blue Agriculture" and constitutes a sustainable agricultural approach in which the agronomic, environmental, social and economic aspects that characterise the agrosystem are rethought in an integrated manner, with the protection of natural resources very clear in the production choices: water, air and agricultural soil. The European Parliament confirms this in a recent declaration, in which it recognizes that «conservative agriculture leads to an increase in fertility and productivity, a more efficient use of water and a better capacity to adapt and mitigate to climate change, as well as a significant reduction in erosion, greenhouse gas emissions and optimal use of plant protection products» (Tabaglio, 2016).

Conservative agriculture (CA), in the context of techniques and equipment, aims to reduce soil tillage, to exclude the inversion of soil layers, up to the total elimination of tillage (no-tillage, direct drill) (Tabaglio, 2016). Compared to the two extremes – arable/conventional and no-till systems, however, there are various intermediate and mixed techniques and forms which for some years have also been contaminating organic farms, in an attempt to hybridise the systems into a new one: ABC, conservative organic agriculture (Fleury et al., 2011).

The three fundamental pillars of conservation agriculture and the promises of greater fertility and resilience of agricultural systems that switch to this approach will be explored individually, bringing the results of some studies.

<u>Continuous soil cover.</u> In conservation agriculture, coverage with crop residue at least 30% of soil surface must be ensured, to protect and provide the agronomic benefits that are proportional to their quantity, being one of the major sources of organic matter, even if not the only one (Kallenbach et al., 2016). In conservative agriculture there are two ways to cover the soil: leaving crop residues after the harvest (dead cover), or sowing specific crops defined as cover crops in the intercultural period in which the land would remain otherwise uncovered (Brugnoli, 2017).

Ensuring permanent soil coverage allows the nourishment of the biological component of the soil which produces and transforms plant residues into organic substance. In particular, earthworms and microorganisms, which are considered the major players in soil fertility.

Furthermore, the porosity of the soil is increased and stabilised, both thanks to the maintenance of important structuring biopolymers of microbial origin (Kallenbach et al., 2016) and due to the physical action of attenuating the beating action of the rain, limiting the formation of superficial crusts and reducing erosive phenomena (wind and water erosion). Furthermore, the temperature is also kept more constant, reducing heating during summer days and the “useless” evaporation of water, i.e. that which does not pass through a plant (transpiration) to build its biomass.

The presence of residue or a herbaceous living coverage (or even both) with the superficial accumulation of organic matter, absorbs the energy that rain has on impact with the soil, limiting the formation of crust and therefore asphyxiation for the plants that grow there (often with problems of emergency, stunted and non-competitive crops with weeds), and decreases the speed of water runoff, slowing it down and therefore giving it time to infiltrate and preventing it from eroding the superficial fertile layer but which is generally also the one most polluted by the products newly distributed plant protection products, with the consequent pollution of waterways. The advantages, therefore, are not only for the hills, where erosion is decidedly more evident, but also in flat conditions, where erosion phenomena can still take on significant values. Thanks therefore to the presence of a protective mulch, together with a greater microbial biomass and the consequent greater bioporosity, which has as its distinctive characteristic that of a strong continuity and interconnection, it is possible to have a conductivity of the saturated soil (K-sat) of up to 123 times greater than soil managed with conventional techniques (Blanco-Canqui and Lal, 2007). However, if we stop only at the mechanical aspect of conservation agriculture and therefore no residues and cover are present on the soil, this parameter can be similar or even lower for a no-tilled soil (Horne et al, 1992, Chang and Lindwall, 1992).

Water management in agriculture is increasingly critical: the climate exposes us to long periods of drought on the one hand (November – December 2016, December 2016 – January 2017; March-April 2020; March 2021; winter and summer 2022) and on the other, to periods of intense and abundant rainfall (May 2013, May – June 2016, November 2016, May and November 2019, May 2021); to which is added the increase in irrigation costs, especially in the most difficult and least supplied areas and finally an increasingly alarmed public opinion about water pollution and its consumption in agriculture.

We understand that the problem is the excesses, extreme events that alternate in an unmanageable oscillation. Mitigation actions must therefore lead to a system that absorbs these peaks and is able to respond by adapting. Much is being done in terms of varietal improvement of crops but we must not forget that there is a tool that every farmer already possesses: soil.

In addition to carbon sequestration, it is thought that conservation agriculture can play an important role in mitigating climate change by also acting on temperature. In fact, forecasts speak of increasingly frequent thermal stress, and we are now getting used to sudden and intense meteorological changes. In conventional agriculture, usually, after the harvest, the crop residues that remain on the ground are buried and mixed with the soil (with deep tillage even in midsummer), used in animal husbandry or burned. Stubble burning, still very widespread in southern Italy but now prohibited by the strengthened conditionality of the new 2023-2027 CAP, is harmful because in addition to leaving the land uncovered, it aggravates the phenomenon of soil erosion (Pisante, 2007 cited in Brugnoli, 2017).

Maintaining soil coverage in the summer period can significantly reduce the amount of water lost through evaporation, bringing the harrowed and uncovered soil, in a single day, to the loss levels experienced in a week from soil covered instead with crop residues (Hatfield et al., 2001). As a result of the loss of humidity, the soil also loses the ability to maintain a certain thermal homeostasis, risking experiencing very high temperatures in the middle of summer with a notable impact on the life present in that soil.

Davin et al. (2014) highlighted another mechanism by which no-till sowing and minimum tillage can mitigate thermal excesses. It is not just a matter of sequestering carbon thanks to organic matter, but of directly reducing the effects of summer solar radiation. In fact, crop residues have the ability to increase the albedo and therefore the quantity of rays reflected by the ground. Added to this is the limitation of water loss through evaporation – which however attenuates the effect of surface residues as it can dissipate a high quantity of heat. On summer heat wave days, the local temperature attenuation effect given by the presence of ground cover is of the order of 2 °C.

The measurements were carried out at an experimental site in southern France, where fields were left unploughed after the wheat harvest and where residues increased the rate of reflection of solar radiation by 50%.

Crop rotations. The presence of a large number of species and even more of botanical families of cultivated plants thanks to the lengthening and diversification of crop changes/rotations, avoiding the repetition of the same crops with too narrow frequencies, allows biological fertility to be preserved and increased, soil chemistry and physics, stabilise and improve yields, reduce phytosanitary and weed-related problems and, consequently, the use of fertilisers, agrochemicals and energy, particularly if legumes are present (Marandola, 2012).

The adoption of "diversified crop rotations, in the case of annual crops, or intercropping, in the case of perennial crops" (Pisante and Stagnari, 2013 cited in Brugnoli, 2017), allows in fact to stagger and interrupt at different times of the year the cycle of weeds so that they never become dominant and do not spread (through dissemination or diffusion of vegetative organs), but also to avoid the proliferation of diseases and insects that share various host plants, acting both directly (periods of absence of host plants) and indirect (favouring predators and parasitoids). «This practice of agricultural activity, since ancient times, was a consequence of the observation of how the failure to rotate different crops on the same field caused serious impoverishment of nutritional factors, decreased fertility, and increased the occurrence of recurrent phytopathologies and growing intensity» (Brugnoli, 2017).

Crop rotation is a technique whose importance has been known since ancient times: from the works on georgic topics of the Roman era which dealt with crop rotations to the chapter books of Charlemagne which prescribed the three-year rotation (Brugnoli, 2017), passing through Columella who he reported how the alfalfa "greased the soil" (and also spoke of a first intercrop of vetch) while instead he spoke less positively about many grain legumes. It can therefore be assumed that, adapting to different climates and soils, crop rotation has always been practised over the centuries. In modern conventional agriculture, crop succession has lost its importance, becoming increasingly simplified until it disappears completely, as in the homosuccession of corn or rice in many areas of the Po Valley or durum wheat in southern Italy. Having machinery, fertilisers, improved cultivars and agrochemicals at their disposal, the majority of contemporary farmers are led to no longer consider it necessary to continue the practice of crop rotation, trusting that they can obtain rewarding and constant results with the use of these modern technologies.

In conservative agriculture, however, from a prevention perspective given the limited means available and the search for better results in terms of efficiency, its undeniable agronomic advantages are once again considered which can also be valorised economically, in this soil management system (Tabaglio, 2013 in Brugnoli, 2017).

This integrated management strategy through rotation acts not only directly on the weeds by staggering their cycle, but also by increasing the competitive capacity of the crops that take resources away from the weeds: crop precession has considerable importance (Anderson, 2008), both directly thanks to the greater vigour of the crop and indirectly, thanks to a reduced seed bank (Anderson, 2006). Furthermore, they also allow rotation of any chemical means used, preventing the onset of herbicide resistance (Anderson, 2014a and 2016a). A large and complex rotation allows weeds to be managed more efficiently, with savings of 50 to 70% in herbicides (Anderson, 2016a).

Reduced or no tillage. Minimising soil disturbance and avoiding the inversion of the layers, decreasing the intensity and depth of tillage until complete non-tillage, together with a fewer number of passages on the land, promotes biological and physical fertility and allows the maintenance of chemical fertility. There is an increase in the activity of earthworms and edaphic fauna, in microbial activity and the balance between the different functional groups; the effects on the structuring of the aggregates and their stability are important with a consequent increase in infiltration (therefore an increase in water stored with each rainfall) and a decrease in erosion.

To be sustainable in the long term, the balance between organic matter consumed (outputs) and constructed (inputs) should at least be in balance: in most agroecosystems this is not possible if the soil is disturbed by tillage. For this reason, one of the dogmas of conservation agriculture is the limitation of mechanical disturbance of soil: by minimising the exposure of the soil to air, in particular of the most protected aggregates, the loss of organic matter through oxidation and degradation is reduced and it contributes to the mitigation of greenhouse gas emissions, while achieving an improvement in soil fertility. Following soil processing, several tens of grams of CO_2 can be lost for each square metre, and remember that in each hectare of our countryside there are ten thousand square metres, leading to a cumulative value 5 hours after processing even at 600 kg of CO_2 lost per hectare, with an increase of over 42 times compared to untilled soil (Reicosky, 2019) and this only in the following hours, but an oxidative condition is maintained for a very long time. Among other things, Reicosky's experiment was conducted with ploughing at 25 cm, something that in the minds of many Italian farmers is already minimal tillage. So, it is better not to think about what happens with plowings that start from 30 cm and often still touch 45.

Ploughing exposes soil organic matter to strong aeration, increasing all oxidative and mineralization processes from which the soil microorganisms also benefit, thanks to the rapid (but transitory) aeration produced by ploughing, rapidly degrading the organic matter and releasing the minerals and the CO_2 contained: «this process leads, on the one hand, to the reduction of the potential biological and biochemical activity of the soil (Doran et al., 1998; Riffaldi et al., 2002) and, on the other, to the destruction of the structural aggregates (Golchin et al., 1994; Bossuyt et al., 2002; Plante and McGill, 2002; Achmed et al., 2003)» (Marandola, 2012).

Mechanical tillage and the continuous removal of crop residues represent the main factors that cause the decline of organic matter, and this inevitably translates into a decrease in porosity and an increase in the bulk density of the soil (Marandola, 2012). The reduction of organic matter can reduce the efficiency of water use, significantly influencing the infiltration capacity and hydraulic conductivity of the soil: factors which in hilly environments inevitably translate into an increase in surface run-off and erosion phenomena. (Marandola, 2012). According to Heard et al. (1988) no-till does not affect porosity so much, which is equal to or lower than those managed with conventional techniques, but what significantly increases is the continuity of porosity between the 10 cm of surface soil and the layers just below, constituting a large useful reserve for the roots of crops, which do not have to spend too much energy to deepen.

CA saves a lot in terms of carbon emissions. Smith et al. (1998) estimate that agricultural emissions could be offset by switching to no-till planting.

Agriculture's commitment to reduce the impact of soil cultivation is therefore essential but may not be sufficient: emissions linked to fuels are reduced by 60% (SoCo 2009) like ones related to the mineralization of organic carbon, but it is necessary to increase the amount of organic carbon entering the soil. In fact, it has been seen that the mere suspension of tillage combined with soil covered only with residues is not sufficient, despite all the benefits seen, for a real increase in the total quantity of organic matter (Mary et al., 2013). This is nothing new: it is unthinkable to accumulate too much C simply by minimising soil disturbance (even if for many farmers it remains the first and most important commandment of Conservation Agriculture).

Plants are the only ones capable of fixing C, so any other activity can only transform or consume it, but with different efficiency. It is therefore necessary to introduce cover crops and optimise cash crops: produce biomass to revitalise soil fertility, with a process that takes time (the well-known transition phase), bearing in mind that each engine has its own consumption, no movement is at zero cost or energy credit. It becomes utopian, therefore, to think of eliminating any carbon emission, with all the biological activity awakened and nourished, but it drastically changes the efficiency of this system.

Healthy and fertile soil, therefore, can accommodate a high quantity of water, while at the same time opposing both laminar and channelled erosion and is able to return water to plants over time. It can absorb large quantities of carbon, to deal with the management of elemental and energy cycles, linked to agriculture but not only – and above all agriculture is not the only one to benefit from it. The role of organic matter in improving the structure towards a situation of stable aggregates is undeniable and crucial, but a great resource are, once again, microorganisms and earthworms, precious allies and generous workers.

Regarding the knowledge of the soil and in particular of its agronomic potential, the concept of soil fertility seems to be as old as agriculture, so acquired and consolidated that Sébillotte (1989) considered it more a shared knowledge than a consolidated scientific knowledge (Marandola, 2012).

Scientific interest in the mechanisms underlying soil fertility took shape at the beginning of the 1900s, with the great discoveries on plant nutrition and root systems, but it is relatively recently that science has dedicated itself to investigating the mechanisms biological, chemical and physical which are the basis of their ability to host agricultural production processes (Marandola, 2012).

Most of the definitions seem to enhance the biological component of soil fertility, a component to which the various organisms that inhabit it contribute, through the reciprocal relationships that they can establish between themselves and with crops (presence/absence of useful microorganisms; accumulation of pathogenic microorganisms; presence of plant parasites) but which are themselves influenced by agronomic management techniques, among which the methods of working the soil, the management of organic matter and crop rotations have a considerable impact (Marandola, 2012).

Even though the microbiota is at the centre of these approaches, it is not easy to directly manage microorganisms and their activity, also due to still limited knowledge. The major control tool therefore remains the agronomic technique, in particular (the absence of) mechanical disturbance of the soil and the diversity of plants that grow there.

90% of the activities carried out by (in) the soil is carried out by microorganisms (Coleman and Crossley, 1996) but 90% of the soil microorganisms are still unknown (Marandola, 2012): billions of bacterial and fungal cells, of several thousand of different species whose names are sometimes not yet known.

In addition to bacteria, fungi play a fundamental role in the biological and physical fertility dynamics of soils. The hyphal network that crosses the soil can induce tolerance in plants to pathologies and drought and improve the structure of the soil. Roots and hyphae stabilise macroaggregates (having a diameter greater than 250 μm); macroaggregation is therefore primarily controlled by agronomic management, which also influences root development and the oxidation of organic carbon (Tisdall and Oades, 1982). The development of mycorrhizae (symbiosis between some particular genera of fungi and plant roots) is encouraged by non-tillage of the soil and diversified rotations, especially those that include legumes (Maché et al., 2012).

«Microorganisms play a fundamental role within the telluric ecosystem. They can, for example, control some phytopathogens (Friberg et al., 2005), improve the physical characteristics of soils (Logsdon and Linden, 1992; Denef et al., 2001) and provide nutrients to crops (Bonkowski et al., 2000; Wardle et al., 2001). The valorisation of useful soil organisms has the potential to offer very long-lasting effects which are based on maintaining and supporting the natural balance of the "soil" ecosystem (Wurst, 2012). The microorganisms present in the soil (number and type) are rapidly affected by changes in agronomic management methods (cultivation systems, fertilisation, control of pathogens and weeds) (Lupwayi et al., 1998; Coleman et al., 2002; Miyazawa et al., 2002) and the decomposition rate of plant residues can be considered a good indicator of their activity (Fließbach et al., 1995; Bradford et al., 2002). In general, the quantity of bacteria, fungi and earthworms present in a soil is positively associated with the carbon and nitrogen content because it is also on these elements that their nutritional activity depends (Frey et al., 1999; Clarholm, 1994; Vreeken-Buijs et al., 1998; Villenave et al., 2004; Nakamoto and Tsukamoto, 2006» (Marandola, 2012).

After sixteen years of cultivation, in a former native prairie soil in Nebraska converted to arable land, total nitrogen in the topsoil (10 cm surface of soil) decreased by 27% when managed with no-till drilling, but by 50% when subjected to ploughing, and the same goes for microbial biomass, which dropped by 43 and 36% respectively (Follet and Schimel, 1989). The respiration rate (CO_2) was proportional to the biomass, but not nitrogen mineralization, which was much lower in the sod treatment. This could suggest that the availability of C for microbial growth is inversely correlated with the intensity of tillage: increasing the intensity of tillage decreases the ability of the soil to immobilise and conserve nitrogen (Follet and Schimel, 1989).

Zuber and Villamil (2016) compared 62 studies from all over the world on fertility in conservation agriculture, in particular with respect to seven microbiological parameters that are considered good indicators of fertility. As seen, the measurement of the biological fertility of the soil is a fundamental evaluation of the soil quality and can be revealing different agricultural managements, in particular soil cultivation. By modifying the microclimate of the soil, tillage (particularly ploughing) exerts the most important factor of pressure on microbial communities. The objective of the work was therefore evaluating the global response of microbial biomass and enzymatic activities. In fact, the factors analysed are microbial biomass carbon (MBC), and the respective nitrogen (MBN), metabolic quotient (qCO2), fluorescein diacetate (FDA), dehydrogenase (DHA), b-glucosidase and urease. Microbial biomass, metabolic quotient and enzyme activities were employed due to their extensive use in assessing soil quality.

Microorganisms, both bacteria but also fungi, "eat" organic molecules deriving from the demolition of plant residues or by reprocessing what is absorbed by root exudates, build more biomass, cells, and structures, which will "encrust" sand, cement silt particles or enter even among the microscopic sheets of clay. Once dead, those cells will leave their vestiges preserved in those spaces, being first emptied of cellular contents and more labile substances, and then progressively consumed and recycled even the most resistant components such as cell walls and membranes which, however, in the meantime will have continued to keep bound and cohesive mineral soil aggregates, thus building a stable structure. However, a part of those "organic matters" will not be easily accessible, and therefore will remain as organic matter stably fixed in the soil. At least until someone releases it, exposing it to oxidation and activity by other microorganisms.

This could be, in a very simplified and quick way, one of the carbon storage dynamics, which therefore requires a significant and continuous increase in carbon input into the system thanks to continuous photosynthesis in order not to starve the soil. But we must also and above all respect the environment and its very fragile balance to preserve everything that has been laboriously produced.

Organic matter, carbon and the nutrients it contains influence biogeochemical processes from the micropore to the global scale and can influence carbon-related climate dynamics. There is now consensus on the emerging idea that microbial materials are an important component of stable organic matter, and new conceptual and quantitative models are rapidly strengthening this view (Kallenbach et al., 2016). However, direct evidence demonstrating that microbial residues contribute to the chemistry, stability and accumulation of organic matter is still scarce. The stabilisation of organic matter of microbial origin by means of abiotic mechanisms is emphasised by some works, while the effects of microbial physiology still remain obscure. Kallenbach et al. (2016) provides the first direct evidence that microorganisms produce chemically different substances that fall into the stable fraction.

Accumulation is driven by distinct microbial communities, more than clay mineralogy; the accumulation of organic matter of microbial origin is greater where the fungi are greater, and the efficiency of microbial biomass production is greater. These polymeric components of microbial origin could strongly play a role in the stability of soil aggregates. This in fact depends on the quantity and nature of the organic materials present, which can be classified into: transient (especially polysaccharides); temporary (roots and fungal hyphae) and persistent (persistent aromatic components associated with polyvalent metal cations and strongly adsorbed to polymers). The significant quantity of rhizodeposed materials in the form of radical exudates, largely simple sugars, and other carbonaceous substrates with low molecular weight (Waligora, 2014), can be used by microorganisms to build more complex polymers of a lipid nature and with important effects on fertility (Kallenbach, 2016).

Organic matter has a primary role in the concept of fertility and its three levels – biological, chemical and physical: there is a strong relationship that links the abundance of life forms present in a soil with a given organic matter content that represents the main vital resource for telluric organisms; constitutes a reserve of nutritional elements which are preserved in the profile that can be explored by the roots; is a fundamental player in the stabilisation of aggregates and therefore the protection of the soil structure and the fight against erosion (Marandola, 2012; Tisdall and Oades, 1982).

Organic matter, and in particular its main constituent i.e. organic carbon, is considered a key indicator of fertility, soil quality and the sustainability of production processes, therefore all practices that go in the direction of its increase and protection are important strategies to disseminate (Reeves, 1997).

Long-term studies have highlighted how in intensive cultivation systems, even in the presence of good rotations and manures, there is an almost inexorable decline in organic C, but that this is strictly dependent on the mechanical tillage of the land, the climate and the type of soil (Reeves, 1997). From this perspective, a particularly strategic significance is assumed by conservation agriculture systems and, in particular, by no-till sowing, as it can support or increase organic C when combined with conservation agriculture systems, with adequate rotations (Reeves, 1997).

The latest challenge facing agriculture is complex: producing better, producing more – especially in degraded and marginal areas, recovering stability and fertility. These two objectives should not be antithetical: in some areas of our planet, it is not possible to produce more, at least not without the introduction of major innovations in the genetic field, while improvements can certainly be made in the quality of production. This takes on an important role in particular in a context of climate change which translates into greater instability and alternation of extreme situations. Increasing the capacity of soils and crops to respond to these situations is therefore fundamental. The agriculture of the "short century" gave too simple answers, losing sight of the agronomic and ecological context, the interdisciplinary exchange and chasing only productivity: «the attention was placed on the product, not on the production process, much less on the system» (Bocchi, 2015).

