The University of South Bohemia in České Budějovice
Faculty of Science

Intraspecific trait variability of herbaceous plants in organic and conventional olive plantations in region at desertification risk

Master's Thesis

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Annotation

This thesis is an ecological study in an agricultural context (agroecological research). It is carried out in olive plantations in south Italy, Apulia, a region at strong desertification risk. The effect of agricultural management (organic vs. conventional) is investigated on i) the herbaceous species composition ii) intraspecific trait variability of five selected herbaceous plants iii) soil conditions. Herbaceous plants are here considered as "indicators" of olive plantations' soil health. Studying if and how species traits and soil parameters vary with management, should reveal how olive understory grasslands are responding to increasing drought conditions and other desertification constraints. Management of grasslands and soils is critical to maintain high ecological functionality and services of olive agroecosystems.

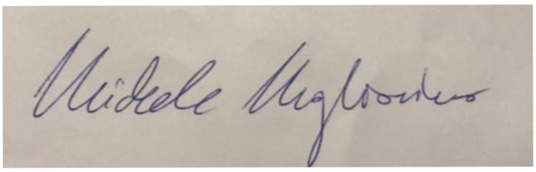
Declaration

I declare that I am the author of this qualification thesis and that in writing it I have used the sources and literature displayed in the list of used sources only.

Date: 07/12/2023

Ceske Budejovice,

Michele Migliorino



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1.Introduction

1.1 Agriculture, Land degradation and Desertification risk in the Mediterranean regions

Mediterranean basin is one of the global hotspots for biodiversity and Italy holds the highest number of species in Europe. At the same time, the Mediterranean basin is a hotspot for Climate Change and is threatened by several human induced problems. Anthropocene have heavy modified species habitats, reducing their quality (de Bello et al., 2016).

Agriculture provokes a radical land-use change which alter ecosystems. Agriculture production is the main driver of the Earth system exceeding planetary boundaries (Campbell et al., 2017). Humans' appropriation of biomass, fibres and food has direct impact on biogeochemical cycles, freshwater use, biosphere integrity and climate change (Rockstrom et al., 2009). Agriculture implies heavy modification of landscape because it "reset" the ecological succession. The great majority of staple crops are annual plants (corn, wheat, rice, etc), therefore, to grow these plants the succession needs to be restarted every year (or more time per year) with consequences on soils and ecosystems. Moreover, agriculture reduces the functional and genetic diversity of an area of land to few crop species or even to monoculture. This induces radical habitat destruction with consequences on species and populations. This process may lead to progressive degradation of the environment and eventually to the desertification of an area or a region.

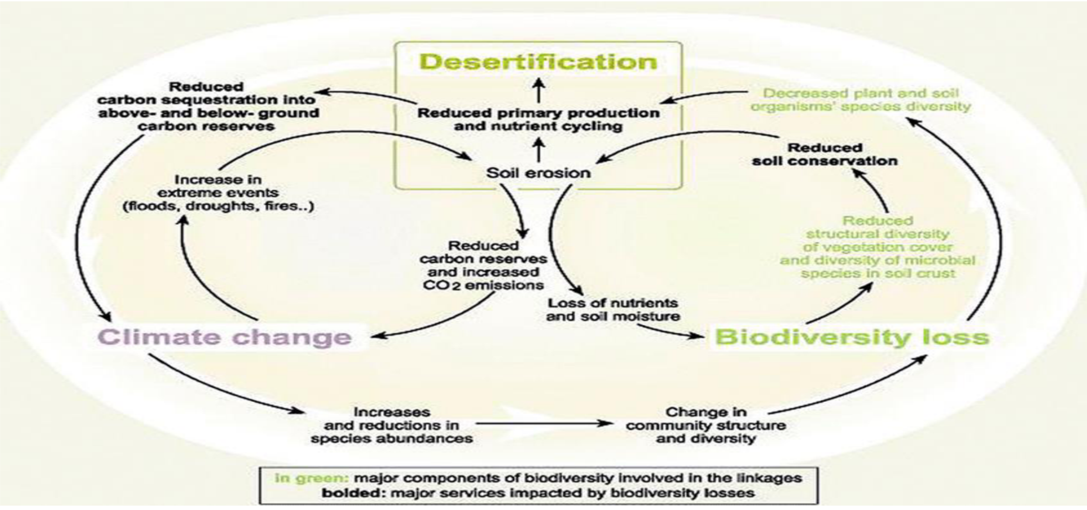

Figure 1. The Millennium Ecosystem Assessment (2005) have provided a framework to comprehend the feedback loops involved in the desertification process.

Climate Change and Biodiversity loss push the system toward a degenerative cascade which may eventually lead to soil erosion, reduced primary production and nutrient cycling. Desertification does not refer to the expansion of deserts. It occurs because dryland (semi-arid and arid) ecosystems are extremely vulnerable to over-exploitation and inappropriate land-use (Boschetto et al., 2019). Some areas and regions of south Europe are considered at strong desertification risk (Cramer, 2020; UNDRR, 2021; Grilli et al., 2021).

1.2 Conventional or organic?

Given the pressure that agriculture imposes to the environment becomes crucial to assess which kind of agriculture is more environmentally friendly. Using words of Tuomisto et al. (2012): “does organic management reduce environmental impact?”. The term “conventional” refer to the most diffuse form of agriculture (in 2021 organic agriculture covered 9.9% of total European agricultural land; Eurostat data). It was introduced after II World War with the Green Revolution (Ritchie, 2017). Through massive use of energy and chemical products it is possible to obtain higher yields. Chemical pesticides have potent active principles that can exterminate almost all organisms present in field. Use of ploughing and chemical herbicides (e.g., glyphosate) ensure high control of weeds. In the terms of the trait-based ecology (ch. 1.3; 2.4), ploughing generate a high degree of disturbance whereas herbicides and insecticides generate stress (Grime, 1999; Tarifa et al., 2021; Terzi et al., 2021). Synthetic fertilizers (nitrates, phosphates, sulphates, etc) can provide very high nutrient addition. For these reasons, it is also called “intensive agriculture”. The main argument in favour of conventional management is considered the higher yields per hectare (or per area unit) that it provides (Ritchie, 2017; Reganold, 2016). Some people claim that if we can produce more food with less land, more space can be left to nature and ecosystem services may be maintained. Given the forecast of an increasing population to 10 billion by the end of the century, many authors sustain its superiority in the capacity to feed the world[1]. However, this popular argument underestimate social and environmental issues connected with increased agricultural intensity (Evenson & Gollin, 2003). A review comparing organic and conventional agriculture have found that organic improves farms’ profitability (even though total costs are the same to conventional); employment of workers; reduce worker exposure to pesticides; it provides a better nutritional quality (Reganold, 2016). Several authors sustain that organic agriculture and other forms of agroecology offer more sustainable solutions for planetary crisis (Altieri et al., 1995). Organic seems a more integrated form of agriculture because it much better sustains trophic webs and interactions (biodiversity); it minimizes energy use and water pollution; it improves soil quality (Reganold, 2016; Altieri, 1999). If it not strictly avoids use of ploughing, available pesticides are much less potent and only preventive. Biological organisms are not seen only as enemies or parasites to destroy but differentiated in “auxiliars” or “enemies” of crops. For these reasons, organic agriculture can be considered a lower-intensive form of management.

1.3 Theory and Methodology

1.3.1 Trait-based Ecology

Functional Traits are often used to assess the effect of Climate Change, Land-use change and other human-induced environmental pressures (de Bello, 2021). Given the pressure that agriculture impose to the environment, they can be used to assess whether there is difference among organic and conventional in terms of environmental sustainability of olive plantations.

Trait-based Ecology have grown in attention during last decades due to the acknowledgment that taxonomical diversity indices were not more considered sufficient to full describe biodiversity (de Bello, 2021). Somebody claims that if methodology based on functional traits will succeed in predicting ecosystem change, this might lead us to the Holy Grail of ecology (Lavorel & Garnier 2002). Index like Shannon (H’) and Simpson (S) take in account number of species (species

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[1] Spain offers well-known examples to discuss issue of intensive agricultural management in Mediterranean region. Almeria holds the biggest agricultural greenhouses concentrations in the world and it is the main horticultural producer of Europe. It produces 3,5 Tons of food per year: “*the production from Almeria’s horticulture provides 40% of gross production while taking up only 3.4% of the land*”. However, which are the environmental costs connected with this management? Almost 31000 hectares are entirely occupied by a “plastic sea”. Spain is also the main global olive oil producer with 1.5 millions hectares covered with olive plantations.

richness) and abundance of individuals (eveness) but do not consider functional or phylogenetic diversity among species (Leps, 2012). Many parts or characters of biological organisms can be considered "functional" in the sense that they manifest specific functions[2]. Different parts reveal different functions. For example, traits linked with plant physiology like Plant Height, Specific Leaf Area (SLA) and Leaf Dry Matter Content (LDMC) are useful to study short-term adaptations of plants to environmental change (ch. 3.3). Morphological or phenological traits, on the other hand, are considered useful to assess long-term effects (Garnier, Navas, Grigulis 2016). Functional traits may reflect the *effect* of the organism on the environment (effect traits), or the *response* of the environment on the species (response traits) (Garnier, Navas, Grigulis 2016). Response traits are particularly interesting for studying drought stress. For example, Leaf Dry Matter Content (LDMC) is often used as proxy to assess drought stress in plants because the increase in dry matter in leaves is correlated with better drought tolerance (Garnier, Navas, Grigulis 2016; Majekova, 2021). Some traits are also effect traits. For example, LDMC reflect the rate of litter decomposition, consequently revealing biological productivity of the community (the higher LDMC, the slower the decomposition; Lavorel et al., 2013).