As seen above, another and more powerful tool can be used, in addition to maintain crop residues, to ensure coverage, increase organic matter but also help plant diversification already seen in the rotation, protecting soil both during growth and at its end, producing a high quantity of biomass: these are cover crops.

Herbaceous covers are intercrops – single species or mixtures, annual or multiannual, having the function of maintaining a vegetal cover during the unproductive periods between two main crops in rotation (Brugnoli, 2017). No direct income is derived from these crops but rather agri-environmental or ecosystem services; for this reason, vigorous and rustic plants are sought after, producing biomass but with the lowest possible management costs (Brugnoli 2017). Species belonging to numerous botanical families can be used, both in monoculture and in mixtures based on different needs and desired effects. The hedging species most frequently therefore belong to the Fabaceae (legumes – peas, field bean, clover, broom, alfalfa, sainfoin, vetch, lentil, grass pea, fenugreek, cowpea, crotalaria); Poaceae (grasses – ryegrass, barley, oats, rye, triticale, sorghum, millet); Brassicaceae (cruciferous vegetables – radish, mustard, rapeseed, camelina) which can be combined with Polygonaceae (buckwheat), Linaceae (*Linum usatissimum*), and other minor ones. The choice between these may concern forage species, minor ones, or simply ones absent from the crop rotation, which they will enter as cover crops.

Cover crops can perform different agronomic roles (reworked by Brugnoli, 2017 and Fasolo and Chiarini 2022):

- nitrogen-fixation and increase in nitrogen availability in the soil;
- carbon fixation and supply of organic matter;
- capture of nutrients and reduction of the risks of percolation in the groundwater;
- decompaction of the soil with the action of the root systems;
- promotion and maintenance of structural stability, porosity and fight against erosion;
- control of weeds and pathogens through direct or indirect interventions;
- stimulation of fertility and biological activity;
- promotion of entomological biodiversity, especially when flowering pastures are scarce;
- containment of temperature and better efficiency of use of soil water.

Focusing briefly on the choice of crops to adopt for a cover crop, you must first of all take into account your pedoclimatic context, followed by the needs sought by the specific cover crop. If there are no particular needs required of the cover crop, its sowing should be used as an opportunity to maximise biomass production and promote biodiversity above and below the soil within the field and not just on its edges.

Legumes are a fundamental element in the construction of soil fertility: they, in fact, play a virtuous role in increasing the overall biological vitality of the soil (Marandola, 2012) thanks to the contributions of nitrogen coming from nitrogen fixation, i.e. from the ability to fix nitrogen atmospheric which can then be transferred to the next cash crop (Tabaglio, 2011) or released into the organic pool.

Effective weed control can be sought in forage legumes, in particular vetches (Brugnoli, 2017) and given the high intraspecific biodiversity it is possible to select the most suitable species for the different sowings during the season.

As regards grasses, both microthermal and macrothermal (summer), in addition to producing a high quantity of biomass, they exert a very fine mechanical action on the superficial layers of the soil thanks to the fasciculated root systems, stimulating the structuring of soil and fighting erosion. Some of them produce allelopathic substances.

Brassicas are famous for their allelopathic and biocidal capabilities, thanks to the production of substances useful for combating pathogenic organisms (fungi and nematodes) and the germination of other plants. Their taproots, perforating the soil, favour an important exchange of water and gas, they manage to decompact soil in depth in the presence of tillage pans with higher efficiency than other species – characteristic above all of some varieties of radish called Daikon (Chen and Weil, 2009), and recover large quantities of nitrates and other nutrients at risk of leaching (Miravalle, 2007 cited in Brugnoli, 2017)

With an intercultural crop it is possible to produce high quantities of biomass, up to 20 t/ha, with notable contributions of organic C. But it's not just about administering C to the soil: it's about reviving, maintaining, promoting the vitality of the soil, microorganisms, insects, animals, earthworms; promote the structuring and conservation of water; recover, recycle and make bioavailable nutrients for next cash crop. The balance of organic matter at the end of this annual cycle may not be significantly positive, especially in the first years, also due to the "priming effect" induced by cover crops, which could induce a mineralization of organic matter, but this allows us to further explore the reserves of nutrients contained in the soil, with enormous long-term benefits (Waligora, 2014, Camarotto et al., 2020).

«In the modern concept of sustainable agriculture, cover crops represent the best tool for managing and preserving the fertility of soils, their quality, the quality of surface and groundwater. The use of specific crops intercropped with cash crops facilitates management of weeds, parasites, and crop diseases. They improve agricultural environment in terms of biodiversity and hospitality for fauna» (Miravalle, 2007 cited in Brugnoli, 2017).

Very interesting, in the context of cover crops, is the possibility of operating mixtures of species, with the aim of maximising the benefits of the cover crop and exploiting an opportunity to introduce biodiversity. Work on multiple root levels, on multiple levels of the air space, biomass with more balanced nutritional characteristics, as well as higher environmental adaptability: mixtures, when well designed, can best offer the advantages of cover crops. However, not everyone agrees, some argue that to get the most out of each species (suppress weeds, or fix nitrogen), they should be left free reign, while others argue that biodiversity is the best way to get the most from ecosystem services sought. Finney and Kaye (2016) confirm this belief that there is a positive relationship between biodiversity and ecosystem services and therefore increasing the latter increases them. Sowing a multi-essence plant cover instead of a monoculture provides greater multifunctionality: this is the result of two years of study on 18 species, combined in different ways in mixtures of one to eight species, on which five ecosystem services were measured. McGurr et al. (2016) found how the presence of strips sown with floral essences (Fabaceae and Asteraceae) around paddy fields made possible to reduce the incidence of some phytophagous in the rice crop, generating savings for the farmer and avoiding the use of plant protection products. Extra-plot biodiversity is an important element of agroecological management, and cover crops can represent the intra-plot counterpart.

The advantages brought by this tool are therefore numerous and should become the foundation of a new agriculture. However, it is not easy to translate these services into a precise economic advantage, against a clearly identifiable expense: from the purchase of the seed to sowing and devitalisation. Estimating the biomass and nutrients brought into play by the cover crop (recycled or newly introduced elements) can therefore become a useful evaluation tool. To do this, a model called MERCI (Méthode d'Estimation des Restitutions par les Cultures Intermédiaires) was developed by the Chambre d'Agriculture Poitou-Charentes and that of the Bas-Rhin. This allows to calculate the quantity of nutrients based on some values of your coverage such as the quantity of dry matter or fresh matter or the height of the plants of the mixture – in a decrescendo of precision (Archambeaud, 2010). Starting from these values and on the basis of the relative composition it is possible to estimate the quantity of nitrogen, phosphorus and potassium and the release dynamics, with a hypothesis of bioavailability for the following crop. The biomass and nutrient values reported in the case studies below were obtained with this model.

Biofuture project cover crops

CIPAN: nitrate catcher cover crops – Azienda Tenuta al Parco

Pedological characterisation of the experimental site (first year was planned to be in another field, where pedological and chemical physical analysis were conducted but after changes in farmer needs this field was selected to continue).

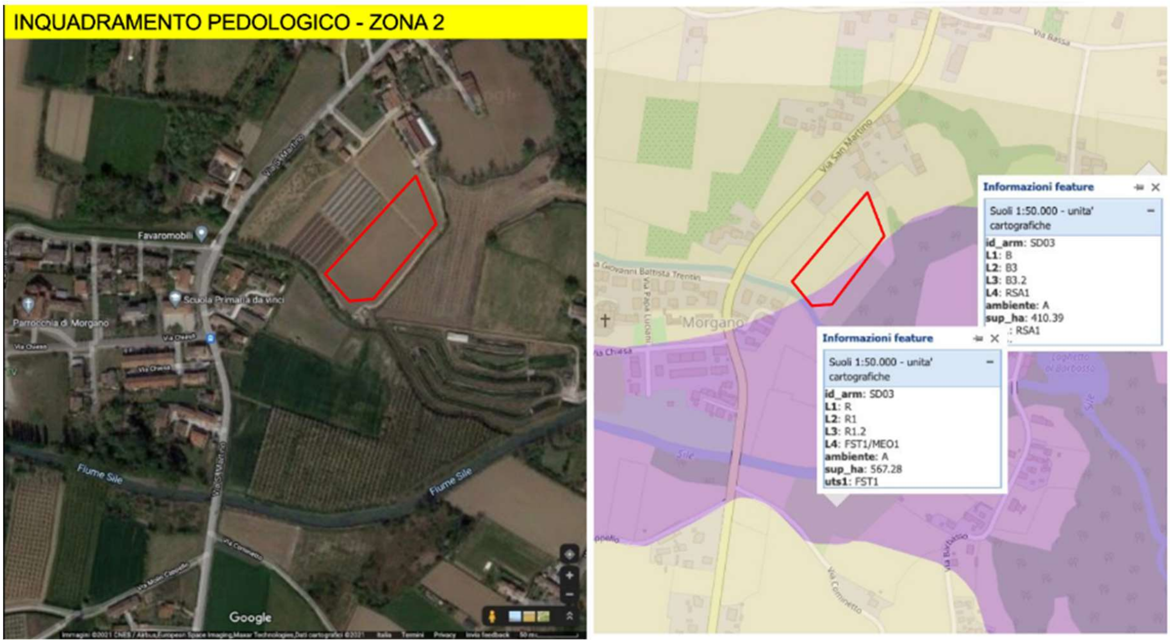
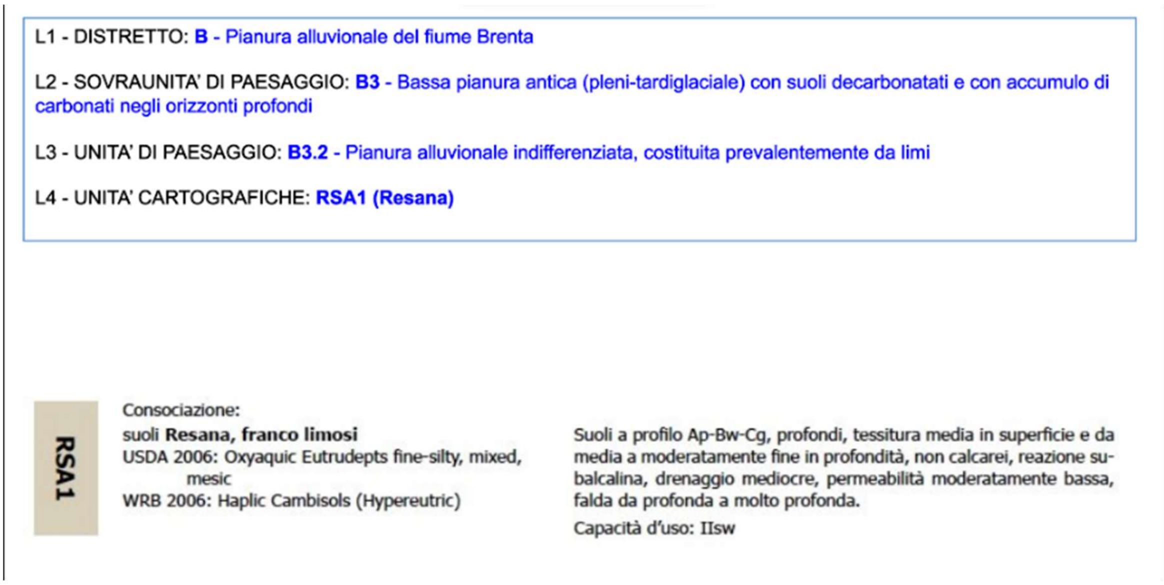


Figure 1, satellite photo and pedological chart (ARPAV, 2022)



L1 - DISTRETTO: **B - Pianura alluvionale del fiume Brenta**

L2 - SOVRAUNITA' DI PAESAGGIO: **B3 - Bassa pianura antica (pleni-tardiglaciale) con suoli decarbonatati e con accumulo di carbonati negli orizzonti profondi**

L3 - UNITA' DI PAESAGGIO: **B3.2 - Pianura alluvionale indifferenziata, costituita prevalentemente da limi**

L4 - UNITA' CARTOGRAFICHE: **RSA1 (Resana)**

RSA1

Consociazione:
suoli **Resana, franco limosi**
USDA 2006: Oxyaquic Eutrudepts fine-silty, mixed, mesic
WRB 2006: Haplic Cambisols (Hypereutric)

Suoli a profilo Ap-Bw-Cg, profondi, tessitura media in superficie e da media a moderatamente fine in profondità, non calcarei, reazione subalcalina, drenaggio mediocre, permeabilità moderatamente bassa, falda da profonda a molto profonda.
Capacità d'uso: IIsw

Figure 2, pedological description of the experimental field soil (ARPAV, 2014)

A profound change in approach was born thanks to CIPAN over 20 years ago in north-west of France, a very rainy area with draining soils right in front of the Atlantic. In those territories the vocation is strongly zootechnical, with widespread meadows and pastures and cultivation of almost exclusively fodder crops, among which corn reigns supreme. This leads to very high nitrogen loads per hectare, both due to livestock waste and corn fertilization, with a long season at risk of leaching. This is where the obligation of CIPAN – Cultures Intermédiares Piège À Nitrates, the nitrate-capturing intercrops, was born. For this journey into cover crops, we start from the lands around Sile, in mid-September 2020 (photo 1).

Photo 1, seeding the cover in September 2020

These intercrops were “born” as mono-specific covers, often mustard, other times phacelia, or ryegrass, or even rapeseed, most often with autumn sowing (early September) and with destruction/devitalization at the beginning of winter (maintenance of vegetation at least until December) for a significant effect on nitrogen conservation.

The “moutarde blanche” (white mustard) approach has spread greatly, also Italy, in Lombardy in particular, an area similar in many respects to the area in which this approach was born.

Small and inexpensive seed, extreme versatility in the sowing technique, early devitalization with frost and therefore free and usable soil already early in spring: these are considerable advantages for covers which must cost little because on systems with soil cultivation and in which benefits on weeds are limited to the competition during growing period and are then lost after tillage. However, the effects on fertility are important: starting by capturing the residual fertility at risk of leaching, with a recovery of 50 to 80% of the nitrogen lost by


Photo 2, the mustard field around mid-October

the naked soil (Briffaux, 2009, AREP experimentation in Thibie, Marne, France), and then also work on other aspects thanks to the powerful root taproots.

In photo 2 there is the white mustard a month after sowing. This choice of cover was proposed because it is very simple to make, inexpensive and frost-killing, to make room for a spring Radicchio, with a mulch that contains weeds, always a big problem for horticultural crops.

Protecting the soil with such a powerful green cover even in autumn months, usually the rainiest ones, is vital, especially where the soil or the ecosystem are more fragile, such as that of the rivers of the high plains. That year it rained little in November, almost too little compared to the previous year: 23 mm in 2020 compared to 220 mm in 2019. Something recovered in October, early, with two very intense events, and then again in December, in a similar way.

On the other hand, November was really (relatively) hot, which therefore means mineralization of the organic matter and therefore nitrogen released. Let's think about what can happen after high-productivity corn, with an efficiency of nitrogen use that is never high and therefore an important residual fertility.

With water and mild temperatures, plants present in the cover exploded, producing good biomass and covering soil well but above all recovering a lot of fertility. On this occasion we finally managed to keep an uncovered plot in order to see differences between covered and naked soil. There were no new expectations, but it's always useful to have confirmation and (re)have new experiences! Above all, being able to make farmers experience first-hand the difference between these two worlds: covered soil or bare soil.

It can be seen in the foreground (photo 3) the development of the weeds in the untreated plot and alongside the weeds under the mustard. Especially in these systems and especially with these weeds, it is unthinkable that a single

Photo 3, experimental field at mid-November seen from the control plot and a detail of it on the right.

cover will solve this problem, and above all that it will avoid the emergence of any weed. But the game is won on biomass: the greater the plant cover, the lower the weeds. However, the effect of the first frost around November 20th on the weeds was impressive (see the central part, the uncovered control in photo 4).

In the following days there were several, with temperatures well below zero at dawn almost until December, when bad weather brought mild temperatures for almost the entire month. The most developed mustard was already well layed and devitalized (photo 5), the other areas with smaller and therefore resistant plants will follow in a few days. Its duty was done: soil covered, nutrients captured, root work done. And, above all, the providing of these services is a few days longer than the weeds, thus doing a more efficient and cleaner job despite the biomass also produced by them.

Photo 4, experimental field at mid-November with a focus on the control plot few days before and after first light frost.

One of the main effects, as mentioned above, is that of a strong recovery of nitrogen, in particular nitrate which is the form at risk of leaching. In concrete terms, it is possible to recover several dozen units of nitrogen, at least, per hectare. All fertility and economic value that remains available to the farmer instead of abandoning the field (and polluting the environment). Of course, it will be made available in shares over several years, so it is a medium-term investment, in a certain sense. However, unfortunately, there is very little else. Brassicaceae, in fact, have very few relationships with the world around them: obviously not with symbiotic nitrogen fixers, but not even with mycorrhizae, and it seems that even earthworms appreciate them little. In short, theirs is more of a physical effect of soil protection and root work, with an important effect on nitrogen given their voracity.

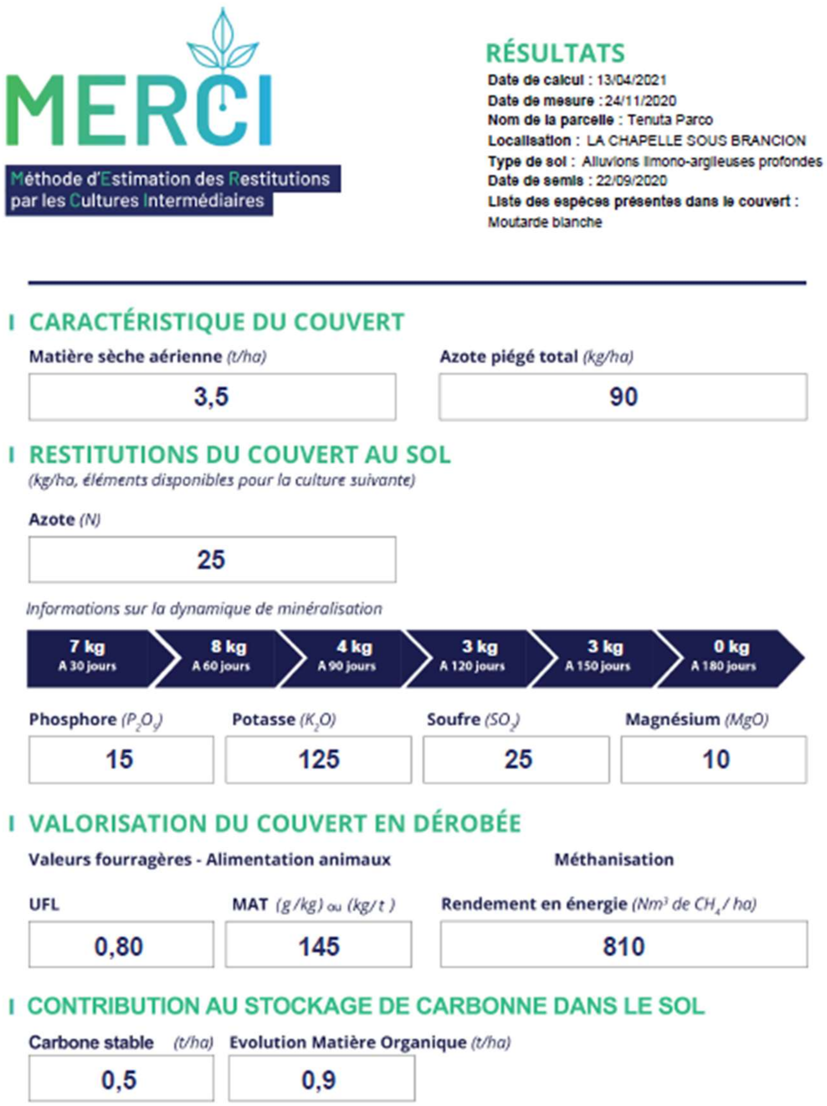
In a recent and substantial meta-analysis, Hallama and his colleagues (2019) evaluated a long series of studies that reported numerous field trials with as many cover crop species, with the aim of better understanding the dynamics of phosphorus in the soil, thanks to its hidden miners. Well, the Brassicaceae stood out only for the concentration of phosphorus in the aboveground biomass, with good results in absolute terms – even if well surpassed by the Poaceae. For everything concerning the complex and fascinating microbial world and the intense relationships at the level of the rhizosphere, the most "odorous" family of plant covers received a severe failure: worse result for abundance of mycorrhizae, phosphatase activity and rather bad also for the availability of the precious phosphate nutrient. In short, a moderate disappointment?

Photo 5, the more developed are mustard plants, the more they are sensitive to frost.

No, but neither they are the Holy Grail that some would like to pass off and in which too many believe. They have undoubted advantages: small and sometimes cheap seed, great versatility in sowing technique and time, easy devitalization and excellent competition with weeds, but above all rapid growth even in marginal seasons, as autumn often is in our climates. In fact, French creativity was born precisely from the desire to overcome the "mandatory CIPAN" – the mustard grown between September and December – seen as a cost, and instead make the vegetal cover become an ally in which to invest because it provides many agro-ecosystem services, works tirelessly

Photo 6, biomass samples, end of November and with important results on the fertility and health of the soil, at the sole cost of one pass of the seeder and a few kg of seed that is much more varied than mustard alone. And the associated rapeseed also arises – in part – from this need and consideration.

Approximately 3,5 tons of dry matter were produced by this cover (biomass samples on the photo 6). It started off a bit slowly due to drought, and several plants were missing. 90 units of nitrogen recovered, of which 25 are available for the next radicchio together with 15 units of phosphorus and 125 of potassium, 25 of sulphur and 10 of magnesium. Not bad, for simple and quick vegetable coverings, and above all easy to manage.