Functional traits are used to investigate assembly rules of communities. It is accepted that common and dominant species exert a disproportionate role due to their "mass ratio" effect which ensure stability of the whole ecosystem (Grime, 1998). Indeed, the more the ecosystem rely on rare species for fundamental functions, the lower its stability in case that some species is lost or goes extinct. Computation of community-weighted mean (CWM) is used to capture the mean value of traits that compose the community (weighed by the abundance of individuals), whereas functional diversity (FD) gives a measure of how traits are distributed around the mean (variance). This approach has been widely used to test the "mass ratio hypothesis" (Garnier, Grigulis, Navas 2016; de Bello et al., 2021) and is recommended to study how communities change across environmental gradients (Leps, 2012). This approach has been adopted also to study biodiversity in olive plantations (ch. 2.2; 2.3). Assessing the effect of management in olive plantations is extremely important. Since humans modifies landscape to cultivate only few species, management quality plays a decisive role to maintain the stability of these agroecosystems (ch. 2.3, 2.4). Olive trees are dominant organisms of these agroecosystems and exert fundamental functions (see ch. 2.2) but to prosper and continue

[2] Biological organisms do not live isolated from their environment but have functions and provide services for the community or the ecosystem. For example, trees emit oxygen in the atmosphere and transpire water which contributes to cool climate (of which humans' benefits). Some species or genera, (e.g., *Nitrosomonas* bacteria) may have a disproportionate role in ecosystems since they are the unique organisms able to provide specific functions (ammonia degradation). Once these "keystone species" disappear or are reduced, the ecosystem might change or suffer consequences. For other functions the situation may be different, and several species might participate to the same function. This "redundancy" ensure that the disappearance of a single species do not affect the function itself, since the "functional group" is maintained.

to exert their functions, they need healthy soils and grasslands (Aranda-Barranco, 2023). Herbaceous plants may have diagnostic role in this, indicating the plantations' state of health (Cano-Ortiz et al., 2020).
Since it underestimates rare species for ecosystem functioning, however, CWM approach might suffer limitations. Some authors have argued that the higher the biodiversity, the more complete the use of resources by species ("niche partitioning hypothesis"; Tilman, 2001). Rare species must somehow exert a role in ecosystems, since generally ecosystems are composed of many rare species (with few individuals) and few common species (with many individuals; Odum, 2004). This has been tested also in olive plantations (Tarifa et al., 2021). Other indices have been developed to capture these aspects of biodiversity (e.g., Functional Divergence, Functional Dispersion).

Herbaceous plants must pass environmental filters to establish and become part of a community. Olive plantations are mediterranean habitats because they are affected by several constraints. The sum of natural climatic constraints (ch. 2.1) and agricultural management (ch. 2.3) impose difficult conditions in which only species with suitable traits can persist (ch. 2.2.1). In addition, species are not only experiencing natural constraints, but also strong environmental changes in Mediterranean regions. Climate Change and desertification (ch. 1.1; 2.1.4; 2.1.5) increase environmental stress resulting in decreased genetic variability which may feedback aggravating Climate Change consequences (de Bello et al., 2021). It is common knowledge that high genetic variability allows species to better adapt to the environment. The higher the variability within a population, the higher will be the fitness and the capacity to persist, other than expand and colonize other environments. Since Darwin, the individual has been considered primary centre of biological variation (Darwin, 1858). Individuals may vary within population by respect of genes (genotypic difference) or by the expression of the same genes in identical genotypes (phenotypic plasticity; Garnier, Grigulis, Navas 2016; de Bello et al. 2021). Short-term adaptations are expressed by phenotypic plasticity, whereas long-term adaptations (acclimation of plants) may lead to modification of genotype (Garnier, Grigulis, Navas 2016). The infraspecific trait variability (ITV) takes both in account (but without possibility of disentangling their specific contribution). Therefore, it is a way to assess how plants are adapting to environmental changes. This approach has been recommended to study aridity in Mediterranean drylands (Nunes, 2017). It appears to be a promising approach to assess how the habitat quality is affected under organic and conventional management.

1.3.2 Functional traits utilized in this thesis

Five functional traits (Plant Vegetative Height, Specific Leaf Area, Leaf Dry Matter Content, Leaf area, Leaf mass) were selected to investigate how plants respond to agricultural management

(respond traits) but also as proxy to investigate soil environment (effect traits). Plant Height, SLA and LDMC are among the most utilized plant traits in ecology. This is because they are relatively easy-to-measure or "soft traits" (Hodgson, Wilson & Grime 1999). They are considered medium variable traits, whereas Leaf area (single leaf area) is considered highly variable (Garnier, 2016). Plant Vegetative Height is measured at flowering time when the plant stops to grow and invest energy for the reproductive phase. This trait has been used to assess competition in plant communities (Hodgson, Wilson & Grime, 1999; Westoby, 1998) and is associated with resource-acquisition strategy (Wright et al., 2004). SLA is the ratio of leaf area to leaf dry mass, hence measurement of dry matter content is a preliminary condition because it is one component of SLA (Poorter et al., 2009). Dry matter content in plants consist of eight classes of compounds (minerals, organic acids, total non-structural carbohydrates, total structural carbohydrates, soluble phenolics, proteins, lignin and lipids; Poorter et al., 2009). SLA is associated with nutrient availability (Westoby, 1998). High nutrients lead to increased ratio. According to Leaf Economic Spectrum, when nutrients are available, plants tend to invest more energy in growth (enhanced photosynthesis) rather than in defence strategies (Wright et al., 2004). Therefore, they tend to "escape" rather than herbivory pressure. The opposite happens in nutrient-poor environments, where plants have few resources to grow and invest their energy in defence compounds. This plant strategy is often assessed through LDMC measurement (Garnier, 2016). Most of plants use one strategy or the other, resulting in a trade-off. A negative correlation among SLA and LDMC is expected (Garnier et al., 2001), hence when SLA diminish, LDMC increase. It is important to notice that whether all classes of compounds increase in content, minerals or organic acids only increased marginally but structural carbohydrates or lignin increase more than twofold (Poorter et al., 2009). These carbon-compounds are structural defence components which decrease leaf palatability for herbivores. They are also used by plants to decrease transpiration during water stress period. As mentioned in the introduction (ch. 1.3) plants have the capacity of rapid respond to environmental changes through physiological adaptations, according to their phenotypic plasticity.
The purpose of this study is to use both response traits and effect traits. Use of effect traits can be useful to investigate soil environment. The slow-fast continuum hypothesis (Kardle & Wardle 2010; de Bello et al., 2021) offers an interesting framework to investigate ecological factors shaping agroecosystems, because it links plant traits with ecosystem properties through soil analysis.

1.3.3 Soil

For the reasons mentioned in the previous chapters, soils are particularly threatened in Mediterranean regions, especially where agricultural practices have deeply modified landscapes (ch. 2.2; 2.3). Gathering data about soils can be used for interpretation or correlation with plant traits.

Habitats can be studied not only by an "above-ground" perspective, but also "below-ground". This can be reached linking trait-based ecology and soil science (Kardol & Wardle, 2010). Utilizing both these approaches may lead to a more integrated comprehension of ecosystems.

Soil is the plant environment. Plants live *on* soils, but at same time they have *formed* them (Brady & Weil, 2017). They use it for anchorage but also nutrition. They obtain water and nutrients by the same soil they have contributed to build, especially through organic matter addition. A well-developed soil has a certain capacity to store nutrients and water which depend on some factors. Soil formation is climate dependent; it is a function of annual precipitation and temperature. For example, Mediterranean soils are formed in low precipitation regime and have high evapotranspiration (ch. 2.1.3); this result in slow horizons formation and shallow soils (ch. 2.1.2). Soil formation depend also on parent material (e.g., limestone, granite, etc). The specific composition of rocks will determinate the physical (e.g., texture) and chemical properties of that soil. Typical parameters (see Appendix) provided by laboratory soil analysis are calcium (Ca), magnesium (Mg) and potassium (K) which give indirect measure of parent rock composition. They may be found bound in minerals and rocks or in ionic forms as positive charged cations (Ca++, Mg++, K+, Na+). Cations exert a fundamental role in soils determining its structure and fertility Electrical conductivity gives a measure of ions concentration in soil solution, which are used as proxy to assess soil structure (Astera, 2011; Brady & Weil, 2017)[3]. Their presence or absence is linked with pH, which measure H+ in soil solution in logarithmic scale (pH 7 is neuter). Calcium is one of the principal minerals in soils. Its main source is calcareous sedimentary rocks. Its double positive charge forms "bridges" which links negative soil charges giving stability and decreasing bulk density (BD). It tends to counteract acidity. Limestone soils have high quantities of calcium, which tends to increase alkalinity. In semi-arid climate limestone soils have specific features which can rise to very high levels (pH >8; ch. 2.1.2; 2.2.2). Magnesium is found mainly in dolomite rocks, bind with calcium. High Mg quantities are associated with decreased bulk density in soils (Astera, 2011). Potassium is a ubiquitous component of many rock types. K-Feldspars are the main source of potassium. Potassium is a structural component of secondary minerals (clays) which contains it folded within their internal sheets (Brady & Weil, 2017).