MERCI

Méthode d'Estimation des Restitutions par les Cultures Intermédiaires

RÉSULTATS

Date de calcul : 13/04/2021
Date de mesure : 24/11/2020
Nom de la parcelle : Tenuta Parco
Localisation : LA CHAPELLE SOUS BRANCION
Type de sol : Alluvions limono-argileuses profondes
Date de semis : 22/09/2020
Liste des espèces présentes dans le couvert :
Moutarde blanche

CARACTÉRISTIQUE DU COUVERT

Matière sèche aérienne *(t/ha)*	Azote piégé total *(kg/ha)*
3,5	90

RESTITUTIONS DU COUVERT AU SOL
(kg/ha, éléments disponibles pour la culture suivante)

Azote *(N)*
25

Informations sur la dynamique de minéralisation

7 kg A 30 jours	8 kg A 60 jours	4 kg A 90 jours	3 kg A 120 jours	3 kg A 150 jours	0 kg A 180 jours

Phosphore *(P_2O_5)*	Potasse *(K_2O)*	Soufre *(SO_3)*	Magnésium *(MgO)*
15	125	25	10

VALORISATION DU COUVERT EN DÉROBÉE

Valeurs fourragères - Alimentation animaux | Méthanisation

UFL	MAT *(g/kg) ou (kg/t)*	Rendement en énergie *(Nm³ de CH_4 / ha)*
0,80	145	810

CONTRIBUTION AU STOCKAGE DE CARBONNE DANS LE SOL

Carbone stable *(t/ha)*	Evolution Matière Organique *(t/ha)*
0,5	0,9

Figure 3, the Methode MERCI output from this cover crop evaluation

Cool season cover crops and conservation agriculture – San Giorgio

Pedological and chemical characterisation of the experimental site

Figure 4, satellite photo and pedological chart (ARPAV, 2022)

Figure 5, pedological description of the experimental field soil (ARPAV, 2014)

The so-called "Linea delle risorgive" identifies territories in the centre of the Venetian plain, with truly unique characteristics: their very particular soils, a transition area between the gravelly soils of the High Plain (and often with decarbonated clays which have a wonderful reddish colour) and the finer and heavier ones of the lower plain; so rich in water, with numerous resurgence rivers that flow there and which make the atmosphere misty and fascinating; so rich in history, now an irregular mix of the provinces of Padua, Treviso and Venice but once an area of important economic activity of the Serenessima republic; in short, these territories are the homeland of Treviso Radicchio and Variegato di Castelfranco (red chicory and hybrids), they are the land of choice for these very particular types of chicory, which takes on multiple shapes and colours (to be honest, almost all shades of a beautiful vinous red) based on the territories: from Treviso, to Chioggia, to Verona, passing through Castelfranco.

Campione n°						6713	6714	8189	8190			
pH			0	4	7	7,28	7,18	8,14	7,96	7		14
Conducibilità		mS/cm	0		0,25	0,08	0,08	0,11	0,11	0,5		2
Carbonio organico		C org. %	0	[illegible]	[illegible]	0,82	0,58	0,85	0,85	[illegible]	[illegible]	3
Sostanza organica		S.O. %	0	[illegible]	[illegible]	1,4	1,0	1,5	1,5	[illegible]	[illegible]	6
Da calcolo (C. org. x 1,724)												
Azoto totale		g/kg	0,5	1	[illegible]	1,0	0,8	1,0	1,1	[illegible]	[illegible]	4
C/N			0		[illegible]	8,51	7,66	8,07	7,51	13		25
Da calcolo												
Fosforo assimilabile		mg/kg	0	[illegible]	[illegible]	77,2	64,5	20,5	20,9	[illegible]	50	100
Potassio scambiabile		mg/kg	0	40	80	187,0	159,3	110,0	113,6	120	[illegible]	240
Capacità di scambio cationico		cmoli/kg	0	[illegible]	10	9,52	6,71	18,55	15,82	[illegible]	30	40
Magnesio		mg/kg	0	50		278,9	179,0	311,4	261,4	[illegible]	200	250
Sodio		mg/kg	50	150	[illegible]	25,6	24,7	5,1	6,6	[illegible]	[illegible]	690
Mg/K			1	[illegible]	4	2,8	2,8	3,0	3,4	6	[illegible]	10
Da calcolo												
Tessitura		Classe				FS	FS	FA	F/FAS			
Sabbia	2-0,05 mm	%				66,8	70,8	44,8	45,8			
Limo	0,05-0,002 mm	%				17,6	16,6	23,6	28,6			
Argilla	<0,002 mm	%				15,6	12,6	31,6	25,6			

Table 1. Chemical properties of experimental site soils.

In this farm, the choice was oriented on a classic winter cover here reported from sowing, on 19 October 2019, recalled in photo 7, to the early stages of winter. The mixture thought up here was a mixture of Brazilian oats, rye, vetch and phacelia, a classic combination for making biomass, but balanced, and with an eye on flowering for useful insects.

Photo 7, cover crop seeding at the end of October and the development after a month.

One month after sowing, towards the end of November (photo 8), this was the situation in the field with the classic enrichment of coverage given by the leaves of the banks and various trees that distinctively enrich this territory of the plain, with a dynamism of the agroecosystem that many regions envy.

Photo 8, trees on field margins leave leaves over the emerging cover.

And this diversification and dynamism are also a peculiarity of this open-field horticultural crop. Horticultural companies, in fact, are often extremely specialized in crops of this kind, alternating botanical families and species of vegetables, between open fields and greenhouses, but with a pressure and a rhythm that the soil often struggles to bear. For some phytosanitary problems such as nematodes, or various rots of both fungal and bacterial origin, the transition from a *Solanaceae* to a *Cucurbitaceae*, or even an *Asteraceae* such as radicchio, does not generate particular adaptation difficulties for the pathogen, so even if it does not manifest disease and generates therefore damage because the species is not sensitive, it can still maintain and conserve itself in the soil.

Therefore, despite (often) good diversification, there is actually a lack of functional (plant) (bio)diversity that nourishes and stimulates a high functional microbial biodiversity in soil.

In the most sustainable agricultural approaches or those that seek sustainability, rotation as an application of the use of plant diversity is an important pillar.

This is not a pressing problem at the moment, especially for some companies that in this area rotate radicchio with corn, soy, wheat and alfalfa. The scarcity of different plant species in succession is a problem that concerns, however, many farms, both cereal and horticultural, and if the contamination between these two systems is often an intelligent expedient, unfortunately for some it is no longer economically sustainable. In fact, some farmers, driven to specialise themselves more and more, tend to increasingly simplify the alternate crops, such as cereals and grain legumes among these tree-lined banks, waiting for the intense green stained with purple flames of the Treviso radicchio.

Photo 9 the development toward winter end, in February 2020

Plant diversification remains fundamental for better and sustainable agriculture, together with the significant reduction of soil tillage. If this second action serves to maintain “the house”, or rather, the houses – entire towns inhabited by useful microorganisms (and not only that... in every city and territory there is a bit of crime, but if kept at bay and under control, there can coexist) for the farmer – it is the presence of different plants that nourishes and stimulates a multitude of microorganisms so complex and articulated that in the first instance it means optimal coverage of all soil activities and cycles, from nitrogen fixation to nitrification, from the mobilisation of phosphorus to that of manganese; but complexity and diversity, together with high activity, also mean little space for the “crime” constituted by bad microorganisms, those pathogenic for our crops.

Recently, an in-depth analysis was published in Science Advances which examined 98 meta-analyses and around 5000 studies, for a total of over 40 thousand comparisons between simplified agronomic systems and much more diversified systems.

Tamburini and colleagues from all over the world who collaborated (Germany, Sweden, United States with California and Iowa, Canada, Italy with the University of Bari) have created a truly impressive work of which here are reported some summaries.

In the meantime (photo 9 and 10), we moved on to the new year, at the beginning of February 2020, when everything was still proceeding more or less normally. Another plot was sown on the same day and in the same conditions, but which will host Radicchio Precoce di Treviso, and a fertilization test.

Photo 10, a detail of the cover.

In the work cited above it was observed that stimulating biodiversity in agricultural systems could promote ecosystem services, thus reducing the dependence on external inputs, while maintaining high productivity unchanged. The impact of different diversification practices on below-ground and above-ground biodiversity and ecosystem services was analysed across a large number of studies, ultimately including 41946 binary comparisons between diversification and non-diversification practices. Overall, diversification improves biodiversity, pollination, disease control, nutrient cycling, soil fertility, carbon sequestration, and moisture and overall climate regulation, without compromising crop productivity. Soil fertility, nutrient cycling and soil moisture regulation are factors stimulated by practices that have underground biodiversity as their object, while practices that are more concerned with what is above the ground influence the rest. Most of the time, the impact of these practices results in a so-called win-win, a doubly successful result both for the services provided and for the crop yield. The variability of the responses and the occurrence of compromises highlight the strong link between the results and the pedoclimatic and agricultural context.

Greater diffusion of diversification practices shows promise in contributing to biodiversity conservation and food security from local to global scales.


Photo 11, the overlap spot in detail.

In an area towards a headland (photo 11), where overlapping passes led to an overdose of seed, no particular deficiencies are noted, but rather a powerful containment of weeds, yet another demonstration of the clearing capacity of plant covers, but also a sign of good fertility present in the autumn period, which absolutely should not be wasted by leaving the ground bare!

Photo 12, cover crop development at the beginning of May 2020

These areas, sometimes involuntary, are fundamental and must instead become common practice. Leaving "uncovered" areas, whether of cover seed or a phytosanitary treatment, or creating doubled areas, both of fertiliser and of seed, is a fundamental tool for reading the response of the soil and crops, over the years, to the practices adopted.

And precisely speaking of practices and returning to diversification, the reduction of soil cultivation, crop diversification and the use of organic soil conditioners are three practices that have highlighted the greatest impact on biodiversity. Obviously the second has a greater impact on what happens above the ground, compared to the others which instead directly affect the soil and therefore influence its characteristics. In particular, the creation of that structure and environmental stability that allow the establishment of complex, diversified, efficient microbial communities. In the article, the researchers also evaluated – as having limited impact – organic farming and the absence of diversification, or even the inoculation of microorganisms: for the latter the only parameter influenced was the crop yield. A well-known effect, but one which must not give false hope: it is unthinkable to repopulate a territory which has been bombed and subject to continuous explosions and catastrophes of all kinds (intense and invasive soil tillage), introducing small groups of colonisers thinking that they can develop important and stable communities. That territory will remain easy prey for looters and criminals capable of living well in those conditions. This is what happens in a soil constantly disturbed by cultivation, often "abandoned" to the elements for many months between one crop and the next one.

Here (photo 13) is instead what can happen to a land which, as soon as it is freed from the cash crop (in this case it was soybean) is immediately sown with other diversified crops, perhaps using species that are rarely present in rotation, with generous blooms such as vetch and phacelia, and with discreet aggressiveness on leachable nutrients such as the two cereals.

Photo 13, flowering of Vetch and Phacelia, a great opportunity to promote functional biodiversity

These aspects should also be taken into account by the institutions that direct agriculture towards more sustainable techniques, and think that establishing ecological islands such as EFAs, which however can also include arable fields, under certain conditions - sometimes a little illogical - to a few months, and then it doesn't matter what happens after, could constitute a set of revolutionary actions when in reality results are very few.

Speaking of the plant biomass results obtained with the MERCI method (Figure 6), cover biomass was sampled at the beginning of May 2020 resulting in 7,1 t/ha of dry matter, 195 nitrogen units captured and produced, of which 75 available for the following crop, together with 35 units of phosphorus and 235 of potassium. As always when intercultural crops are successful, the fertility numbers put into play are considerable.

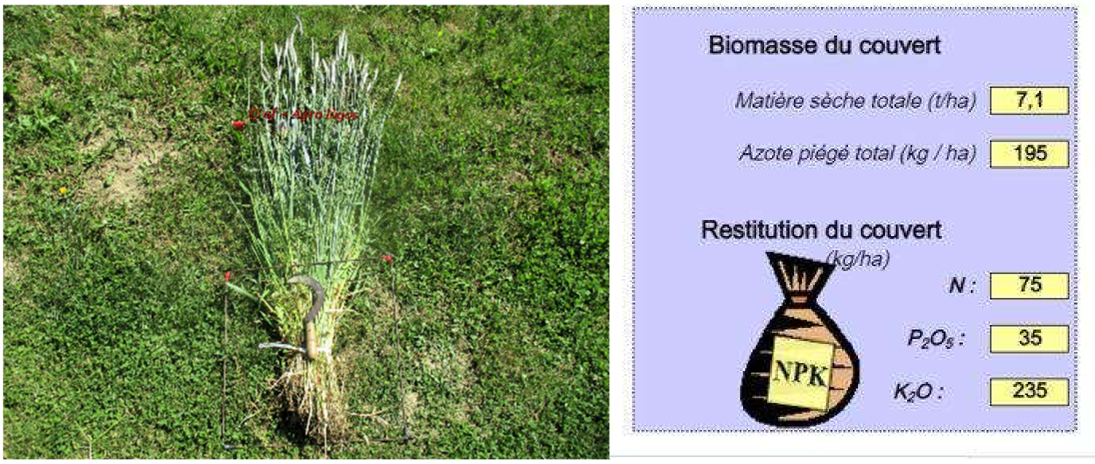


Figure 6, the Methode MERCI output (version 1.0) from this cover crop evaluation

Covers, therefore, are an excellent ally in the diversification of crop rotations even in horticultural companies, especially in those which for management or economic reasons struggle to grow (many) other crops in rotation. And obviously they are the engine for regenerating soil fertility, in particular combined with a progressive reduction in soil cultivation.

Photo 14, seeding a relay cover crop of faba beans on the previous crop already mulched but still alive.

Precisely in this regard, the idea arose of a sort of combination between a cover relay (a definition that usually refers to a winter crop grafted onto a summer one, while in this case it was the opposite) and a sort of "green tillage", with the sowing of field beans on future Radicchio transplant lines. This second sowing was carried out on May 29, 2020 (photo 14), on sod soil. Unfortunately, given the period which, with the exception of the heavy rainfall in the following days, was hot and then quite dry between mid-June and early July, they compromised the optimal development of the field beans, which in any case reached a development of around 40 cm at the moment of the preparation of the Radicchio transplant: not much but some additional units of nitrogen definitely arrived.

Photo 15, from summer preparations and transplanting to the crop ready to be harvested.

Some photos of the prepared soil (on the left, photo 15) while at the bottom of the field there is still the field bean present (large on the right), which will only be passed with a rotary harrow. Following, the seedlings to be transplanted and the final development, in November 2020, when the results of this first year of work were finally collected (photo 16). At the moment the strong reduction in tillage for a plant that needs a significant flow of fertility and that needs a good soil structure to develop its root, a key factor for the subsequent bleaching process in spring water, does not seem having given particular impacts, indeed, the plants grown with minimal tillage appear to have yielded equally (or even a few grams more, but without statistical significance). Further analyses and tests will confirm this first good result!

Photo 16, Radicchio collected after cleaning and weighting.

Radicchio tardivo San Giorgio - prove lavorazione			
Campo via Pitter, radicchio tardivo			
Prova 2020			
Tesi	Cover	Cover+ACS	
Peso in	790	1119	Cover invernale e lavorazione "standard"
grammi	1416	1038	e cover invernale+cover relay estiva e minima lavorazione
	1077	1282	
	1250	1258	Nessuna differenza visiva particolare legata a fallanze o marciumi.
	1213	1099,5	Pianta sterrata, lavata e non toelettata
Media	1149,2	1159,3	

Table 2, results from weighting Radicchio with the simple winter cover crop ("cover") or with, in addition, also the faba bean and the reduction of soil tillage ("cover+ACS").

In conclusion, some food for thought left between intercropping, coverage and tillage. Because everything happens down there, around the roots of the plants, and who knows what still unclear complexity exists around the exchanges between the roots of Radicchio and some reborn vetch plants. For now, these covers have been carried out with minimal tillage, perhaps a little intense but, in any case, without ploughing and with reduced depth. The path to be taken to optimise these techniques remains long but the premises of these first experiences give confidence.

Photo 17, spring 2021: seeding of a new cover crop on the same field.

Taking up the chronological thread of the second vegetal cover presented, in the photo 18 is the situation of the field in Quinto di Treviso in mid-April 2021, about a month after sowing the second year and cover of this farm and project. There seem to be some emergency difficulties, several plants are missing.

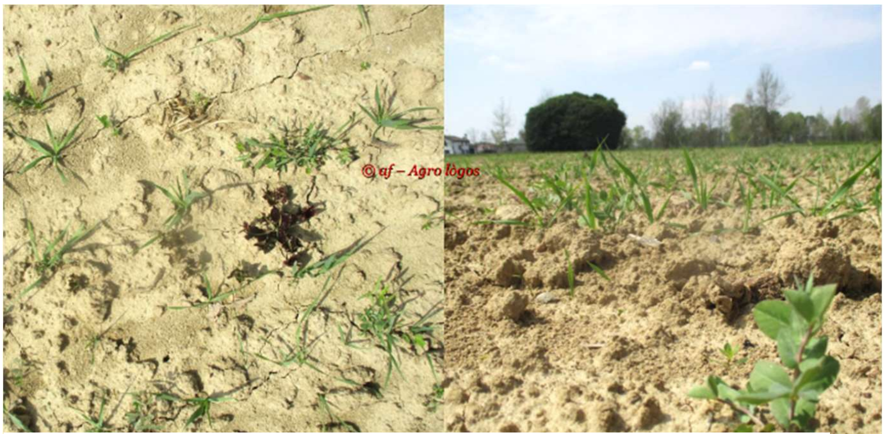


Photo 18, mid-April, development of the cover crop after quite a month after seeding.

It's a basic mixture common to another farm of the project and composed of oat, common vetch, forage pea, field bean, phacelia – with self-produced seed and, for comparison, an area with brown mustard alone and an overlapping band of the two covers (photo 19).

In addition to the drought of the period (March-April), even two big frosts as April often brings us, around Easter, do not help the plants which started with a certain difficulty. Here the soil had been bare much earlier than in the other farm test field and the land tilled in advance has perhaps dried out too much.

Photo 19, situation of the field at the end of April 2021: lots of soil coverage is lacking due to poor emergence.

Another recurrence of recent springs is also confirmed: the rain comes but often all at once. From April 25th to June 10th there has been a maximum of 2 and a half days without rain. When it finally dried, after almost 45 days, the heat came suddenly. Stresses that are not good for crops, and by now those in the Quinto field have taken a certain (bad) path and will not be able to produce large biomasses (photo 20). Anyway, soil is still covered, the potentially leachable nutrients remain retained in the soil and there is some competition towards the weeds.

Photo 10, end of May 2021, cover mixes in full blooming.

On June 15th, after the first part of the covers had already been shredded, biomass samples were taken. Pure brown mustard produced 4,3 t/ha of dry matter (Figure 7) with a total quantity of nitrogen of around 95 units, however very little amount available – only 17% with an initial phase of “nitrogen starvation” following incorporation with tillage.

Figure 7, the Methode MERCI output (2.0 version) from this cover crop evaluation

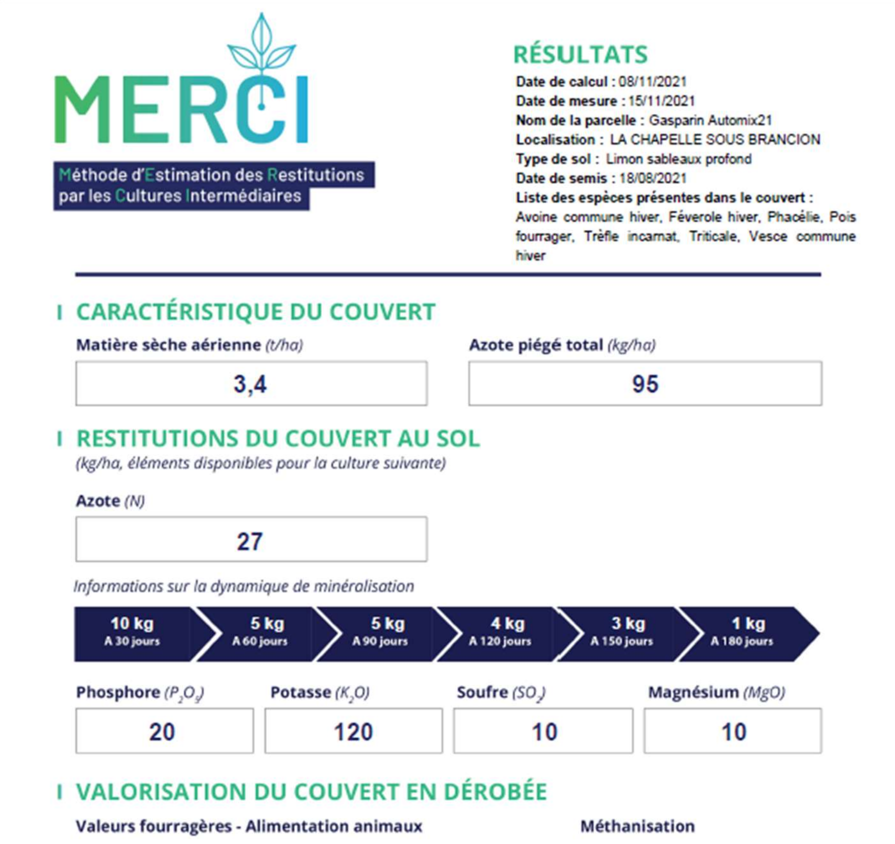


Figure 8, the Methode MERCI output from this cover crop evaluation

The self-produced mixture (like the seed) stopped at 3,4 t/ha of dry matter (Figure 8) with a quantity of total nitrogen however equal to the previous mustard, 95 units: the same quantity in lower biomass means a lower C/N ratio, and in fact the return fertility rate rises to 28% with greater availability already from the initial phase.

The overlap of the two covers probably "fell" in a particularly fertile area of the field, given that an increase in biomass like the observed one is anomalous (Figure 9), but is reported for completeness. 8,1 t/ha of dry matter with a quantity of nitrogen of 195 units, with an intermediate situation compared to the previous ones, both in terms of return rate and C/N.