Clay minerals and humus (stabilized organic matter) have colloidal properties which act as negative magnets (-), attracting positive charged cations and water. High quantity of humus is related to high WHC[4] and soils' buffer capacity of counteracting soil degradation processes. These two factors are

[3] "low salt (ion) concentrations and weakly attracted ions (e.g., sodium) encourage soil dispersion and puddling, while high salt concentrations and strongly attracted ions (e.g. calcium) promote clay flocculation and soil permeability" (Brady & Weil, 2017, p.462)

[4] It is estimated that 1% SOM have the capacity to store approximately 150.000 litres of water.

main responsible of soils' water-holding capacity (WHC) and cation exchange capacity (CEC), which hence determines the amount of plant available water and nutrients, which are crucial in desertification risk regions.

Grilli et al. (2021) have proposed Soil Organic Carbon (SOC) as indicator of soil degradation. Carbon (C) is the main constitutive element (almost 60%) of soil organic matter (SOM). It is linked to fundamental soil parameters like total nitrogen (N), bulk density (BD), cation exchange capacity (CEC). They have found that 2% SOC is to be considered the threshold below which other soil parameters decrease rapidly, affecting soil quality. Moreover, carbon sequestration has raised as fundamental issue in order to mitigate Climate Change (Toensmeier, 2016). Soils are often carbon-sinks, however inappropriate agricultural management can transform soils into carbon-sources (Smith, 2004).

Nitrogen (N) is the main plant nutrient and is found in soils as component of organic matter (total nitrogen) or mineralized in inorganic forms like nitrates (NO_3) and ammonia (NH_4). Nitrogen becomes available through organic matter mineralization (Madigan et al., 2020). This process is provided by specific microorganisms, but solely if soil organic matter has a specific carbon to nitrogen ratio (Madigan et al., 2020). Therefore, this ratio, rather than absolute values is utilized as a measure of soil fertility. Phosphorous (P) is the second main plant nutrient after nitrogen (Brady & Weil, 2017). Building up soil phosphorous levels brought farmers to overfertilize fields to counteract tendency of this element to bind to iron oxides and clays in soils (especially at acidic or alkaline pH; Brady & Weil, 2017). For this reason, it is typical to see high P levels in conventional management due to inorganic P-fertilizers.

Agricultural practices tend to degrade soil environment. It is acknowledged that intensive practices (e.g., ploughing; ch. 2.4.5) tends to reduce SOM and this may lead toward a path of degradation. Globally half of arable land has been lost (Pravalie et al., 2021) and it has been calculated that agricultural soils in EU have reduced SOM levels from 5 to 1%. However, agricultural management may differ in their capacity to preserve soils. The link among soil conditions and desertification appears to be clear. Soil erosion and degradation lead to reduced plant productivity (Figure 1) since plants will be less able to resist increased drought and aridity conditions. Mediterranean regions suffer of high rate of soil erosion and degradation therefore it is crucial to assess how this occurs in olive plantation, given their prominent ecological and economic role in these regions.

1.4 Research Questions

In this study I hypothesize that conventional/intensive management of olive plantations reduce herbaceous plants' adaptation to desertification. I assume that organic management provide a better habitat for herbaceous plants living as "understory" and inter-rows of olive plantations. I expect that organic/lower-intensive management should decrease drought-stress and provide better soil conditions. I expect higher SLA, higher Plant Height and lower LDMC in organic plots. These were selected as primary traits. Expectations about secondary traits (Leaf area and Leaf mass) should follow similar pattern, i.e. both being larger under organic management. These traits should also help to interpret the pattern observed on SLA and LDMC. Regarding soil parameters, I expect higher soil organic carbon levels (SOC) and higher total nitrogen (N) levels. Other soils parameters are used to assess soil conditions; however, expectations are not formulated.

I therefore aim to test the following questions: 1a) Is there significant intraspecific trait variability between organic and conventional management?1b) are SLA and Plant Height higher; LDMC lower, in organic plots? 1c) Does it reduce drought-stress and desertification risk ("aridity hypothesis")? 2a) Are there significant differences in terms of soil parameters between organic and conventional management? 2b) Are SOC and total N higher on organic plots? 3) Do species composition change between organic and conventional management?

2. Study Site

2.1 General features of the region

2.1.1 Physical geography

Italy has a wide variety of climate regions and ecosystems. Considering a north-south gradient, the alpine climate of the Alps gives place to the temperate climate of the Padana lowland, which in turn gives place to mediterranean climate going toward south. The Apulian region is the south-eastern "heel" of Italy. It develops in length for approximately 400 kilometres. Almost half of the region is shaped by hills and mountains. Hills dominates centre-west of the region ("Le Murge hills") and mountains in the northwest ("Monti Dauna"). Lowlands are present in the north ("Tavoliere delle Puglie") and in the southern part of the region ("Salento").