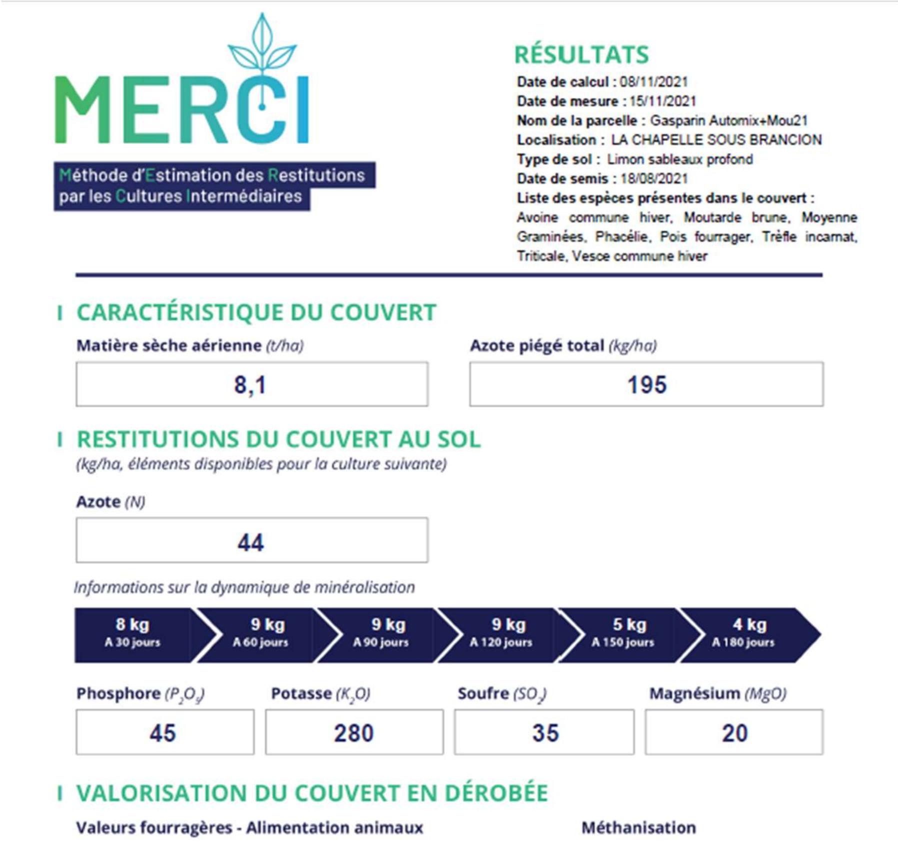


Figure 9, the Methode MERCI output from this cover crop evaluation

Correctly governing these "constitutive" parameters of the mixtures is essential to maximise the benefits of plant covers in terms not only of recovery and retention of fertility (nitrogen) but also of recycling and processing other nutrients and stimulation of biological activity.

Correctly planning the sowing period and technique can also allow to maximise the biomass produced and therefore mineral fertility brought into play. But this also concerns the subsequent processes, as can be seen in the following photos, concerning a unique experience.

Photo 11, cover crop biomass sampling.

In this plot, in fact, in addition to the different soil covers, different conservative soil tillage techniques were also tried. Together with continuous soil coverage, the reduction or elimination of tillage remains one of the few effective techniques in regenerating soil fertility, from a physical (structure), chemical (organic matter) and biological (earthworms and microorganisms) perspective.

In addition to very superficial tillage with the rotary harrow alone, in summer 2021 a strip-tiller was used (photo 22) in one of the first Italian experiments to prepare the strips for the future transplant of Radicchio.

Photo 12, soil preparation with strip tillage on different areas of the field, July 2021

As can be seen (photo 22, bottom right), the soil is worked only in some bands approximately 15 cm wide and approximately 25 cm deep by a series of idle working parts – row residue cleaner, pre-cut disc, cultivator anchor and containment discs, finishing roller.

Tillage operation was done on 17 July, on temperate soil and chopped cover crop. Rotary harrow passages were carried out transversally (photo 24) to have the strip-tiller and rotary harrow overlap to manage the different portions of soil (inter-row, intra-row), compared with the individual operations on the different bands of cover (mustard, mixture, mustard+mixture, untreated control).

Photo 13, different plots after soil preparation with the strip-tiller, on cover crop or uncovered soil.

The radicchio was transplanted at the end of July and some surveys and monitoring were carried out during autumn, both on soil and on crop (photo 25). Radicchio crop, as already from the first experiences of the previous year, did not show any problems in the face of a strong reduction in soil tillage, and given the nature of the soil and the production of biomass of the plant covers, the cultural interventions (fertilizations, weedings) were not found to be more difficult in the presence of firm soil in the inter-rows. However, in the presence of more performing covers, in particular as seen during the 2021 demonstration day on similar mixtures but higher biomasses on another farm, the machine may find itself in difficulty managing the residue.

Photo 14, after transplantation, several soil managements can be seen, with differences between inter-row and intra-row tillage.

The radicchio samples were collected in mid-

December, in total on 8 diverse plots differing in terms of soil tillage technique and coverage in the intercropping period. The bleaching began on 17 December 2021 and ended with the grooming and weighing of the samples on 17 January 2022.

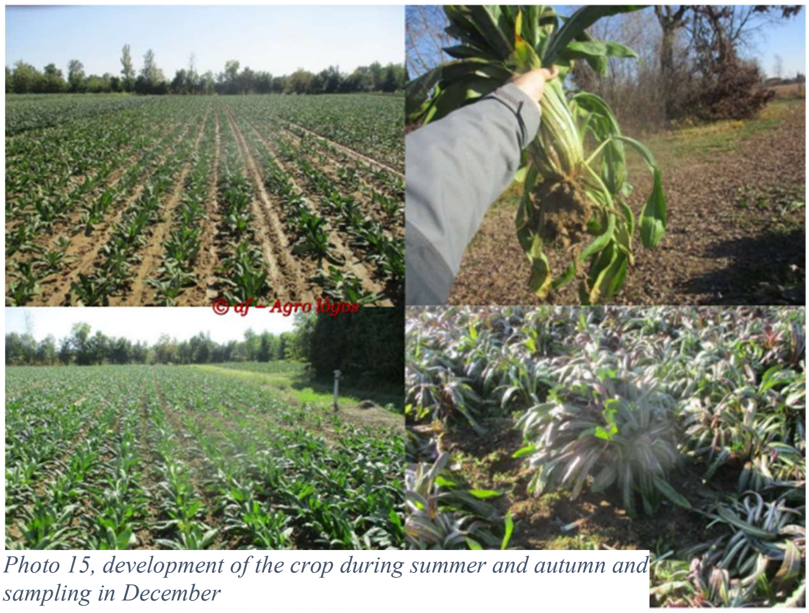


Photo 15, development of the crop during summer and autumn and sampling in December

No statistically significant differences emerged in the average weight of the 12 tufts collected in the different areas, which included also the comparison with standard farm management (heavy cultivator and refinement with rotatory tiller or power harrow).

On 28 March 2022, again in the same plot, a new cover was sown, after the radicchio harvested between January and February (photo 26). The mixture is the result of the experience of the previous two years: oat, forage peas, vetch, field bean, brown mustard, triticale, red clover and phacelia. The innovation introduced was the sod sowing of the mixture.

Photo 16 seeding of the third cover, always on the same plot, on minimum tillage or with direct drill.

In the easternmost part of the plot, the early harvest of radicchio favoured a certain development of weeds (chickweed and veronica) despite without a strong biomass produced and with a certain vegetative stasis due to drought (photo 27). Two strips of the plot, in addition to the headers, had recently been harrowed to accommodate some ruts produced during the harvest, and thus served as a “tilled” comparison compared to no-till sowing.

Photo 17, seeding on a very dry soil; it can be seen the difference between tillage or no tillage (left) and weed development between to dates of Radicchio harvest.

It was particularly useful, even if it represented an agronomic strain, to maintain the same plot fixed for the 3 years, being able, on some occasions, to consolidate the work done the previous year. Unfortunately, having underestimated the presence of that infestation turned out to be deleterious and compromised the good progress of the 2022 cover. These, and often especially mixtures, are effective tools in the management of weeds but the essential condition to succeed is that they have to establish themselves without competition and be able to gain a certain margin of advantage.

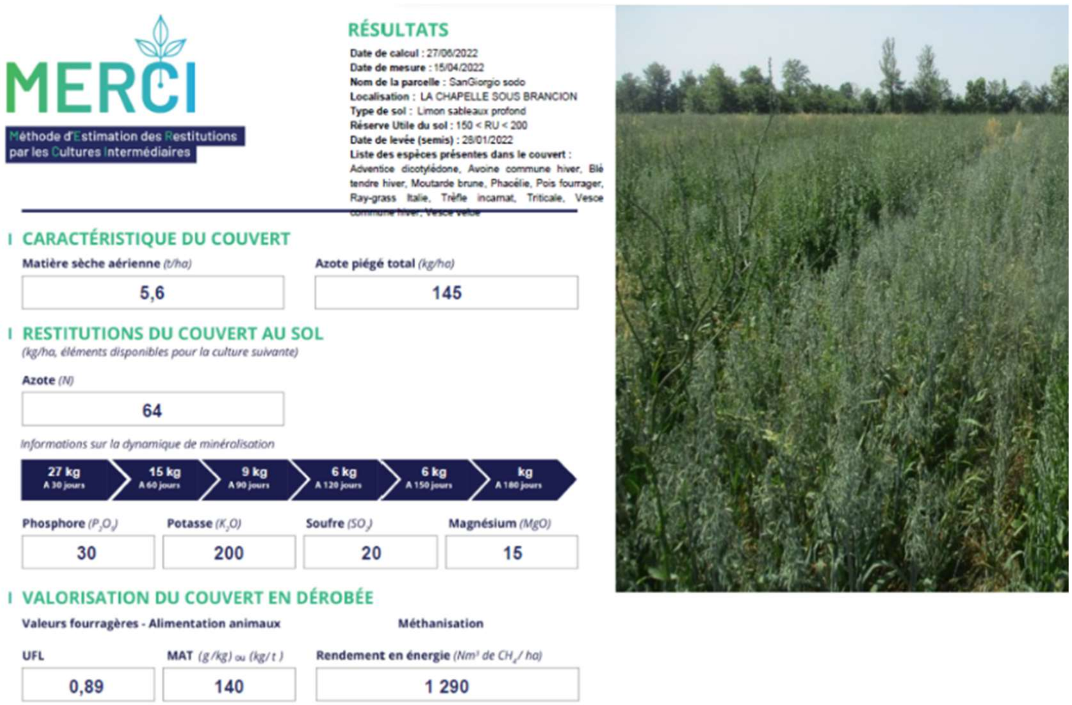


Figure 10, the Methode MERCI output from the no-till cover crop evaluation.

Furthermore, with yet another critical spring, starting with a certain disadvantage certainly did not pay off, and the difference in terms of biomass was there at around 2 t/ha of dry matter (5,6 versus 7,6 t/ha; Figure 10 vs Figure 11), especially with an advantage of grass species in the more productive cover. Correlated to the biomass is therefore the total quantity of nitrogen (about 30 nitrogen units of difference) but not the quantity returned (substantially identical between the two coverages), as the composition of the biomass itself has changed.

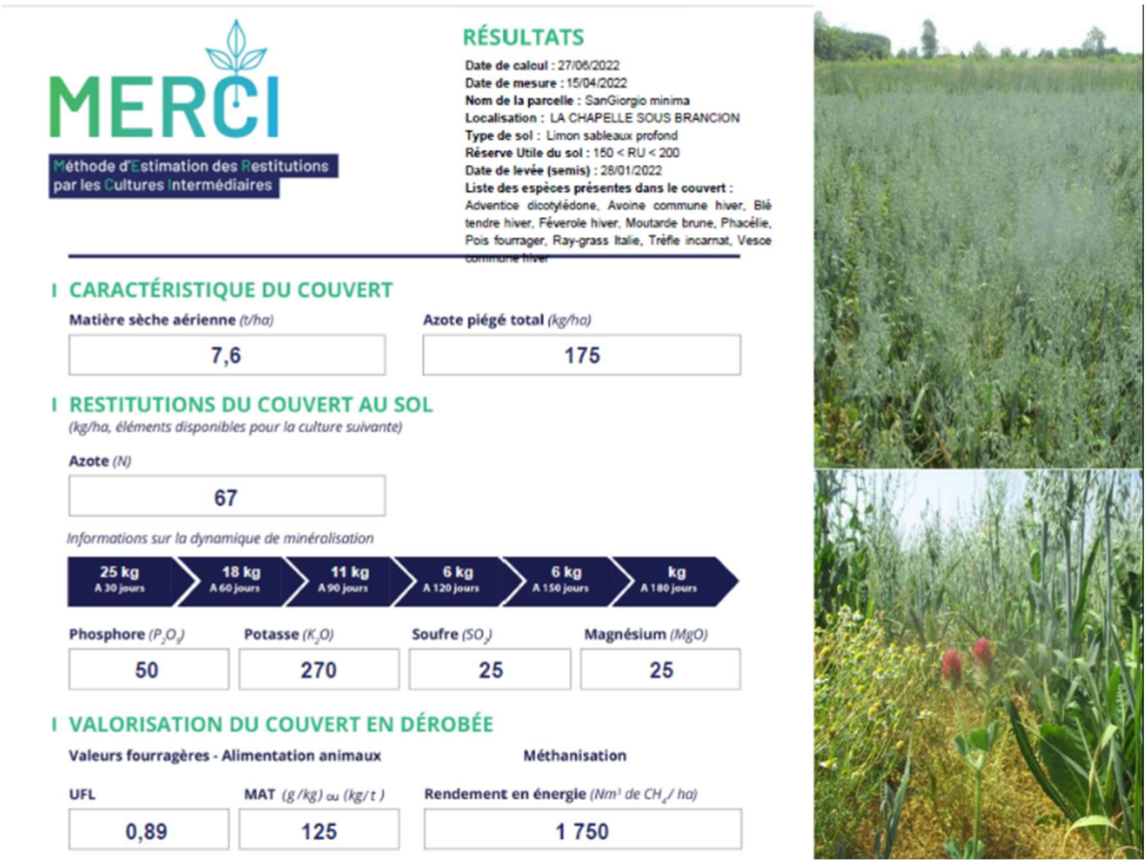


Figure 11, the Methode MERCI output from the minimum tillage cover crop evaluation.

After 3 years of radicchio, production capability was maintained, without the need for heavy fertilisation or any particular phytosanitary problems arising. Some difficulties remain in the management of typical horticultural weeds, but to resolve this issue it will be necessary to return to crop rotation, perhaps also taking advantage of some conservative techniques, in particular direct sowing under cover crop, a well-established technique in cereal crops, to better manage the seed bank and pests present. Radicchio has proven to be a crop with a very aggressive and rustic root system, capable of withstanding the reduction in soil tillage, necessary to regain lasting fertility together with plant diversity and permanent soil cover.

Cover crops: complex mixes and C/N ratio – Agricola Brognera

Pedological and chemical characterisation of experimental site soil (second year the experimental site changed).

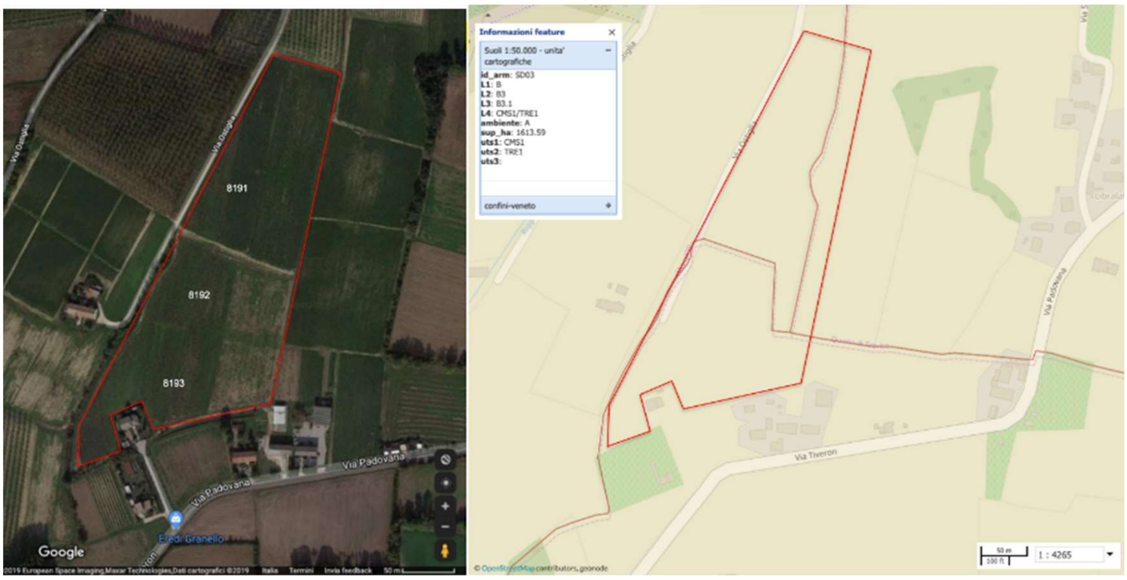


Figure 12, satellite photo and pedological chart (ARPAV, 2022)

L1 - DISTRETTO: **B** - Pianura alluvionale del fiume Brenta

L2 - SOVRAUNITA' DI PAESAGGIO: **B3** - Bassa pianura antica (pleni-tardiglaciale) con suoli decarbonatati e con accumulo di carbonati negli orizzonti profondi

L3 - UNITA' DI PAESAGGIO: **B3.1** - Dossi fluviali poco espressi, costituiti prevalentemente da sabbie

L4 - UNITA' CARTOGRAFICHE: **CMS1/TRE1 (Camposampiero 1 / Treville 1)**

CMS1/TRE1

Complesso:

suoli **Camposampiero, franco sabbiosi**
USDA 2006: Dystric Eutrudepts coarse-loamy, mixed, mesic
WRB 2006: Haplic Cambisols (Hypereutric)

Suoli a profilo Ap-Bw-C, da profondi a molto profondi, tessitura moderatamente grossolana, da non calcarei in superficie a moderatamente calcarei nel substrato, reazione da subalcalina o neutra in superficie e alcalina nel substrato, drenaggio buono, permeabilità moderatamente alta, falda da profonda a molto profonda.
Capacità d'uso: IIsc

suoli **Treville, franchi**
USDA 2006: Dystric Eutrudepts fine-loamy, mixed, mesic
WRB 2006: Haplic Cambisols (Hypereutric)

Suoli a profilo Ap-Bw-BC, profondi, tessitura media, da non calcarei in superficie a scarsamente calcarei in profondità, reazione da subalcalina in superficie ad alcalina in profondità, saturazione molto alta, drenaggio buono, permeabilità moderatamente alta, falda molto profonda.
Capacità d'uso: I

CMS1 - suoli CAMPOSAMPIERO, franco sabbiosi

AMBIENTE
Dossi fluviali poco espressi della bassa pianura antica (pleniglaciale) del Brenta, con suoli decarbonatati ed accumulo di carbonati negli orizzonti profondi. Il materiale parentale e il substrato sono costituiti da sabbie fortemente calcaree. **Uso del suolo:** mais, seminativi avvicendati.

PROPRIETÀ DEL SUOLO
Sono suoli a moderata differenziazione del profilo, decarbonatati, con un orizzonte di alterazione (Bw) e a granulometria franco grossolana. Hanno profondità utile alle radici da elevata a molto elevata limitata da bassa ritenuta idrica, drenaggio interno buono, permeabilità moderatamente alta, capacità d'acqua disponibile (AWC) moderata; la falda è da molto profonda a profonda.

CLASSIFICAZIONE
USDA (KEYS 2010): Dystric Eutrudepts coarse-loamy, mixed, mesic
WRB (2006): Haplic Cambisols (Hypereutric)

Figure 13, pedological description of the experimental field soil (ARPAV, 2014)

Campione n°			8191	8192	8193
pH			7,36	5,84	6,55
Conducibilità		mS/cm	0,13	0,20	0,04
Carbonio organico		C org. %	0,47	0,51	0,49
Sostanza organica		S.O. %	0,8	0,9	0,8
Azoto totale		g/kg	0,63	0,65	0,59
C/N			7,5	7,9	8,4
Fosforo assimilabile		mg/kg	79,0	83,6	48,9
Potassio scambiabile		mg/kg	81,9	144,8	61,8
Capacità di scambio cationico		cmoli/kg	6,63	3,80	3,93
Magnesio		mg/kg	221,0	98,1	93,8
Sodio		mg/kg	23,2	15,8	12,5
Mg/K			2,8	2,8	3,2
Tessitura		Classe	FS	SF	SF
Sabbia	2-0,05 mm	%	75,0	80,0	82,0
Limo	0,05-0,002 mm	%	14,8	9,8	9,8
Argilla	<0,002 mm	%	10,2	10,2	8,2

Table 3. Chemical properties of experimental site soils

It was March 2020, about a week after the almost total closure of activities. Walking around the semi-deserted country roads, but teeming with agricultural activity, was pretty strange (photo 28). Agriculture, in fact, has never stopped, even if it has suffered greatly from the blockage or strong slowdown of related activities: from those of assistance and supply for the agricultural company and its operations, to the commercial ones where the agricultural product is brought to the end customer.

Photo 18, seeding the demonstration field on 20^{th} March 2020.

But the particular sensation was also linked to the explosion of blooms which contrasted with the general climate we were experiencing at that moment, and which at least, thanks to the activity in the field, we could continue to enjoy.

In short, it was the time to sow some spring covers. The fields freed from radicchio (photo 29), a winter flower, were crying out for another type of flowering, which would also provide a nice covering for a while, perhaps until the next radicchio.

Photo 19, same field, different period: from the first visit and soil sampling in October 2019 to spring 2020

This plot is called “Valle del radicchio”, a beautiful countryside a few hundred metres from the Zero river, and a few km from the Sile River, in the heart of the “Linea delle Risorgive” and the production area of the Radicchio di Treviso (or rather, of “Radicchi”). Soils generated by limestone silt and sand once brought by the Piave and other rivers and which clearly separate the Upper Plain, with the red and gravelly soils (“Ferretti”) of the ancient Piave from the Lower Plain, with its light and often finer and heavy soils. The line of the springs is a border and a hinge, with a truly unique landscape, which in summer is a green oasis, but also a crossroads of provinces and many stories.