2.1.2 Geology and soils

The geology of the region is composed of a calcareous basement, formed during Cretaceous period, upon which sedimentary formations stand, formed after quaternary period (Enipower). Karsism occur (especially in Le Murge) leading to very low retaining and permeable landscape, with very deep aquifers. The calcareous bedrock give rise to the typical alkalinity of Mediterranean soils (>7 pH). Soils are typically "mediterranean red earths" (iron oxidized in hematite) but vary with the sub-regions. There are three main soil sub-regions: "Tavoliere" lowland in the north, "Le Murge" hills and "Salento" (map of soil of Apulia, Regione Puglia). Similar soil classes may be found in all of them. Cambisols and Luvisols may be find across all the regions. Vertisols are a feature of

“Tavoliere”, due to its wide winter-summer temperature variability, whereas Regosols (not developed soils) may be find in “Le Murge” or in “Salento” (Steduto et al., 2001).

2.1.3 Climate

Climate is Mediterranean semi-arid (Ladisa et al. 2007) with 15-16°C mean annual temperature depending on the elevation and site. Summers are hot and dry. High pressure system (“Azzorre anti-cyclon”) moving from lower latitudes bringing with them dry trade winds during summer. During autumn and winter an opposite trend occurs. Prevailing winds brings rain from higher latitudes. Apulian climate is particularly dry compared to nearby regions. A-pulia means “no rainfall” in ancient italian. Precipitation is concentrated during autumn but differ widely from northern areas like Gargano (>800mm to 1100mm extreme) and Tavoliere lowland (<500mm). A water deficit occurs during summer months. This is a typical pattern for the Mediterranean basin, generated by the global air circulation (Prach, 2020). This condition determines the climate fragility of the Mediterranean basin. This is the reason why the basin is a Climate Change hotspot. The Figure 2 below shows water deficit of Apulia considering historical climatic series.

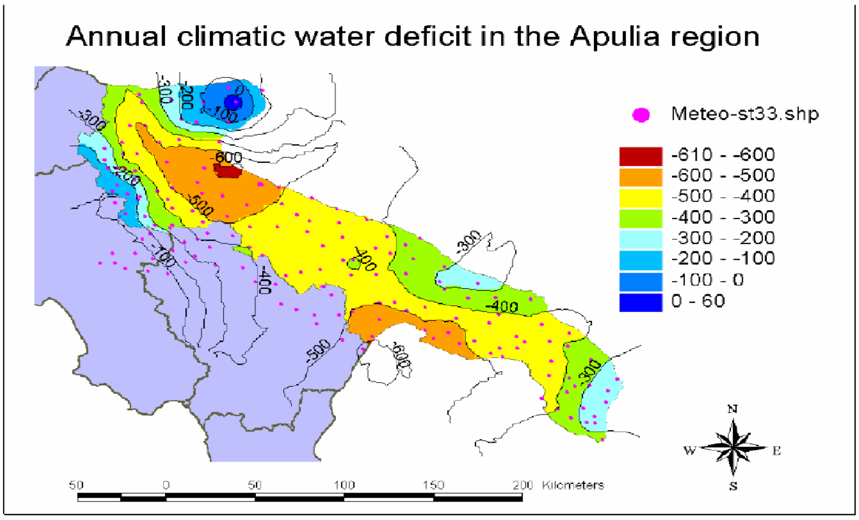

Figure 2. Spatial distribution of climatic water deficit in the Apulia region on an annual average basis. Picture taken from Steduto et al. (2001).

2.1.4 Climate Change in Apulia

Hotter and dryer climate is occurring in south Italian regions, with increasing heavy rainfall events and long drought periods (Polemio et al., 2008). Mediterranean drought is caused by the persistence of high-pressure systems which normally would leave the way at the end of summer (ch. 2.1.3). The change of position, duration and intensity of anticyclones determines rainfall and temperature anomalies. An increase in drought frequency and intensity is expected (UNDRR, 2021) as well as a general aridification of climate (IPCC, 2023; Cramer et al., 2020).

Polemio et al. (2008) have studied the relation between Climate Change, drought, and groundwater in south Italy. Data from 1821 to 2003 describe a decreasing trend (-0.8mm/year) of annual precipitation in 97% of the region. The groundwater situation is dramatic. What is the specific situation about drought in Apulia? Marini et al. (2019) have investigated this issue comparing two drought indices (SPI and RDI). They have found an increased drought frequency in west Apulia. The opposite in the east. However, the severity of drought events appears to have increased in the east. Mediterranean vegetation is well adapted to high frequency drought events (Braun-Blanquet, 1954) but decreased precipitation, and heavy agrarian modification of the landscape (land-use change; see ch. 2.2.3, 2.3), have increased aridity (Gouveia, 2016). The risk of damages to the vegetation have increased from 2000 to 2010.