This field trial from the first year of the Biofuture project was certainly the one of the most articulated, complex, but also beautiful and flowery, and in which fortunately we managed to organise the first demonstration day of, precisely on the occasion of its devitalization.

Thanks to the willingness of this farmer, we had the opportunity to try different mixtures and single species, and also from this opportunity came the desire to make as many farmers as possible aware of this beautiful showcase field but also some particular aspects of the covers, also and not only in horticulture.

Quick and easy soil preparation. Cover crops always live between the need to cost little, from the seed to the devitalization through tillage, and the need to make the most of it, following the mantra that the cover must be treated as a cash crop. Some Radicchio plants survived tillage operations (photo 29) and perhaps will give an unusual spring flowering, as a biennial plant.

Photo 20, several plot with single species (brown mustard) or mixtures.

Some underestimations of the sowing density led to less surface area being sown than expected, and so the proposal to close the field with brown mustard (photo 30 on the left) for further comparison with a species that is starting to be widespread in the area due to its biofumigation capabilities and its versatility as a sowing period, was gladly accepted. In photo 30 it can be seen the various stripes with different mixtures separated by strips without coverage as control.

Complex mixtures of 10 or more species are always very fascinating, up to 15-20 proposed by some seed companies! But are they really effective?

In particular, one of the two “ready-to-use-mixtures” purchased – both, however, suitable for horticulture and spring sowing – was composed of 10 species while the other 15. The MERCI biomass data are immediately presented, before moving on to some reflections on covers, among the lessons learned from this first year of experiences and new international stimuli on the truly central topic.

Photo 21, the Ecopro03 at the end of development.

Starting from the mixture called Ecopro03 (photo 31), with 10 species represented by triticale, field pea, common vetch, field bean, Alexandrian clover, squarrose clover, white mustard, radish, phacelia, safflower, the results in terms of biomass show 4 tonnes of dry matter per hectare, 120 units of organic nitrogen produced (retention + fixation) of which 35 units made available in a decreasing manner over 6 months (from 15 kg to 1 kg), 25 units of phosphorus, 155 of potassium, 20 of sulphur and 10 of magnesium. The stable carbon contribution is around 0,6 t/ha.

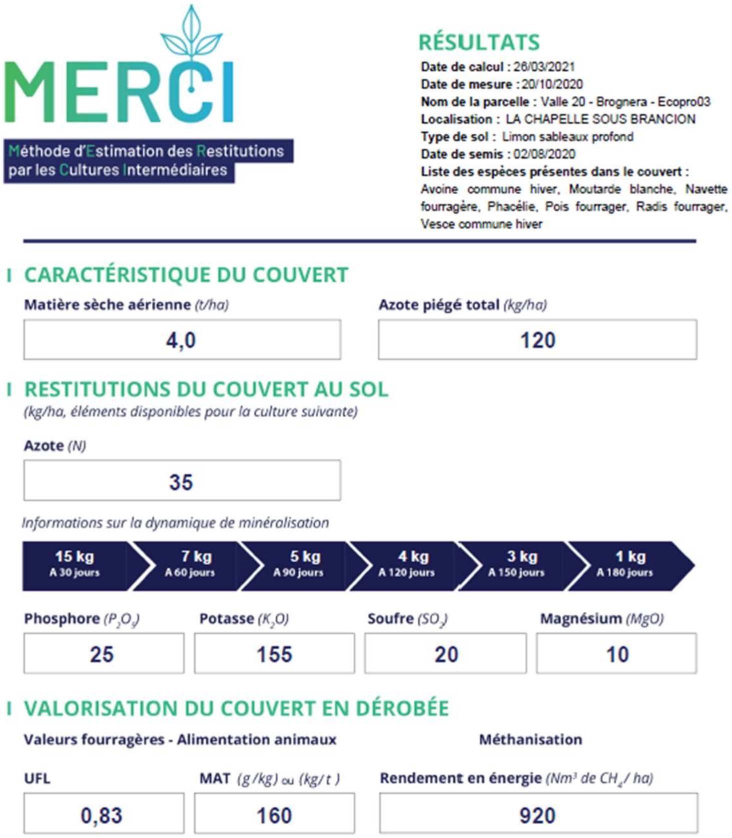


Figure 14, the Methode MERCI output from the Ecopro03 evaluation.

The latest version of the MERCI Method was presented at the beginning of 2021 when this data was processed and saw the introduction of interesting new information. There has been a very slight review of the average nutrient contents for each species, a small reduction in the nitrogen produced but also a different consideration of root coefficient. The real novelty is the dynamics of the transfer of that amount of nitrogen which, based on the C/N, is estimated as available for the crop cycle following the crop.

To this an interesting complement was added with two “meso-nutrients” such as sulphur and magnesium, very important in plant nutrition, and finally a focus on the fate of organic matter, with a whole series of options to make this estimate as plausible as possible: it can be indicated, in fact, the type of soil, water holding capacity in mm, the management of cover crop biomass (removed or left, and therefore buried or rolled) and values of any secondary valorisations of


Photo 22, cover crop development at the end of April 2020

biomass, within a livestock activity or methanization. A complex and detailed system, which must be learned to read in its relative uncertainty, but which can be really useful in managing processes in order to better understand which path a "soil system" is taking and to be able to see its evolution over time.

Ecopro04 (photo 33) is the other ready mix, again for horticulture and always for spring sowing. Its composition, of 14 species, is: triticale, common oat, field pea, blue lupine, cowpea, common vetch, Alexandrian clover, squarrose clover, white mustard, radish, field mustard, phacelia, buckwheat, safflower. In this case (Figure 15) slightly higher production of dry matter, 4,3 t/ha, but slightly lower in nitrogen, 110 units, with a quantity similar to the previous one relatively to nitrogen returns, with 31 units available spread over 5 months, 30 units of phosphorus, 160 of potassium, 20 units of sulphur and 15 of magnesium. Organic carbon values instead equal to the previous cover, despite a few kg of additional biomass.

Photo 23 the Ecopro04 mix at the end of his development.

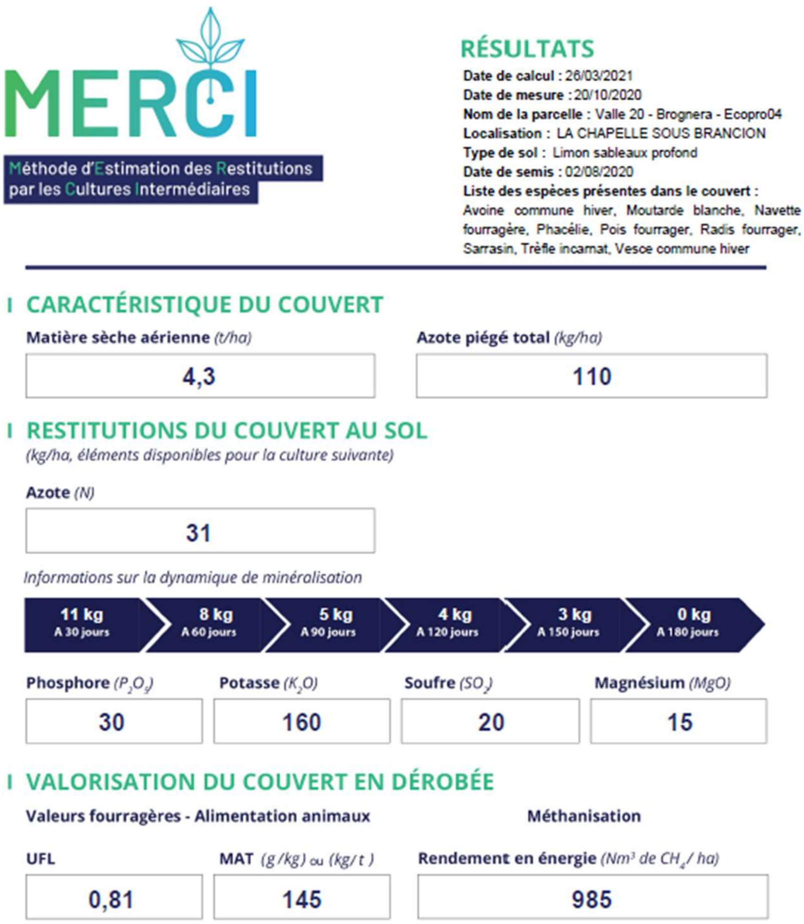


Figure 15, the Methode MERCI output from the Ecopro04 cover mix evaluation.

The differences between the two mixes are really slight, but it is interesting to see how the change of mixture, under equal conditions, gave a different response. In fact, in this second mixture it was above all the mustard and phacelia that stood out in terms of productivity and therefore brought the biomass values high but without high nitrogen contents, indeed lower.

In fact, here a profound reflection opens up which has long generated a certain debate on the effectiveness or, better yet, on the greater interest in cultivating (very) complex mixtures compared to simpler mixes or even single species. This reflection is also the basis of two works published by Stéphane Cordeau together with colleagues from INRAE.

In order not to leave the field open to weeds, the strategy is well known: we need to occupy space. Sowing a cover contributes to this but its management and its benefits continue to be discussed. Which species to choose/how many and with which agronomic path? Some answers can be found in the recent works published by the INRAE of Dijon together with its fellow countryman GIEE Magellan, a sort of operational group of farmers, among the references in the use of CDI (indefinite duration covers, also called permanent covers) and very great inspirations as far as is about direct sowing under vegetal cover, with the creation of spectacular guides and tools in building a mixture like the ACACIA spreadsheet.

«Nature is afraid of emptiness: leaving an empty place means encouraging the development of weeds» says Jérôme Séguinier, president of GIEE Magellan.

The introduction of covers into the rotation, whether temporary or multiannual, can satisfy numerous needs and respond to various objectives, what we call ecosystem services: providing organic substance, capturing nitrogen, limiting erosion, producing fodder, promoting biodiversity, but the often-desired effect of reducing weeds should not be forgotten.

An idea that has now made its way among many farmers, especially interested in conservative and regenerative techniques, is to increase the number of species so that each one can contribute to the ecosystem services listed above, and therefore that a very complex mixture is able to respond to these needs effectively. But diversity and effectiveness don't always go together.

With these photos (31, 33, 34) it can be seen the development of the different plot at the end of May. In April plants had laboriously covered the ground, waiting for a bit of rain. Meanwhile their work intensifies below, so even if the conditions seem adverse, it is always worth sowing. As we entered in May, there has been even too much water, but also heat has not failed to make the plants work intensely.

Photo 24, a view of the demonstration field the afternoon before field day and the end of May 2020.

In “Are cover crop mixtures better at suppressing weeds than cover crop monocultures?” published by Cambridge University Press in January 2020, Cordeau and his colleagues studied different mixtures of 5-6 species, other very complex ones that reached up to 14 or some monocultures, in three growth periods: summer, autumn and winter. The three monocultures - buckwheat, radish and triticale - were the best, with decent results even from the simplest mixtures compared to the more complex ones, with oscillations between 66-70% and 90-99% of the result obtained from the respective ones (per season of development) individual species.

In fact, it is first of all the rapid coverage and the high biomass that are effective in suffocating the weed flora. If the species that are mixed compete with each other for resources, and the most aggressive and performing in terms of cover, biomass or ability to obtain resources, win over their mixing companions, weak points could be created in the cover, leading some plants competing with other species in the mix with the risk of being overwhelmed by weeds. In all of this, to prevent the war on weeds from being lost, we must refrain from the temptation to exaggerate, unnecessarily, with the number of mixed species, which are easier to increase in summer “biomaxes”, where the opportunity is tempting to introduce little explored plant biodiversity such as buckwheat, flax, sunflower.

Photo 25, detail of cover crop self-made mix complexity.

A Friday afternoon at the end of May, right before the demonstration day. In the quiet of the sunset, so much beauty to be enjoyed almost in silence, because bees, bumblebees and other pollinators were making a noisy mess in such a beautiful bloom that had invaded the countryside.

Returning to the topic of which is the best solution, mixtures or single species, it is worth underlining that, at the same time, there is (almost never) a miracle plant, but building a mixture correctly by also playing with proportions, with 4-5 or 6 highly performing species such as those also shown in other mixtures on this project, can constitute a winning strategy for significantly suppressing weed flora. And now more and more experiences demonstrate this.

And it is precisely the field, with its heterogeneities and the difficulty of living under the sky, with rain, heat, sun or cold - which one is never sure of how much and when it will arrive, and perhaps with a timing completely wrong, arriving when it shouldn’t (how we would like) and failing when they should (we would like!), field experience teaches us that it is important to focus on mixtures: to intercept the heterogeneity of a field - more fertile areas and others less, more compacted areas and others more structured, more humid or shaded areas compared to drier ones - but also to protect themselves from uncertainty or, in a certain sense, from the heterogeneity of the seasons. The 2020 drought which affected the entire second half of March and almost the entire month of April, saw the Brassicaceae explode, with early flowering even with little biomass, and who knows what the increasingly "unusual" upcoming seasons will give us this year.

Obviously agricultural management also intervenes: time and technique of sowing both the cover and then the cash crop and the construction of rotation, a succession of plants over time that has a reasoned and balanced architecture. Without denying ourselves those little tricks such as light irrigation, or fertilisation, perhaps organic, or finally some light tillage of the soil, such as decompaction to help the roots create and consolidate a good soil structure. In short, the coverage must be done quickly and with a lot of biomass, and then the game with the weeds can be said to be won. Or maybe not?

And so comes the second work published by Cordeau and his INRAE colleagues, together with some Pisan researchers, "Cover crops promote crop productivity but do not enhance weed management in tillage-based cropping systems". It always deserves a moment of admiration when taken for granted improving soil fertility and health, as often seen in the United States. In reality, the title of the work also tells much more.

In fact, these results refer to weeds that arise in the cover crop, therefore during the same vegetative period. But the desired effect is instead linked to the subsequent cultivation period, that of cash crops. Weeds are an aspect, like fertility, that must be managed and planned over time, throughout the agronomic rotation, but you cannot start off on the right foot and fall foul from the second.

Photo 26, several approaches of biomass and soil management was showed: mulching, rolling, shallow ploughing.

The work was carried out using 3 cover species – *Brassica juncea*, hairy vetch and squarrose clover – in an agronomic system which included, following cover, sunflower and durum wheat and then corn and durum wheat, using two tillage techniques: the conventional one and a “reduced” one. Unfortunately, it is known that any mechanical intervention on the soil activates weed seeds which become active and start germinating again. And so even in this case, a balance, or compromise, must be sought between various factors, including tillage. In another work, the French researchers reported that in the absence of tillage, the germination of weed seeds remaining on the surface is reduced by 26% due to lack of burial, by 17% due to the darkening of the cover and by 19% due to water stress, hoping they are cumulative numbers!

Photo 27, the uncovered strips between the different mixes after almost two months after tillage.

Above is the result of the uncovered bands between one mixture and another. There is no need to comment much on them other than to bring one final reflection: is it better to have to deal with one biomass or another? Which of the two is preferable to have in the field, and possibly which is preferable to let some seeds escape, a bit of mustard or pea or *Chenopodium* or soft rag? Considering that in any case it was about 1 t/ha of dry matter from those weeds, capable of indicating more the agronomic system in which they develop rather than particular pedological conditions.

This explains how, with a correct construction of the crop rotation in which to also deal, in advance, with future weeds, rotations in which to intelligently introduce plant covers and also accurately position direct interventions (mechanical or chemical), it is possible to bring the infestation levels at such a low point that there are several examples of direct sowing under cover without any type of weeding intervention (chemical, physical or mechanical). The problem remains the stability of the system over time, but it is a very long and still open chapter.

One final consideration remains, before moving on to the last mixture on the field. That is what Anderson, in the United States, discovered 20 years ago now: a living and fertile soil gives crops so robust that they can tolerate a moderate level of infestation without any consequences. Which, at that point, we realise will have to be taken into account in the future, but this too is part of the art of knowing how to best manage a system as complex as a well-cultivated field!

Last mixture, the one that gave the most satisfaction and maintains a guarantee. Mixture made by always buying all the seed (separated) and mixing it. The cost is at least halved, and maybe there is some budget left to add something more expensive like the phacelia. It was composed of oats, phacelia, forage peas, white mustard, brown mustard and radish (three brassicas purchased in a ready-made mix, for convenience, given the small quantity), vetch and finally red clover to achieve a good sowing density.

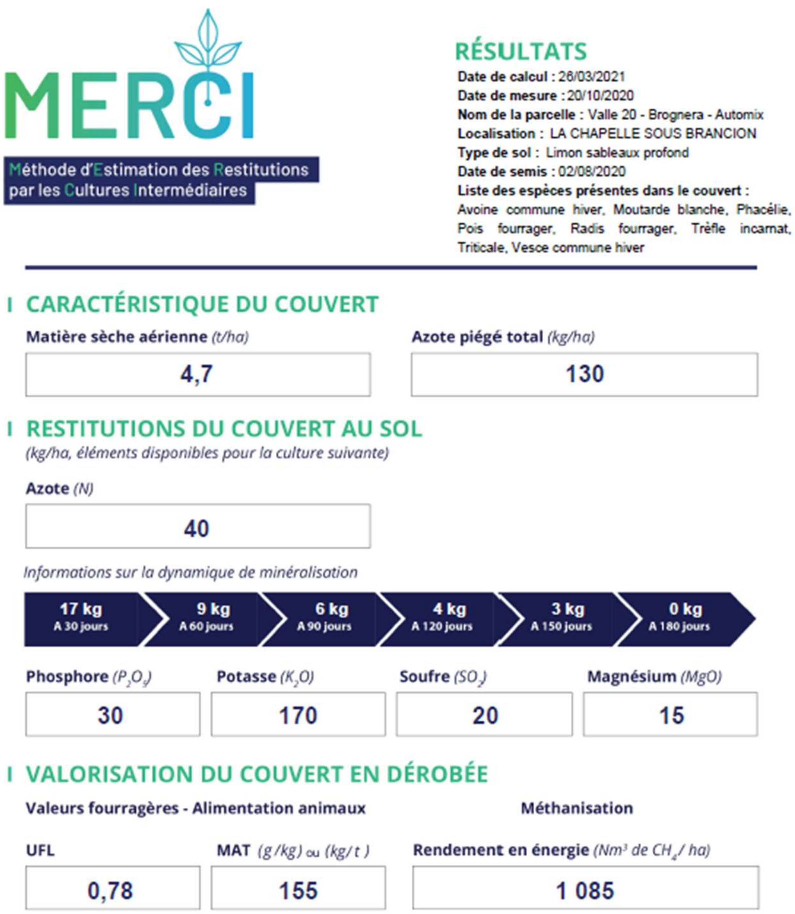


Figure 16, the Methode MERCI output from the self-made cover crop mix evaluation.

The results: 4,7 t/ha of dry matter, 130 units of nitrogen, of which 40 are returned for the following crop, and also 30 units of phosphorus, 170 of potassium, 20 of sulphur and 15 of magnesium (Figure 16).

The increase in organic matter, in this case, reaches 1,1 t/ha. Sometimes it is worth spending something to learn how to construct suitable mixtures, saving something on the seed, but learning a lot about the role of the various plants, of varying the proportions of the individual species, of the role of some minor plants or of the complementarity between species with different morphology.

The important thing, as Steve Groff (Cover Crop Coach) always says, is to ask yourself what you want to get from your crop, which ecosystem service(s) you want. In fact, even more than one, combining the most suitable species, sometimes with some small sacrifices or compromises, more often with some pleasant surprises.

Photo 28, the first field day of the Biofuture project, 30th May 2020.

As taught, for example, by associations between brassicas and legumes, of which we have the spectacular example of associated oil rapeseed. Many insects are attracted by the odours emitted by host plants and sometimes move from plant to plant with visual aid. The concept could therefore be explained in one sentence: the rapeseed field must not look like a rapeseed field. The presence of vigorous, flowering plants, which can even overwhelm the rapeseed, keeps them protected from the attack of *Altica*, especially in autumn, and instead attracts useful insects.

The biomass produced also keeps weeds at bay in a period when rapeseed is uncompetitive and could become infested. By using winter-killed plants – some grain legumes, Asteraceae or even buckwheat – this biomass will be destroyed at the first frosts and slowly degraded, releasing the nutrients captured or supplied as in the case of legumes: this contribution can be around 30-40 nitrogen units per hectare, with good-sized plants. Late autumn and winter attacks of *Altica* lead to poorly developed plants, with the characteristic bushy shape, with consequences on yield. The results of many experimental campaigns, such as the 2020 one by GIEE Magellan in collaboration with Terres Inovia, confirm the effectiveness of the “colza associé” technique.

It is, in fact, in the management of phytophagous parasites, which really seem to adore this fragrant botanical family, that intercropping shows the most impressive results: the presence of field beans and lentils in rapeseed maintained the same quantity of weevil *Ceutorhynchus picitarsis* as non-intercropped rapeseed but chemically treated (in short, intercropping is worth as much as an insecticide, around 10-15% in both cases), and at levels not far from the treated even intercropping (while the absence of both treatment and fertilisation has given disastrous results, around 70-80 % damage).

Photo 29, brown mustard ready to be terminated.

Similar dynamics were also found in the intercropping between Alexandrian clover and cauliflower, with notable positive effects on both *Altica* and cauliflower moth substantially halved, with some other interesting side effects on weeds and nitrogen.

Lastly, biomass produced by mustard (photo 39) was also really good, with around 4,4 t/ha of dry matter and considerable nutrient values with 100 units of nitrogen, 25 of phosphorus, 180 of potassium, 35 of sulphur and 15 of magnesium, thanks to a small contribution from some other seeds that "dirtied" the cover.

This cover deserves a little consideration: the 18 units of nitrogen available are spread over 6 months, or rather: over 5. This is because, for the first month, the result gives even a -1, meaning that the soil system releases nitrogen to the activity biological because of the need to digest the biomass. The return, however, is slow and limited. Therefore, compared to a biomass similar to the mixtures (4 and 4,3 t/ha and then 4,7 t/ha), and also a similar value of total nitrogen (120, 110 and 130 respectively compared to 100 for mustard), only 18 are available, half if not less of the mixtures. However, if biomass was left on surface, simply

Photo 30, comparison between rolling and mulching for ploughing rolled (photo 40), the nitrogen units released would be as many as 25 with constant release from the first to the fifth month following the conclusion of its activity. The results of the simulation with the MERCI Method are reported (Figure 17), highlighting the availability of nitrogen in the case of rolling the plant biomass compared with the practice of green manure. However, as it will be seen when talking about biofumigation, if this is the objective for which Indian mustard is used, green manure has its own reason for being.