Whereas drought is a temporary condition, aridity is permanent. The aridity is measured as the increase in frequency and duration of drought events (Colantoni et al., 2014). The Aridity Index (AI) is the ratio between annual precipitation and evapotranspiration.

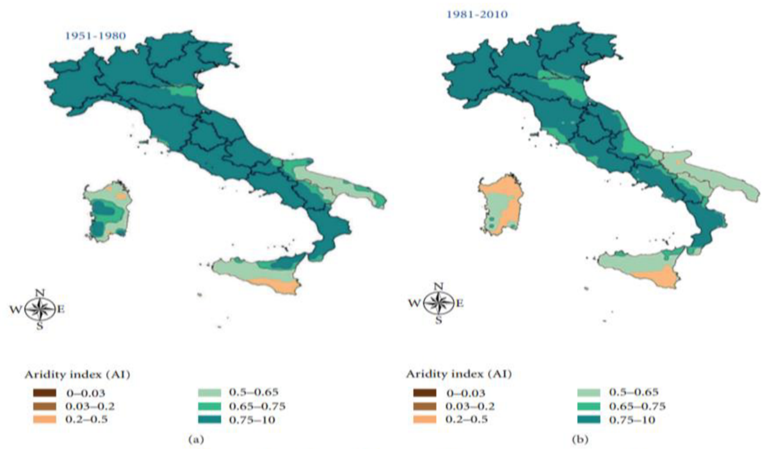

FIGURE 3: The spatial distribution of the Aridity Index in Italy by time period.

Figure 3 shows the spatial distribution of the Aridity Index in Italy by time period. Picture by Colantoni et al. (2014).

Colantoni et al. (2014) confirm the tendency of increased aridity during the last forty years (picture on the right). Whether previously some parts of the region (like Gargano) were not considered arid, now all Apulia appear to be homogenous in this respect. This increases risk, because desertification can be accelerated under severe drought conditions (Boschetto et al. 2010; UNCDD 2005).

2.1.5 Land degradation and desertification in Apulia and “Le Murge” hills

For the reasons explained, *Apulia region is particularly prone to desertification risk.* Climate features predispose the region to be threatened by processes of land degradation when intense human activities take place (ch. 2.3). A set of indicators have been proposed by Ladisa et al. (2007) with the aim to identify the areas sensitive to desertification and subsequently develop action plans to combat land degradation. The picture below shows which kind of pressures make constraints on the Apulian environment.

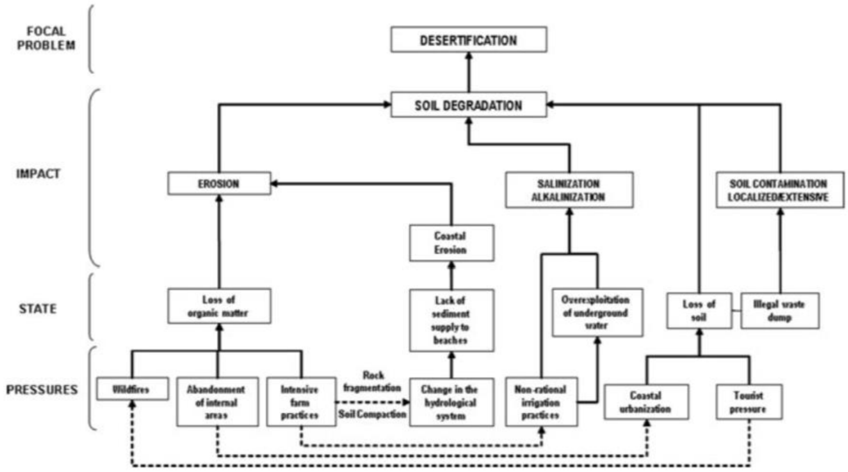

Figure 4 shows problem-tree of the main causes of soil degradation and desertification in Apulia region (by Ladisa, 2007).

Including human-induce factors in developing this index, authors conclude that 80% of territory is critical, 12.9% is fragile, the rest is potentially affected and non-affected (0.2%). 60% of Bari province, part of which is located in ”Le Murge hills” (where the study site is located), is critical but compared to other provinces human pressure is lower.