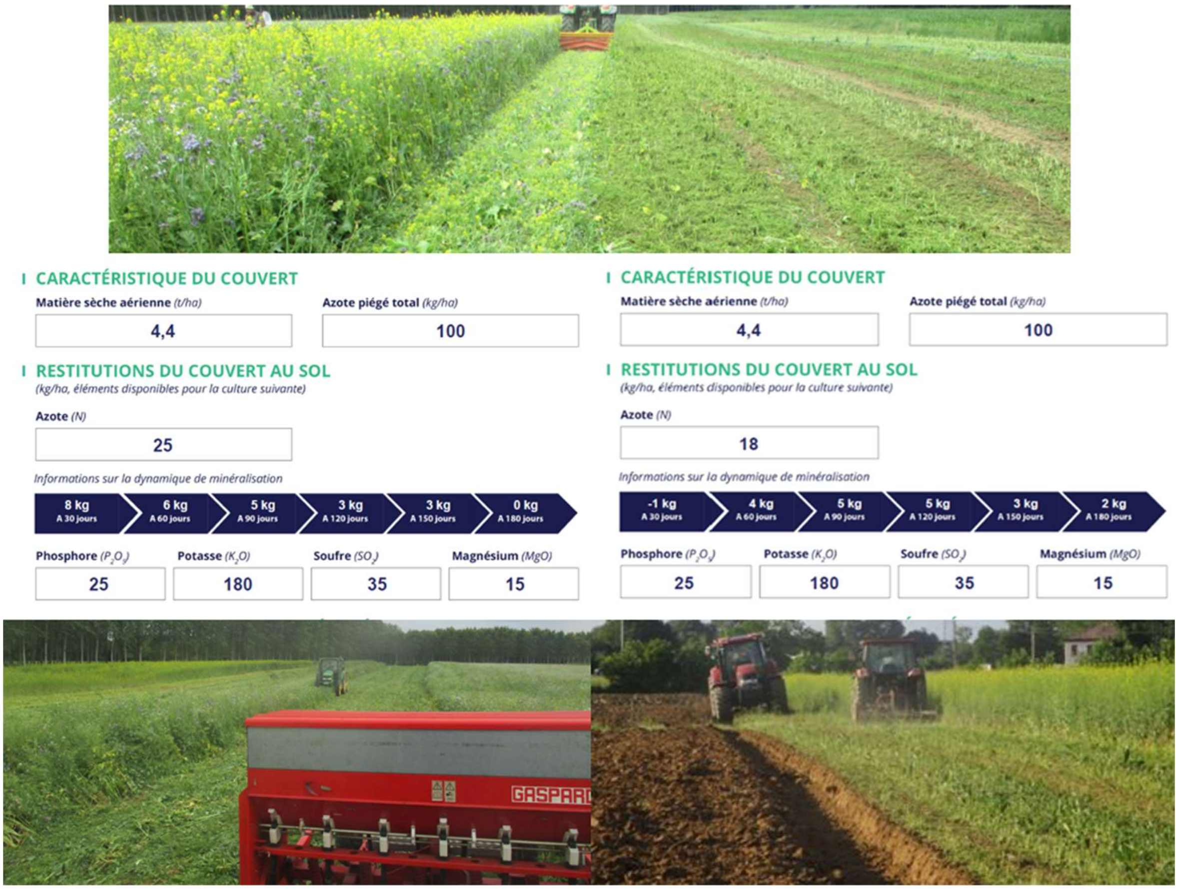


Figure 17, comparison between conservation cover crop management approach on the left and classic cover crop approach on the right, simulating the differences with the Methode Merci on the same biomass data (brown mustard).

The succession of plants, and their possible co-presence, is a complex system to build but obviously not impossible. It leaves room for a certain creativity and a certain elasticity in combinations. There is still a lot to discover and experiment, but it may be farmers that, with little concrete effort but a lot of mental commitment, could produce well done scientific experiments even if perhaps not with all the statistical rigour of certain experimental farms. But unlike these, the dedication in the search for practical solutions, the ability to observe as well as the knowledge of their own crops in their own fields and finally the most important thing: the commitment to wanting to bring home concrete results, allow participatory research, in the field, to proceed sometimes faster and more validated on farm scale than research conducted in experimental farms. Above all, obviously, if it’s all accompanied by the basics of the scientific method such as some replies, the fundamental untreated plots and a bit of organisation of comparisons and theses.

Some photos (photo 41) of the first Biofuture demonstration day, a Saturday morning at the end of May. Finally, everyone can enjoy the spectacle of this immense flowering and so much biomass produced: shredding, crimping, burial or direct management with a direct drill at work. We had fun (but obviously there was also real work on the part of the farmer who was responsible for managing this biomass).

Photo 31, first field day in May 2020

"Making things work instead of just giving tests a chance."

What a beauty to roll in such a mass! At some points the seed drill seemed to cling to the biomass and slow down, but it's a wonder to find the tractor's nose and ballast all dirty with coloured petals (photo 42)!

In short, the world of cover crops is as rich in challenges as it is in lessons. We can choose which species to grow based on which ecosystem service we want to obtain: nitrogen recovery? Nitrogen production? Weed cover, perhaps with some allelopathic effect? Biomass? Low C/N for rapid return of fertility? Biocidal actions? And much more. Most of the time it is mixtures that can cover a good part of these actions, to which is added the sequestration of carbon, which is the fuel of all the biological activity of our soils, that solar energy transformed into biochemical energy thanks to photosynthesis of green living beings.

Photo 32, some shoots of the preparation of the field day, in the morning

It is a topic that has recently become aware of, but which risks being dangerous, like all the other services mentioned above. They aren't always explained, they aren't always understood: not everything always works as we would like or should.

The objective for the farmer must be to produce biomass, biodiversity and coverage. In a certain sense, some of the ecosystem services have been discovered as unexpected side effects, but, as we have seen for the present and future management of weeds, it is often the entire system that must be managed in a coherent manner, according to a new agronomic paradigm. Who knows, maybe the next generations will do agriculture that is totally different from ours, or perhaps just a more advanced and ecological form, in the noblest sense of the term.

And just like with weed management, we don't have very high hopes of significantly increasing organic matter in a system with systematic tillage. We will see later how it can actually be detrimental working soil for each sowing, both for cover and for cash crop, despite much public funding to sow covers on (doubly) ploughed, and doubly ineffective, systems.

Nematocidal and biofumigant cover – Azienda Pavarin

Pedological and chemical characterisation of experimental site soil (every year the experimental site moved from field to field).

Figure 18, satellite photo and pedological chart (ARPAV, 2022)

L1 - DISTRETTO: **A** - Pianura alluvionale del fiume Adige, a sedimenti molto calcarei

L2 - SOVRAUNITA' DI PAESAGGIO: **A2** - Bassa pianura recente (olocenica) con suoli a iniziale decarbonatazione

L3 - UNITA' DI PAESAGGIO: **A2.3** - Dossi fluviali ben espressi, costituiti prevalentemente da sabbie

L4 - UNITA' CARTOGRAFICHE: **SAB1/LUS1 (Sabbioni 1 / Lusia 1)**

SAB1/LUS1	Complesso: suoli **Sabbioni**, *sabbioso franchi, a tipo climatico da subumido a subarido* USDA: Typic Ustipsamments, mixed, mesic WRB: Haplic Fluvisols (Calcaric, Hypereutric, Orthoarenic)	Suoli a profilo Ap-C, moderatamente profondi, tessitura grossolana, molto calcarei, alcalini, non salini, drenaggio moderatamente rapido, falda molto profonda. Capacità d'uso: IIIc
	suoli **Lusia**, *sabbioso franchi* USDA: Aquic Ustifluvents sandy, mixed, mesic WRB: Endogleyic Fluvisols (Calcaric, Hypereutric, Orthoarenic)	Suoli a profilo Ap-C-Cg, profondi, tessitura grossolana, molto calcarei, alcalini, fortemente alcalini in profondità, non salini, drenaggio mediocre, falda profonda. Capacità d'uso: IIIc

Figure 19, pedological description of the experimental field soil (ARPAV, 2014)

We move to Lusia, one of the capitals of Venetian horticulture, on the sands brought by the Adige River. Farms here are very specialized, many surfaces covered by greenhouses, and unfortunately rotations are often very tight, several cycles of the same crops repeated and, even if interrupted with other crops, a similarity between the species often persists from a phytopathological point of view. Unfortunately, in this way phytosanitary problems can only increase over time and become truly serious. Once upon a time, fumigation could be used more easily, but following the removal of the chemical products reserved for this use, the bio-fumigant capacity of plant used for covers, in particular some species, has strongly emerged.

Campione n°		8184	8185
pH		7,64	7,76
Conducibilità	mS/cm	0,09	0,10
Carbonio organico	C org. %	0,93	0,65
Sostanza organica	S.O. %	1,6	1,1
Da calcolo (C. org. x 1,724)			
Azoto totale	g/kg	0,97	0,86
C/N		9,54	7,60
Da calcolo			
Fosforo assimilabile	mg/kg	141,1	135,9
Potassio scambiabile	mg/kg	157,0	164,5
Capacità di scambio cationico	cmol/kg	9,77	9,60
Magnesio	mg/kg	162,0	160,7
Sodio	mg/kg	26,4	25,1
Mg/K		10,7	9,7
Da calcolo			
Tessitura	Classe	S	S
Sabbia 2-0,05 mm	%	91,9	91,9
Limo 0,05-0,002 mm	%	4,3	4,3
Argilla <0,002 mm	%	3,8	3,8

Elementi minerali			8184	8185	8184	8185
		Limite sup.	Dotazione pseudo-totale		Dotazione Mehlich 3	
Ag	mg/kg		< 1	< 1	<0,1	<0,1
Al	mg/kg		15926	17721	207	214
As	mg/kg	30	7	8	1,00	1,01
B	mg/kg		8	9	63	67
Ba	mg/kg		81	92	22	23
Be	mg/kg	7	0,52	0,59	0,05	0,05
Ca	mg/kg		37566	38558	8259	8559
Cd	mg/kg	5	0,24	0,25	0,12	0,12
Co	mg/kg	30	8	8	0,88	0,87
Cr	mg/kg	150	23	27	0,25	0,25
Cu	mg/kg	200	90	55	32	32
Fe	mg/kg		21254	22623	289	298
Hg	mg/kg	1	< 1	< 1	<0,02	<0,02
K	mg/kg		4365	4942	157	165
Li	mg/kg		24	25	0,10	0,11
Mg	mg/kg		14592	15039	387	401
Mn	mg/kg		394	421	82	83
Mo	mg/kg		0,4	0,42	0,01960784	<0,02
Na	mg/kg		310	323	107	111
Ni	mg/kg	120	15	16	1,33	1,32
P	mg/kg		1095	1193	376	376
Pb	mg/kg	100	16	17	8,31	8,20
S	mg/kg		177	186	72	75
Sb	mg/kg	10	< 1	< 1	<0,2	<0,2
Se	mg/kg	3	< 1	< 1	<0,2	<0,2
Sn	mg/kg		2	2	<0,2	<0,2
Sr	mg/kg		49	54	14,93	15,51
Ti	mg/kg		1049	1122	2,34	2,50
Tl	mg/kg	1	< 1	< 1	<0,2	<0,2
V	mg/kg	90	31	34	0,86	0,87
Zn	mg/kg	300	70	73	20,00	20,06

Table 4. Chemical properties of experimental site soils

But what is biofumigation? Why is it done and with which plants? From the photos presented in this chapter, it's easy to understand what the main plants of this technique are: Brassicaceae. On this farm, a commercial mixture of white mustard, brown mustard and anti-nematode radish was used for all three years, sown at 15 kg/ha, in different plots.

Photo 33, plot few weeks after seeding, March 2020

For this purpose and given their clarity, we recall the words of Giovanna Curto, Loredana Antoniacci (of the Phytosanitary Service of the Emilia Romagna Region) and Luca Lazzeri (of CREA-CIN).

Biocidal plants and biofumigation

Plant species with a suppressive action are generally defined as biocidal plants and develop the toxic effect with different mechanisms depending on the compounds contained in their tissues (glucosinolates, terpenoids, alkaloids, glucosides, phenols, tannins, etc.). These compounds, as they are or following biochemical reactions, release bioactive substances with a nematicidal or nematostatic action capable of interrupting the feeding of the parasite, inducing the hatching of the eggs even in the absence of the host plant or simply having a repellent effect.

How they act

Biocidal plants are divided into trap-plants and plants with biofumigant action; they are cultivated and chopped or distributed and buried in the form of flours or pellets in their organic matrix without being subjected to procedures for separating the active substances, as is instead required for plant extracts and essential oils.

Photo 34, poor light and thick biomass means very low weeds presence.

Trap plants or catch crops

They are plants with a high content of toxic compounds in the roots. The larvae of endoparasitic nematodes are attracted by root exudates and after having penetrated the roots, they begin to feed on a substrate poisoned by hydrolysis products or toxic metabolites, and consequently are unable to complete their development cycle. This action causes a significant reduction in the nematode population in the soil. In this case, the subsequent burial of the plants has only a secondarily biofumigant effect. Furthermore, they also have an effect on some weeds that are suffocated by the fast and vigorous growth of these plants.

Photo 35, cover crop development at the end of May 2020, quite after two months of growth.

Plants with biofumigant action

They are plants that contain high concentrations of toxic compounds especially in the epigeal part; their biocidal action manifests itself mainly following green manure. In this case, the hydrolysis products perform a mainly biofumigant and soil improver action. They have an action in some ways similar to that of a chemical fumigant, against nematodes, early larvae of Elaterid beetles, fungi responsible for “soil fatigue” and in the devitalization of weed seeds.

Effectiveness in the containment of harmful organisms

Biocidal plants can be effectively used for the containment of root-knot nematodes (*Meloidogyne* spp.), cyst nematodes (*Heterodera* spp. and *Globodera* spp.) and longidoid nematodes (*Xiphinema index*).

Biofumigation is also active in the containment of early age larvae of Elaterid beetles and especially in the medium term of soil fungi such as *Gaeumannomyces*, *Rhizoctonia*, *Fusarium*, *Helmintosporium*, *Pythium* also considering the contemporary increase in some antagonistic fungi following biofumigation interventions.

As regards the production estimated with the MERCI model, it is just over 5 t/ha of dry matter with 140 units of nitrogen, of which 29 are assumed to be available for the following crop, with a dynamic over approximately 5 months. 30 units of phosphorus, 215 of potassium, 40 of sulfur and 15 of magnesium then entered through the roots and made organic fertility for future crops. In this case, in particular, the focus was on lettuce.

The impressive thing was the buzz of bees and bumblebees that animated that vegetal cover. And not only hymenoptera find beauty nearby, but also birds and beetles, which meanwhile live on the ground protected by the forest represented by all this biomass. The weeds, on the other hand, find themselves well suffocated: some seeds continue to emerge, but the plants remain truly stunted.

Photo 36, biomass sampling for MERCI cover crop evaluation.

Unfortunately, and also due to the operational needs of many horticultural crops, regardless of the species used and the purposes, the preferred technique is that of the classic green manure with chopping and burial in close proximity, whether it is a horseradish or of a *Brassica juncea* below. Unfortunately, this botanical family is known for being unfriendly towards other living organisms, as described with the first plant cover. In short, a useful and valid technique, but which has many limitations, including the need to allow several days, up to two weeks, to pass before the next transplant, or the phenomena of nitrogen starvation.

Cover crop: produce biomass to produce fertility. Green manure? No thanks - Azienda agricola Basso

Pedological and chemical characterisation of experimental site soil

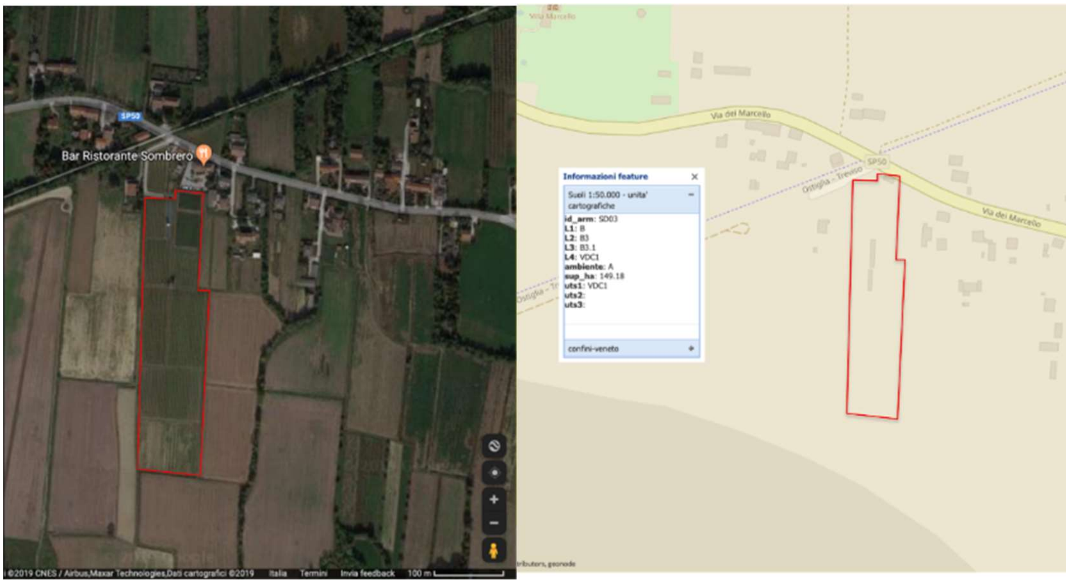


Figure 21, satellite photo and pedological chart (ARPAV, 2022)

L1 - DISTRETTO: **B** - Pianura alluvionale del fiume Brenta

L2 - SOVRAUNITA' DI PAESAGGIO: **B3** - Bassa pianura antica (pleniglaciale) con suoli decarbonatati e con accumulo di carbonati negli orizzonti profondi

L3 - UNITA' DI PAESAGGIO: **B3.1** - Dossi fluviali poco espressi, costituiti prevalentemente da sabbie

L4 - UNITA' CARTOGRAFICHE: **VDC1 (Villa del Conte 1)**

VDC1

Consociazione:
suoli Villa del Conte, *franchi*
USDA: Oxyaquic Eutrudepts fine-loamy, mixed, mesic
WRB: Endogleyic Cambisols (Hypereutric)

Suoli a profilo Ap-Bw-Cg, da profondi a moderatamente profondi, tessitura media, grossolana nel substrato, scarsamente calcarei, molto calcarei in profondità, alcalini, drenaggio mediocre, falda profonda.
Capacità d'uso: IIw

Figure 22, pedological description of the experimental field soil (ARPAV, 2014)

Campione n°		6716	6717	6718	6719
pH		8,21	8,13	8,06	8,41
Conducibilità	mS/cm	0,12	0,13	0,11	0,11
Carbonio organico	C org. %	1,65	1,62	1,71	1,24
Sostanza organica Da calcolo (C. org. x 1,724)	S.O. %	2,9	2,8	2,9	2,1
Azoto totale	g/kg	0,90	1,03	1,01	0,98
C/N		18,29	15,62	16,99	12,76
Fosforo assimilabile	mg/kg	34,5	26,7	17,0	18,8
Potassio scambiabile	mg/kg	85,3	101,9	87,0	108,9
Capacità di scambio cationico	cmoli/kg	15,50	16,71	12,48	16,26
Magnesio	mg/kg	257,7	252,4	185,8	282,4
Sodio	mg/kg	27,9	23,5	39,8	38,8
Mg/K		5,8	5,2	4,9	3,4
Tessitura	Classe	FAS/F	FAS/F	FAS	FAS
Sabbia 2-0,05 mm	%	52,8	50,8	56,8	55,8
Limo 0,05-0,002 mm	%	24,6	24,6	22,6	22,6
Argilla <0,002 mm	%	22,6	24,6	20,6	21,6

Table 5. Chemical properties of experimental site soils

"The series dedicated to vegetal covers continues and began last month. We move further north, along the line of the resurgences, an area of fertile lands and clean waters, which among the many crops also nourish the late radicchio of Treviso and the asparagus of Badoere. And it is precisely on these two "IGP" crops - together with lettuce and kiwi – that the activity of the Biofuture operational group is based – Biodiversity and valorisation of fruit and vegetables and ecosystems in typical production areas, conducted by OPO Veneto together with 9 agricultural companies spread across the entire Region, but with a strong representation of companies that cultivate that territory. Among others, there is one entirely dedicated to asparagus, both white and green."

Photo 37, the plot described in this chapter, in autumn 2020 (just seeded with a winter cover crop) and then summer 2021 (with the emerging summer cover crop).

Thus began the first of the articles dedicated to each of the cover crops of the Biofuture project, of which only the most significant have been reported here. There was talk of a winter-killing vegetal cover of sorghum sudan, radish and faba bean sown in September

2019 in anticipation of the asparagus planting at the end of winter 2020. An asparagus whose results we would have seen perhaps in 2021, more likely in 2022, at the end of the project. It seemed like a long time, but the months actually flew by, covering around 10 thousand km between farms and experimental fields in dozens of inspections, visits, meetings and sampling in the 4 provinces involved.

However, on several occasions, the companies involved in the Biofuture project did not limit themselves to introducing innovative techniques only in the fields initially designated for the experiments, but also added other plots, tests and ideas, “exploiting” the presence of experts and researchers in their farm, with the aim of being able to expand their knowledge.

During the last two winter seasons that the project has gone through, in this farm, both in autumn 2020 and 2021, some vegetable covers were also introduced in other plots compared to the one identified in 2019. In these plots the asparagus has been uprooted and will return only after a few years but above all some operations aimed at regenerating the soil and creating an interruption, including a sanitary one, in the asparagus monoculture.

What can be seen so far are photos of the 2021 sowings, both on autumn (2020) and on summer (photo 47). Here we are in July 2021 (photo 48), returning from the strip tillage test for radicchio in Agricola San Giorgio, to bring the strip tiller back to those who had kindly lent it to us.

Photo 38, July 2021, the development of the second consecutive cover crop on this plot.

The covers used were in all cases complex mixtures (6-8 species) but balanced for the botanical families present, without particular purposes other than the simplest and most important one of producing aerial and root biomass, and therefore providing significant inputs of carbon and organic matter, in addition obviously to nitrogen.

The abundant presence of legumes, in fact, guarantees important nitrogen fixation thanks to their root symbiosis. The mixtures have therefore always seen the co-presence of grasses, legumes, brassicas and also other botanical families (*Hydrophyllaceae*, *Polygonaceae*, *Asteraceae*).

The 2021 summer “biomax” covers were truly a spectacle! Here (photo 49) we are with a sowing of the second half of June. We also sowed the same mixture between the rows of green asparagus, however in the upper Marca Trevigiana, in the gravelly clays of the Pedemontana.

In fact, in addition to the winter sowings, in the summer of 2021 and then also in the summer of 2022 (and you will see a very particular thing in this sense), sowings were carried out to follow the winter coverings, and in these the last ones were also present two botanical families mentioned, with precisely summer behaviour.

Photo 39, development of the cover crop, the first summer "biomax", July 2021

Returning to winter covers, this farm has sown a rather classic mixture of oats, vetch, fodder and protein peas, barley, and phacelia, with some additions of red clover or field beans based on the availability of the year. The summer mixture was instead based on sorghum, sunflower, buckwheat and cowpea, all rapidly developing "macrothermal" species.

These plant covers have almost always been sown on tilled soil, in particular to manage the old asparagus patch, sometimes still present in the summer cover or originating from parts of surviving root systems. Sometimes, for a good establishment of a vegetal cover on poorly structured soil, a light tillage before sowing can be effective.

Does it really make sense to work the soil to sow vegetal covers, but above all to devitalize them and conclude their activity by incorporation into soil?

In particular, in the plot near the entrance to the farm building portrayed in the previous photos, three consecutive vegetal covers were sown: two summer and one winter (2021), and a particular technique was adopted on

Photo 40, the third consecutive cover in spring 2022 this winter one. The occasion was the demonstration day 2022, which had as its theme the presentation of the results of the mycorrhization and cover test before the asparagus planting which took place in spring 2020 on the 2019 cover.

It was a real satisfaction to see the enthusiasm of some of the farms involved, including this one despite the small surfaces, in wanting to add more surfaces to sow more each year. And it was an even greater satisfaction to see them accept (after the usual strong hesitations) to apply innovative techniques such as strip-tillage for radicchio or direct sowing under cover for the summer cover, directly sown under the winter cover. The innovative proposal was in fact not to shred and bury the biomass of the winter cover, carrying out the classic green manure, but to directly sow the summer cover without tillage or interventions either on the ground or on the green and living biomass still standing.

Photo 41, detail of this self-made mix with self-produced seeds, and its complexity at full blossom.

The biomass production of this cover crop was truly remarkable, and in a conventional management of shredding and burial, this would have cost the farmer a lot in terms of time and energy (diesel).

Results of the last Methode MERCI is reported below (Figure 23): 13 t/ha of dry matter, with over 300 nitrogen units contained and highly available, given the not excessively high C/N: these are the extraordinary results of this winter mixture. High fertility and biomass values mean high soil coverage, high quantity of roots and ease of devitalization of the cover often even with a single mechanical passage.

Figure 23, the Methode MERCI output from this cover crop evaluation.

But there was talk of avoiding green manure (also terminologically! In Italian it's "sovescio" and often farmers talk about the cover crop mixture or even the seeding itself with this term) and instead maintaining the cover on the surface: a theme that comes up every time we talk about vegetal cover crops and interlayers. We saw well in the introductory chapters how ploughing (and to a lesser extent all other tillage operations, in proportion to their intensity) destroys our soils. It oxidizes the organic matter, breaks down the structure and porosity, exposes the organisms that lived comfortably in the cool and dark layer, with the right humidity and degree of oxygen to hotter, drier and unsafe conditions to predators, to talk about the effects on the weeds that they suddenly find clean and uncovered soil, with fertility available in mineralization and perhaps with seeds brought to the surface thanks to tillage.

In addition to the direct impact that soil tillage such as ploughing could have on the living component of the soil (earthworms, arthropods, invertebrates in general but also microorganisms) which has developed thanks to over seven months (in this case) of protected and nourished soil, what is the impact on organic matter?

In recent experiences conducted in Veneto (RECARE project), the green manure technique – as well-known as it is considered beneficial and natural – has shown worrying gaps also from the point of view of maintaining the carbon sequestered by the biomass, leading to a strong and sudden mineralization and oxidation not only of the "young" organic matter produced by the cover, but also of the "old" organic matter with a reduction, in short, of the organic carbon stock.

Photo 42, winter cover crop development, beginning of May 2022

The experimentation involved the three plain companies of our regional authority: Vallevecchia in the upper Venetian area, Diana in the central plain of the lower eastern Treviso area and Sasse Rami, finally, in the lower Rovigo plain. The monitoring involved 240 georeferenced sites and sampled twice, in 2011 and 2017. The crops, initially, in all the farms were wheat, corn, soybean and rapeseed, while subsequently (2015) rapeseed was removed from the crop rotation, remaining however homogeneous in all farms.

Three different agronomic systems were compared: conventional (CV), which involved the use of classic soil tillage (ploughing at 35 cm) and the absence of intercrops between cash crops; a conservative system (CA), i.e. direct sowing (only usage of no-till drill, photo 53) and cover crops between cash crops; finally, an intermediate system (CC) which saw the union of conventional tillage and sowing of intercrops (classic "sovescio", or green manure). These were represented by sorghum, which often produced very high quantities of biomass, and a barley-vetch mixture (almost always more modest and in fact resulting in just 20% of the C contribution to cover biomasses).

Photo 43, a no-till drill can be used both for seeding a new crop (both cash or cover) and terminate the previous vegetation present at the moment.

The contribution of carbon to the soil was quantified both from crop residues, from the root systems and from the possible contribution of intercrops. The conservative system had a lower carbon input from residues than the conventional one, a difference however compensated by the contribution of the vegetal covers. The intermediate system, however, benefited from both the productivity of the conventional system and the contribution of intercrops. The expectations become truly remarkable: in fact we are talking about almost 25 t/ha/year of C added!

The concentration of organic matter was then measured, diversifying the 3 major profiles: 0-5, 5-30 and 30-50 cm depth. It is not surprising that an accumulation and stratification of organic matter has been seen, in the absence of tillage that periodically mixes soil profile.

In fact, in the first centimetres a large quantity of organic carbon accumulates such as to often modify the characteristics of this layer and essentially make it possible to identify it as a horizon. Below, up to 30 cm, most of the roots and biological activity are located and a discontinuity often persists for several years (in the once ploughed soils) in correspondence with the ploughing sole, even if perhaps this has been progressively fractured.

In depth, the differences between the managements were gradually more nuanced and not very significant. But this is for the concentration, i.e. the quantity of carbon or organic matter (which we remember: it is composed on average of 58% Carbon) in a fixed quantity of soil. Another thing is if we talk about carbon stock which is, in a certain sense, the quantity of carbon "weighed" in a hectare of soil, and this is a very important value, also and above all if we want to be interested in the world of credits or carbon certificates.

A first sowing (photo 54), on the morning of May 25, 2022, was carried out on a post-radicchio field, and in the afternoon instead in the winter vegetation cover. In the background, the field that was sown in autumn 2020 and then summer 2021 shows the rows of asparagus in its first year.

Photo 44, a first sowing in the morning on a plot after Radicchio harvest

"The results reveal that changes in SOC (soil organic carbon) stock were influenced by both tillage ($p < .01$) and carbon inputs ($p = .05$), while texture had no obvious effect."

"Management strategies to accumulate SOC in agricultural stocks can act both by increasing carbon inputs and by promoting SOC protection mechanisms to minimize mineralization."

"Variations in the organic carbon stock emerged with varying depth, estimated at -143% in the 0-30 cm profile and -203% in the 0-50 cm profile in the CC thesis, indicating that some mechanism of disturbance of the SOC had occurred. According to several authors, (Chenu et al., 2018; Fontaine et al., 2007), a priming effect is possible when fresh biomass with carbon at a high decomposition rate enters a soil, this induces a consumption of carbon with a negative final balance. Therefore, providing fresh plant material from a vegetation cover can accelerate the SOC mineralization dynamics of an “old” and recalcitrant carbon, especially in deep horizons, where microbial activity is limited.

Blagodatskaya and Kuzyakov (2008) saw that the triggering action depends on both the input of plant biomass and microbial biomass, highlighting that when this ratio is unbalanced, SOC can be strongly affected.

Furthermore, the decrease in SOC is emphasized by carbon-rich biomass associated with low nitrogen availability (both in the soil and in the added biomass); the microorganisms

Photo 45, results of seeding a summer cover crop on a winter cover crop with a no-till approach and equipment.

will obtain nitrogen by decomposing organic substance and therefore triggering this priming effect. In fact, only the CC thesis saw the burial of fresh biomass with high C/N, in particular Sudanese sorghum, which represented approximately 80% of the total fresh biomass input. This phenomenon is supported by observations of nitrogen stock variations that remained essentially stable in CC over 6 years, whereas they increased in both CA and CV. Instead, the soil C/N ratio did not distinguish microbial-mediated C dynamics among treatments, because differences in the microbial diversity did not alter the rate of C and N mineralization (Nannipieri et al., 2003).” Citations above are from Camarotto et al., 2020

“Ogle et al. (2012) highlighted that transition to a no-tillage system can increase the mean residence time of SOC by 15%, and therefore assumed that an input of C could decrease by 15% before causing a reduction in SOC stocks” (Camarotto et al., 2020) therefore the ability of conservation practices to protect and increase SOC is a mixture of reducing carbon mineralization (less tillage possible, attention to biomass quality) and a strong increase in carbon inputs.

Photo 46, a view from the tractor cab while seeding.

“If these results are confirmed by further experiments, a biomass production greater than that observed experimentally here, and similarly the use of legumes in cover crops, will be required, which would limit the microbial-driven SOC mineralization and soil N depletion”.

There is therefore no need to reiterate that the fundamental strategy is to make both cash crops produce well – for commercial production and therefore income and for the biomass released as residue – and vegetal covers, both in terms of biomass produced and complexity in the mixtures – often too scarce, only grasses and therefore very far from the “biomax” also presented in these pages. Furthermore, thanks to the MERCI Method widely used in the 30 or so experimental fields of this project, there was several occasions to show how biomass left on the surface rather than buried behaves differently with regards to nitrogen.

Below (photo 56), the situation at the beginning of June, with temperatures already since July and now a month without serious rain.

However, returning to May 25, 2022, and the sowing of the summer cover, the operation was effective both in the felling and devitalization of the existing vegetation cover, and in the sowing of the summer vegetation cover: for those who have never seen these things, especially in their own fields, it is not at all obvious!

Photo 47, field view few weeks after seeding, with emerging plants from the summer cover crop.

This summer vegetation cover, however, had a very slow start and was no longer able to develop correctly, in light of the extremely hot but above all exceptionally dry summer that we also experienced in our Region during 2022: this problem is increasingly relevant, and the question of the best summer coverage strategy is still open.

In any case, the soil remained protected and covered by the residue of the winter vegetation cover, and some plants managed to develop anyway. Returning to the experiment described above, however, the average emissions of the field managed with green manure was approximately 3116 kg CO_2 equivalent per hectare, 4,14 times more than the conventional one and 6,6 times more than the conservative one. Further evidence that should push to ban rather than oblige farmers to bury vegetal covers, regardless of the type of mixture, sowing time, frost resistance or any other aspect.

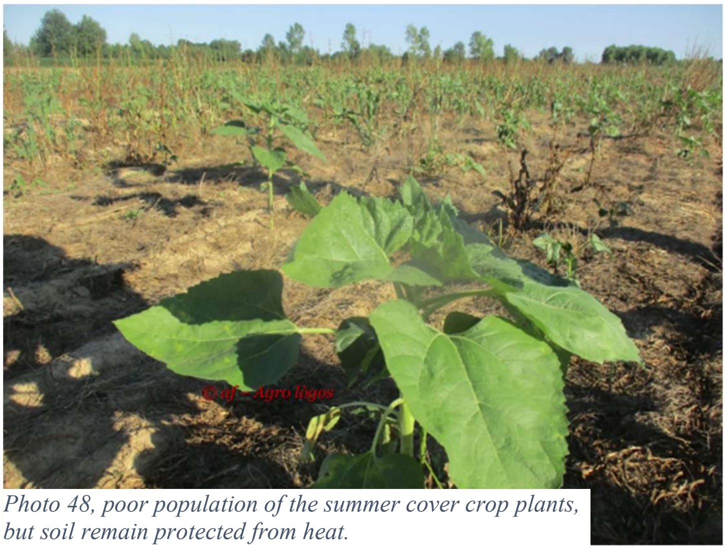


Photo 48, poor population of the summer cover crop plants, but soil remain protected from heat.

Below, the development at the beginning of July in the field that had been cultivated with radicchio in the winter of 2021-2022, and then the plot sown under the winter cover, on the right, at the beginning of August.

As could be seen further back, in fact, the same summer mixture was sown in two plots: the first, where radicchio had been grown, in anticipation of further winter coverage and subsequently again another crop (cash or cover based on the farmer’s needs), while the second where there was winter coverage, in anticipation of planting asparagus in spring 2023.

Photo 49, summer 2022, plants have well developed in both plots and conditions despite a lack of plants per m^2.

In mid-August, following light irrigation and the arrival of the first – of a long series of – rains, in the field that followed radicchio, plants finally grow and produce biomass, in addition to the blooming of the first sunflowers. This type of typically summer covers is increasingly difficult to create in our contexts. Later sowing is much more effective, towards the end of summer as in other more validated summer "biomax", although this must obviously be contextualised for each individual farm, plot, crop rotation and need.

Keeping the soil fertile and productive, and one's business profitable, is the true objective of every farmer: beyond the crops, the machines, the contexts, the science of the living and fertile soil always remains the same. The difficulty in knowing all the complexity that we have under our feet, in the agricultural soil, always remains the same, but the more we insist, try and experiment, the more we continue to build a constellation of knowledge that guides us, shedding light on what is right for soil fertility and his life, and what not.

The soil is alive, the soil is life. The thin skin of earth of our Earth is what supports us, nourishes us. Let's keep it covered in greenery for now: it will be stained with blooms in spring! And let's be kind when we enter a cultivated field.

Note: all the photos presented in this chapter and work, as been originally published on Agro-lògos (https://agrologos.tumblr.com/) during the three-year period of the project, presenting every single cover crop and field developed within the project. In the handbook published at the end of the Biofuture project and from which this chapter has been taken, only a selection of all the cover crops done have been presented.

A great thanks for all the farmers that have participated, contributed and let us make this great and full of teachings experience.

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Contribute 2

ASV vs OTUs clustering: effects on alpha, beta, and gamma diversities in microbiome metabarcoding studies

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Abstract

In microbial community sequencing, involving bacterial ribosomal 16S rDNA or fungal ITS, the targeted genes are the basis for taxonomical assignment. The traditional bioinformatical procedure has for decades made use of a clustering protocol by which sequences are pooled into packages of shared percent identity, typically at 97%, to yield Operational Technical Units (OTUs). Progress in the data processing methods has however led to the possibility of minimizing technical sequencers errors, which were the main reason for the OTU choice, and to analyze instead exact Amplicon Sequence Variants (ASV). We have tested the two procedures on the same dataset encompassing a series of samples from 17 adjacent habitats, taken across a 700 meters-long transect of different ecological conditions unfolding in a gradient spanning from cropland, through meadows, forest and all successional transitions up to the seashore, within the same coastal area. This design allowed to draw from a high biodiversity basin and to measure alpha, beta and gamma diversity of the area, to verify the effect of the bioinformatics on the same data as concerns the values of ten different ecological indexes and other parameters. Two levels of progressive OTUs clustering, (99 % and 97%) were compared with the unclustered ASV data. The results showed that the OTUs clustering proportionally led to a marked underestimation of the ecological indicators values for species diversity and to a distorted behaviour of the dominance and evenness indexes with respect to the direct use of the ASV data. Also multivariate ordination analyses resulted sensitive in terms of tree topology and coherence. Overall, data support the view that reference-based OTU clustering carries several

misleading disadvantageous biases, including the risk of missing novel taxa which are yet unreferenced in databases. Since its alternatives as *de novo* clustering have on the other hand drawbacks due to heavier computational demand and results comparability, especially for environmental studies which contain several yet uncharacterized species, the direct ASV based analysis appears to warrant significand advantages in comparison to OTU clustering at every level of percent identity cutoff.

Introduction

The stages of development of high-throughput and Next Generation Sequencing methods have unfolded at such a fast pace in the past two decades, that nowadays the term NGS itself is sounding obsolete. Within the *in silico* methods the processing of the raw data has seen an important shift as regards the increasingly acknowledged value of handling the reads as amplified single variants (ASVs) to infer directly their taxonomy (Eren et al., 2013; Tikhonov et al., 2015); without clustering them first into Operational Technical Units (OTUs) packages of shared 97% homology (Dunbar et al., 1999; Quince et al., 2009). The 97 % threshold had been originally chosen as it approximates the species cutoff homology boundary (Stackebrandt and Goebel, 1994). But the advancement in sequence denoising steps (Callahan et al., 2016) has enabled a minimization of the sequencing errors which was the main reason for which the clustering of sequencing reads into OTUs had been originally adopted. In the early stages of the metagenomics era, the use of alignment algorithms against a known reference template was hampered by the risk that, even a limited number of single nucleotide variants due to the background sequencer error in base calling, would confound an aligner and cause mistaken final attributions. Such issue was felt particularly relevant in targeted sequencing (e.g. aiming at 16S or ITS metabarcodes) when working with community DNA from unknown environments, in which the focus is the comparison of multiple similar sequences, as opposed to other approaches as the alignments across multiple genomes of single isolates of certain origin. The metabarcoding context was thus prone to misattribution of a given sequence, causing either the false detection of a close, but incorrect taxon, or the false discovery of a new one. The OTU clustering strategy was initially the workable walkaround to circumvent this potential bias. The clustering rationale rests upon the idea that related microorganisms have similar target gene sequences, over which, rare sequencing errors would have a negligible contribution to a given consensus sequence, whose cutoff could be arbitrarily imposed by grouping reads into operating taxonomic units (OTUs) sets (Blaxter et al., 2005).

Nevertheless, generating OTUs using similarity thresholds of pre-set sequence identity is not risk-free, as evident by the inherent consequence that multiple similar but different species would be grouped and blurred into a single OTU, losing their individual identifications. The assumption that the shared sequence identity border in prokaryotes for the 16S gene should be "near" 97% (Stackebrandt and Goebel., 1994), or to 98.65 % (Kim et al., 2014) or to 98.75 (Stackebrandt et al., 2006), shows how relative this concept is, and is increasingly seen as a rather arbitrary and unstable default choice, whose weaknesses are also evident from the incongruencies that the databases are continuing to reveal (Beye et al., 2017; Rossi-Tamisier et al., 2015) which made the 97 % value, on which the OTU 97% clustering is referred, just a mere relic of a surpassed convention, being not a reliable nor unambiguous set point for the bacterial species discrimination.

As alternative approaches to the binning into OTUs some had proposed to of require extremely high levels of sequence identity to minimize the loss of diversity when clustering, which however was recognized to potentially mistake the sequencing errors as grounds for false new species attribution (Kunin et al., 2010).

The OTU clustering that was traditionally adopted by the sequencing studies is the reference-based type, which, as opposed to the *de novo* clustering, is a closed-reference operation that draws within the available database of target gene sequences. While a sequence that has a high number of discrepancies would prevent any clustering to known subjects and will be discarded by the process, this represents at the same time both a measure against sequencers errors but also a condition that hampers the discovery of genuinely new taxa due to the self-referenced nature of the comparative process. Moreover, if errors exist in the reference database itself, which does indeed occur at a basal rate, a further level of bias adds up. These caveats made clearer the necessity to develop open-reference clustering, adopting both the principles of the closed-reference method and those of the *de novo* clustering, to avoid loss of the novel taxa. Within this rationale the ASV-based approach was developed to pursue a process which starts as the opposite of the clustering. Rather than blurring reads into an averaging consensus, the method focuses straight on exact sequences, and determines how many times each variant occurred, combining the result with an error model for the sequencer's run and working out a probability of exactness with a statistical confidence with a p-value for the null-hypothesis that each given sequence were due to a sequencing error. Such choice has also the added benefit that any given target sequence, being an exact variant, is bound to generate the same ASV, which makes the results far more comparable to those from other studies, and endowed with a higher resolution for a more precise identification at species level and beyond (Callahan et al., 2019).

Moreover, while OTUs are generally considered to be a suitable protocol to retain sequences that are rare in a sample, they pay the toll of risking a higher rate of picking spurious OTUs (Edgar, 2017). From the ASV side on the contrary the issue has been efficiently addressed since the advent of a variant determination software as DADA2 (Callahan et al., 2016), which is particularly suited for low-abundance reads (Nearing et al., 2018).

In general the ASV approach has been recognized to be advantageous also for its better performance in the presence of confounding factors as contamination issues where, using community standards at known amounts, it was demonstrated to accurately tell apart sample DNA from contaminant biomass proportions (Caruso et al., 2019).

Also as regards the technical drawback of chimera occurrences, being the ASVs exact sequences, they do not require to deal with a fuzzy consensus of lumped sequences as the OTUs, because a chimeric ASV, being the 'exact daughter' of two exact parent sequences usually prevalent in abundance within the sample, is easily spotted upon alignment due to its neat junction (Callahan et al., 2016).

Within the scientific community the light on the need to catch the relevance of the newly available resolution offered by ASV was casted rather eagerly by a paper published in ISME Journal with the title: *Exact sequence variants should replace operational taxonomic units in marker-gene data analysis* (Callahan et al., 2017). The debate has also seen arguments as regards the inference of correct ecological information from sequence data processed with either method. While some authors indicated a substantial reliability of the OTU-based conclusions due to their strong correlation with the ASV (Glassman and Martiny, 2018), others pointed out the biases bound to the use of OTUs in estimating community diversity when compared to the exact sequence variants (Chiarello et al., 2022), although in that case the methods used that introduced other variables in the parallel protocols, ending up in a condition in which the number of resulting ASV was even lower than the one of the OTUs, which is in itself a contradictory outcome, .

In this work we sought to address particularly this aspect which is critical for the applied aspects of environmental impact assessment, or to measure the effects of different agricultural management practices on soil ecology, as well as to compare between natural and anthropized landscapes and their biodiversity gradients.

As elements of novelty in our present analysis, the following apply: (a) differently from prior reports, to avoid pipeline-related proportional alterations, as e.g. DADA2 denoising filtering for ASV vs. straight Mothur-based clustering for the OTUs, we kept constant all aspects of the two parallel processing routes, and had as the only differing variable the presence or absence of the clustering step. The same denoising with DADA2 was thus performed in both cases. Additionally, (b) we analyzed 10 different ecological indicators, most of which are usually neglected in microbial community surveys; (c) we analyzed also the effect of clustering reads on the correlation between the different ecological indicators; (d) as site of investigation we chose a transect in a single vegetational gradient landscape in order to assess also gamma diversity, leading to the possibility of reporting the effect of the bioinformatics choices on each of the three levels of ecological diversity (alpha, beta and gamma diversity); (e) we included multivariate approaches as cluster analysis, that visually showed aspects as the dendrogram tree topology collapse, which occurred rather evidently at 97% OTUs clustering.

The working hypothesis was that the ASV based analysis, being endowed with a higher statistical power conferred by the higher number of data points within each sample, and consequently by an inherently higher resolution of the actual sample diversity, should yield data whose consistency would be progressively affected by the compromising custom of melting the available diversity data into discrete packages of Operational Taxonomic Units.

Materials and Methods

Samples origin and collection

The 34 samples analyzed covered a vegetational gradient through 17 connected adjacent habitats and their ecotones, from cropped fields through meadows, riparian hedges, forest, floodplain, prairie, sand dunes to seashore, located in the same coastal area of North-Eastern Italy. The transect extended within a 700 m length. The choice of a series of very different (bur spatially connected) habitats was meant to encompass a wide diversity in the resulting database, i.e. a high gamma diversity of the whole area. Such choice was envisaged as a way to maximize the chance of observing diversification, in terms of micro-evolutionary variation within the whole community. A thorough description of the site and of the sampling campaign has been described in our prior report in which the account on the overall taxonomical comparisons and other methods to extract the concealed diversity within data, has been previously presented (Fasolo et al., 2020).

Sequencing and Bioinformatics

Microbial community profiles were determined using the 16S rRNA gene V4 hypervariable region with universal primers (515F/806R). Sequencing (paired ends, with reads length of 2 × 250 bp) was performed with an Illumina MiSeq platform at the Ramaciotti Centre for Genomics (Sydney, Australia) generating a total of 3 million reads.

Raw fastq reads were imported into Qiime2-2022.2 (Bolyen et al., 2019), and primers were trimmed using the Cutadapt Qiime2-2020.2 plugin. Following primer trimming, an average of 95826 reads per sample were obtained and the downstream analysis was split into two approaches. The first one aimed to analyze sequences based on the operational taxonomic units (OTUs), where the trimmed sequences were denoised using the qiime DADA2 plugin, followed by OTU clustering with a 97% or a 99% sequence similarity cutoffs using the Qiime vsearch plugin. The representative sequences from OTUs were then classified using the classify-sk-learn plugin with the SILVA 138.1 16S SSU database (Quast et al., 2012).

In the second approach, the analysis was performed using an amplicon sequence variants (ASV) approach, where the primer-trimmed reads were denoised using the qiime dada2 plugin, followed by taxonomic classification using the same classify-sk-learn plugin with the SILVA 138.1 16S SSU database (Quast et al., 2012).

The pipeline scheme adopted for the different processes is shown in Fig. 1

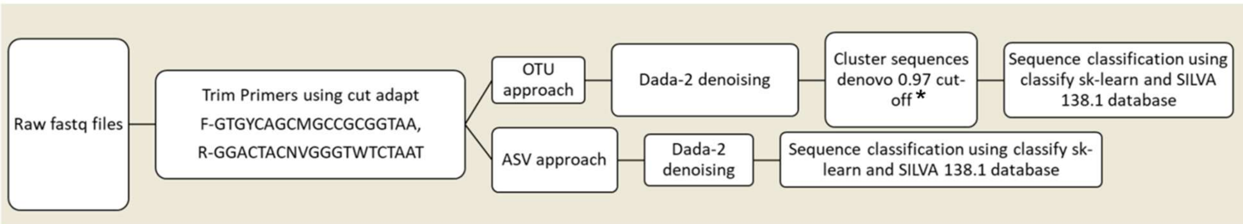

Fig.1 Bioinformatics processing strategy for the two parallel approaches.

*For the OTUs 99% clustering the 0.99 cut-off threshold was used at this level.

The sequence data were deposited in the NCBI Sequence Reads Archive (SRA) under code PRJNA608631.

Results

Alpha diversity and community evenness underestimation

Once the customary quality filtering steps with the denoising tools were performed, clustering the passed reads into discrete subpackages, as the OTUs are, the result caused, as inherently expected, a reduction of their numbers with respect to their full list of single sequence variants. The corresponding datasets in these procedures include therefore fewer and fewer sequences as a function of the chosen percentage of shared homology cutoff, which was regularly observed in the present data. The verified consequence was that of a blended level of identity that in turn, reduced the resulting diversity since the achievable resolution was deliberately reduced. The indexes that are linked to taxa presence and abundance patterns are therefore, in theory, expected to be susceptible to these changes, which was confirmed by our analysis. Table 1 reports the effects of the progressive clustering through 99% and 97%, which is, as mentioned, the commonly used OTU standard. Data are stemming from the total of 13073 ASV distributed across 34 sampling points covering an ecological gradient of habitats from cropped fields to the seaside and consequently warranting a wide range of diversity variation. Data are expressed as percentages of the values scored by the ASV dataset.

	ASV	OTU 99	OTU 97
n. taxa	100 %	95,7 %	83,6 %
Simpson_1-D	100 %	99,9 %	99,5 %
Shannon_H	100 %	98,0 %	92,3 %
Evenness_e^H/S	100 %	93,7 %	76,3 %
Brillouin	100 %	98,1 %	92,4 %
Menhinick	100 %	95,7 %	83,6 %
Margalef	100 %	95,6 %	83,5 %
Equitability_J	100 %	98,8 %	95,0 %
Fisher_alpha	100 %	94,9 %	80,5 %
Berger-Parker	100 %	119,3 %	183,3 %

Table 1. Alpha diversity result loss caused by each of the two clustering choices in comparison to full ASV data analysis. The percent values with respect to those stemming from the taxa counts of the ASV table are shown. Values are the means from 34 samples collected across the different habitats of the habitat type gradient.

Besides the straight reduction of over 16 % of the number of different taxa, the behaviour of the ecological indexes appears very uneven. While the Simpson 1-D index (Simpson, 1949) was only minimally affected (being based on the complement of dominance, and essentially concerning the probability that two taxa taken randomly from the community, represent the same kind), conversely, the equally popular Shannon-Wiener index (Shannon, 1948) is barely reaching the 92% of its corresponding value when the full variants are considered. The latter index, in comparison to the former, is recognized as the one more closely reflecting the community structure as it takes into account both the number of taxa and that of individuals. The Shannon-Wiener index however starts from the theoretical assumption that individuals are randomly sampled from an 'infinite' population and that all taxa would have to be featured in the sample An inborn source of bias in such index arises therefore from the failure to possibly have all taxa in a sample, and this error increases progressively as the proportion of species discovered in the sample declines (Peet 1974; Magurran 1988).

Even more dramatic is the effect on another major indicator used in community ecology, which is the Evenness value (e^H/S, Buzas and Hayek, 2005), dropping to just 76.3 %. Other indexes are all variably affected; the Brillouin measure (Brillouin, 1956) takes into account the number of observations and the number of individuals belonging to the most abundant taxon and the total number of taxa, and results underestimated by a factor similar to that affecting the Shannon values. A further higher discrepancy results also for the Menhinick richness index, which is the number of taxa divided by the square root of the individuals (Menhinick, 1964) and Margalef's richness, computing the number of taxa -1 divided by the natural logarithm of the number of individuals (Margalef, 1958).

The Equitability J parameter is instead the Shannon diversity divided by the logarithm of the number of taxa, and accounts for the partitioning level by which individuals are spread among the species present (Shannon, 1948), while the Fisher alpha index is tied to a slope constant of the distribution (Fisher et al, 1943).

Besides the underestimation of richness-proportional measures, it is relevant to remark that the Berger Parker indicator, based the number of individuals in the dominant taxon relative to the total number of individuals in the community (Berger and Parker, 1970), which is the only one that regards dominance, and has therefore the opposite meaning in ecological terms (the lower the better), displays an extremely inflated effect that boosts it to nearly a double value (183 %) when using the 95% clustered OTUs.

The data shown in Tab. 1 are moreover the average ones as the situation can be far more extreme depending on the community structure of a given habitat. In this respect the minima observed can be as low as 64%, as e.g. for the case of community evenness, which, conversely, is known to suffer of an overestimation for samples in which the total number of different taxa is particularly low (Buzas et al., 2005). The full dataset of ecological indexes is available as Supplementary Material (Supplementary Dataset S1 Ecological Indexes).

Effects of clustering on ecological indexes correlations

A further aspect on the alpha diversity context emerged by the pairwise correlation analyses among the different ecological indexes obtained from each of the three progressively clustered taxa tables. The sequences abundance and the number of resulting taxa were also included in the crossed comparisons, whose results are shown in Fig. 2. As a premise it needs to be recalled that the statistical power of an analysis is a function of the sample size and that therefore using ASV which are always more numerous of the OTUs, is bound to guarantee a higher accuracy and a consequently higher possibility of finding true correlations when those exist.

The analysis unraveled differences in their significance stability as a function of clustering and also unexpected inversions of sign in the correlative direction, i.e., positive correlation turning negative as in the case of community evenness.

In first instance a weak relationship between sequencing depth (reads abundance) and all indexes' outputs appears, displaying in most cases a positive sign with the expected exception of Berger-Parker dominance and the less intuitive exception of the Menhinick richness, that is linked to the denominator position of the square root of the individuals.

As regards the positive correlations (blue spots) it can be noticed that in many instances the strength of the correlation is weakened along with the clustering intensity and in some cases a significance that was recorded using the ASV data is lost when OTUs are the clustered units. It is the case of Equitability and of both Shannon and Simpson indices, and even of the latter index itself in its relationship between the ASV data with either the number of OTUs at 99% or OTUs at 97%, which by definition ought to be strongly correlated with it.

As regards differences in the correlation direction, an opposite sign in pairwise comparisons among these indexes (red spots) is expected only between dominance indicators (i.e. Berger-Parker) and all the others which are instead representing diversity or evenness. The data comply, but at the same time show also an equally opposite phenomenon in terms of effects of the clustering: the significance tends in this case attributed to the comparisons involving the progressively most clustered cases (OTU 97%) and excluded in crosses involving the indexes that were calculated with more available data (ASV) and that are therefore endowed with an inherently higher statistical power. The fact that those significant-scoring correlations would pop up just in crosses involving the Berger-Parked Dominance is tied up to the above signalled bias of inflated estimation of the index itself, as seen in Table 1, where an off-scale value of 183% was occurring for the 97% OTUs as a fraction of the same index when calculated from the more data-rich ASV dataset.

But the most striking incongruency observed from the correlation table is the behaviour of the Evenness parameter, as that is the only one that even showed a sign inversion along with the clustering percentage cutoff reduction displaying the whole gradual change from an extreme to the other. That is well visible (Fig. 2) in the nine crossed comparisons between the three evenness values (ASVEv, O99Ev, O97Ev) and the three taxa numbers resulting from each set (ASV, O99, O97), in which a positive correlation (blue) occurs with ASVEv, an absence of correlation (blank) with the O99Ev and a negative correlation (red) with the O97Ev. Analogous inversions are visible across the same Evenness parameter and the indexes of Brillouin, Fisher alpha, Margalef, Menhininck and Shannon, for all of which the correlation with the evenness calculated from the OTU 97% clustered units paradoxically assumes a negative correlation outcome unlike the cases of its ASV and OTU 99% counterparts.

The corresponding non-parametric version of the same correlation analysis was carried out using the Spearman Coefficient (Supplementary Figure. S1) which yielded correspondingly analogous results.

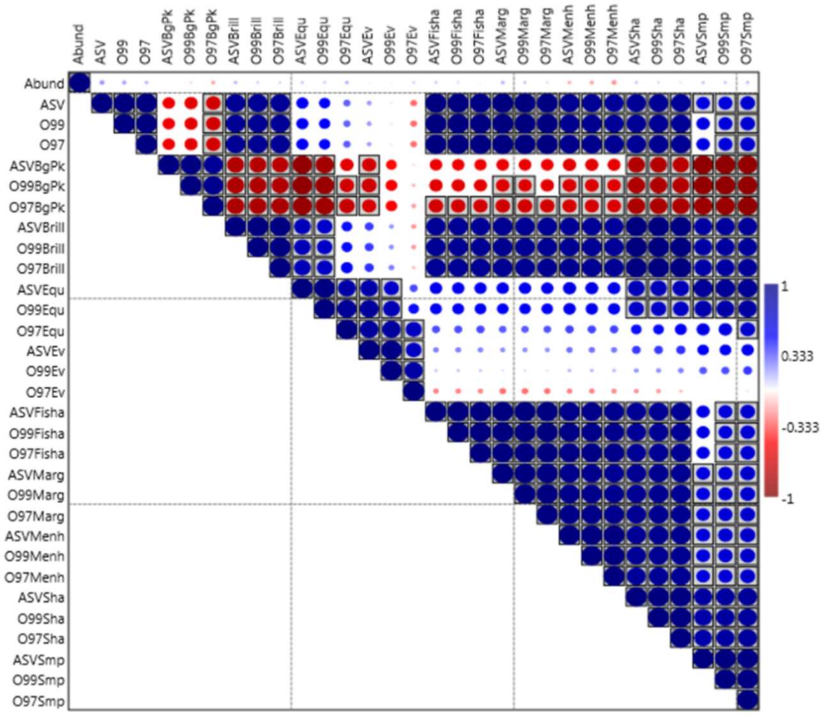

Fig. 2. Correlation matrix (Pearson Coefficient with Bonferroni-corrected p values) of the **pairwise comparisons across numbers of taxa and ecological indexes of the three sequence clustering approaches.** Boxed cells with grey background indicate significant differences for p<0.05. Abund: sequence reads abundance; ASV, O99, O97: number of different taxa resulting from the full ASV analysis or from the OTU clustering at 99% or 97

% shared homology, respectively. These three prefixes apply for the remaining correlation indexes' abbreviations, whose suffixes indicate the following: BgPk: Berger-Parker Dominance; Brill: Brillouin Diversity Index; Equ: Equitability J; Ev: Community Evenness e^H/S; Fisha: Fisher alpha Diversity Index; Marg: Margalef Richness Index; Menh: Menhinick richness index; Sha: Shannon-Wiener H Diversity Index; Smp: Simpson 1-D Diversity Index.

Beta diversity: effects of clustering on apparent distances in multivariate ordination

To analyze the between-samples difference, the pairwise Bray-Curtis distances from each of the three cases have been used in a multivariate cluster analysis ordination with the Neighbor Joining criterion to produce the resulting dendrograms. As can be seen by inspecting the resulting phenons (Fig. 3) the grouping of samples, when comparing the ASV-based ordination with the one obtained by the minimal clustering (99%), is substantially consistent, while the adoption of the standardly used 97 % OTU clustering, results instead in a number of changes in the samples relative positions, in an alteration of the horizontal distances, and in a marked overall change of shape of such distance/similarity-based dendrogram, underlining once again the effects of the clustering choice.

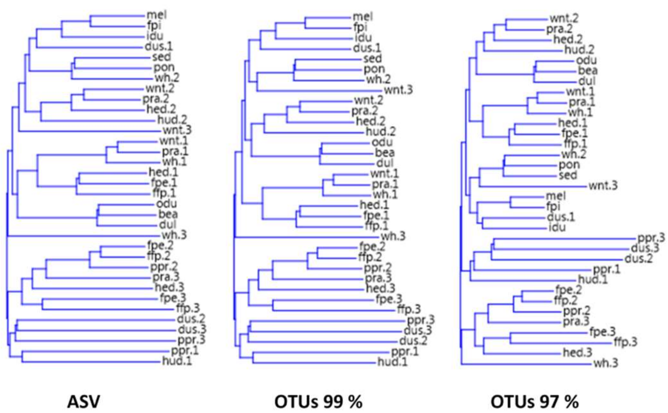

Fig.3 Neighbor Joining dendrograms obtained by the Bray Curtis-based cluster analysis of each of the three different data matrix tables. The holding consistency of the tree topology at OTU 99% clustering is visible, along with its evident loss when assembling sequences in the 97% clustered packages. Samples nomenclature is drawn from Fasolo et al., 2020.

Gamma diversity drops upon reads clustering

Having chosen for the present survey a well-defined area encompassing a continuous transect of several adjacent habitats and their respective transitional ecotones, we can take into account the full range of diversity arisen from the metabarcoding through the Valle Vecchia Oasis i.e. the resulting Gamma diversity of the whole site. In this respect the total number of non-redundant different taxa counted among the whole series of samples amounted to the following values: ASV: 13073 taxa, OTU 99%: 10515 taxa (80.4 % of the ASV value corresponding to a -19.6% richness); OTU 97%: 7275 taxa (55.6% of the ASV value corresponding to a -44.35% richness). These figures further emphasize the severe detection limitations that the practice of clustering into OTUs is causing.

Discussion

The analysis performed has evidenced several critical differences that endorse the use of exact Amplicon Sequence Variants as preferable with respect to Operational Technical Units. The clustering mechanism that underlies the latter, besides the consequent reduction of the diversity output which is inevitably assumed by the procedure in itself, shows limits in the estimation and comparability of ecological diversity indexes which should instead constitute the actual goals in metabarcoded microbiome comparisons in applied studies. The underestimation of richness-based parameters when compared to the corresponding values stemming from ASV, and the inflation of the ones based on dominance, as well as the loss or reduction of significance and correlations among indexes and data outputs, suggest to apply care when dealing with the clustered packages of data which are the basic units in the OTU-assembling procedure. The inversion of correlation coefficients direction observed for community evenness across the two sequence analysis methods is in this respect particularly emblematic of the existing discrepancy and of the ensuing interpretational risks.

Our data are in essence very consistent with the arguments in favor of moving the field towards the choice of ASV (Callahan et al.,2017, Caruso et al., 2019) due to the more precise identification of microbes within communities as well as in providing a crisper picture of the actual environmental diversity within each sample. On the contrary, an OTU, embodying a cluster of multiple reads could contain both real sequences or errors lumped together into an arbitrarily assembled unit, introduces a process bias that can be avoided by the handling of the separate single variants.

The trade-off between the computationally easier generation of OTUs appears surpassed by the issue of reference-related biases, and in particular when the comparison involves the traditional closed-reference OTUs which are prone to miss novel sequences. While that could be acceptable in projects whose target is an already well-defined database of records, as, e.g., in human microbiome surveys (Gevers et al., 2012) where the expected taxa are nowadays at the most within acquired knowledge, the situation is very different for the explorative environmental microbiology, where soils, oceans and most yet poorly-known habitats, contain arrays of uncultured and unknown taxa that we can not afford to loose due to closed self-referencing annotation methods. Alternatives as the complex and machine memory-intensive *de novo* OTUs clustering or mixed open referenced and closed references OTU-based approaches appear less advantageous that the ASV approach, both for the implementation of databases with new data and in terms of accuracy, since the choice of accepting only high-confidence exact variants can be exerted.

Undoubtedly OTUs have contributed enormously to the build-up of our microbiome knowledge and for some scopes as the gut microbiomes of well-characterized species as humans, livestock animals, or laboratory model species, they will keep being preferable in terms of big data handling from population wide studies and other designs. But on the other hand, the maturity reached by bioinformatics applications as the mentioned DADA2, or the improved denoising procedures (Nearing et al., 2018) and finishing tools as Deblur (Amir et al., 2017) along with error corrective ones as UNOISE (Edgar 2016) have paved the way for an increasingly trustworthy adoption of the amplicon sequence variants in microbiome metabarcoding.

In conclusion, we have put in evidence from different standpoints the advantages that support the use of amplicon variants. As advancements and novel elements over previous reports we have here chosen to adopt fully parallel protocols for the two approaches on the same dataset, by applying the same denoising operations to the original FASTq outputs and using the clustering at the two levels of similarity as the sole variable. Moreover we explored the behaviour of ten independent ecological indexes based on rather different formulas, to point out the variable extent of bias that occurred with each, to which we added a multiple pairwise correlation analysis, including the relationships among the different indexes themselves, which, to our knowledge, had not been precedented in literature. The visual observation of the consequences of clustering via tree topology restructuring, culminating at the 97% threshold, in spite of its wide use as routine clustering cutoff, is a further element of novelty. Furthermore, the chosen site offered an extremely variable but spatially-continuous gradient of habitats across a vegetational transect from agricultural crops through forest and seashore, all within the same iso-climatic and iso-geological setting. The elements that arose concur against the null hypothesis of a possible non-significance of differences between results based on OTUs vs. those obtainable by ASV and support the preference of the latter in this type of environmental studies.

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Conflict of interest.

The authors declare no conflict of interest.

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