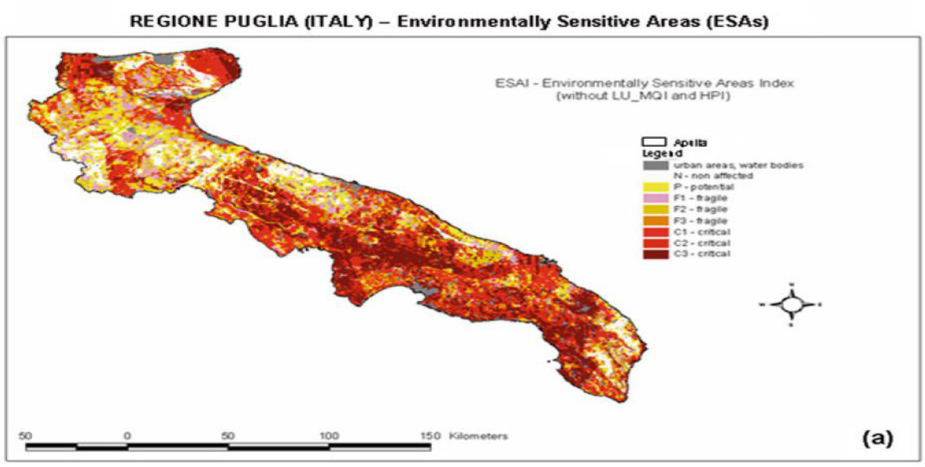

Figure 5 shows spatial distribution of environmentally sensitive areas in apulia. Source: Ladisa et al. (2007).

Soil Quality Index (SQI) indicate "Le Murge hills" as "low-quality land" being made of calcareous terraces (ch. 2.1.2) and shallow soils. What is relevant for the purpose of this thesis is that "*probably, such results from a large extension of annual crops (e.g. cereals) with low resistance to drought and presence of high fire risk areas*" (Ladisa et al., 2007). Boschetto et al. (2010) confirm that Apulia have medium to high sensitivity to desertification.

2.2 Ecosystems and agroecosystems of the region

2.2.1 Ecological characters of apulian ecosystems

The region holds over 2000 vascular plant species, of which many are rare and endemics. The biological richness of this region is due to the different microclimatic conditions and diversity of its environment. Due to water scarcity (ch. 2.1.3; 2.1.4) soil water is the main limiting factor for vegetation and determine two growing season, spring and late summer, when the water is more available from precipitation and evaporative loss is reduced. Fire is also a limiting factor for the vegetation of the region (which its dramatically increasing due to human activity). This disturbance factor may lead to degradation or reverse succession, leading to garigue ecosystem dominate by fire-adapted plants. The vegetation is shaped by the physical and climatic elements mentioned above. For example, trees like *Quercus ilex* manifests typically xerophytic adaptations (small, evergreen leaves) to reduce transpiration during hot summer (Prach, 2020). This feature moreover retards nutrient cycling through slower leaves decomposition. Low rainfall and oligotrophic soil conditions determine slow plant growth.

2.2.2 *Olea europaea*

The most prominent semi-natural ecosystem of the region pertains to the OLEO-CERATONION alliance[5]. Olive orchards and plantations sometimes mixed with other woody crops species (like cherry and almond), are by far the dominant vegetation of the region. Ancient secular olives constitute a fundamental part of the landscape and are considered monumental trees of great historical value. Unfortunately, due to the Xylella pandemic affecting millions of secular trees, there is an increasing tendency to substitute ancient trees with Xylella-resistant varieties (e.g., F17). The olive tree (*Olea europaea*), is the domesticated version of *Olea europaea ssp. sylvestris* which is not

[5] "Br.-Bl. *ex* Guinochet & Drouineau 1944 em. Rivas-Martinez 1975 is an Alliance of the Order Pistacio lentisci-Rhamnetalia alaterni Rivas Martinez 1975 of class Quercetea ilicis Br.-Bl. *ex* A. & O. Bolòs 1950". Source: Prodromo della vegetazione d'Italia.

endemic of Apulia but it is present here since the second millennium a.C. After the end of Roman Empire and the Norman conquer during the 11th century, the calcareous hills of central Apulia ("Le murge hills") have been cultivated and become important economic activity of the region (Caractura, 2019). The olive tree is very adapted to arid and semi-arid conditions given its capacity to tolerate high alkaline soils (up to pH 8,4). It can withstand very low soil water potential compared to normal range of tolerance (Brito, 2019). Olive plantations are expanding toward north, because the general warming of temperature tends to favour species shift toward northern countries (Parmesan, 2006). Mediterranean grasslands are very rich in species (120-180 x 100 mt2; Fernandez, 2017). Other species like *Ceratonia siliqua*, *Pistacia lentiscus*, *Oputia ficus-indica*, *Arbutus unedo*, are typical occurring in this alliance and this sub-region. Grasslands are the semi-natural vegetation forming the understory of olive plantations. However, it is rarely left grown by farmers and owners for traditional reasons (ch. 2.4.1).

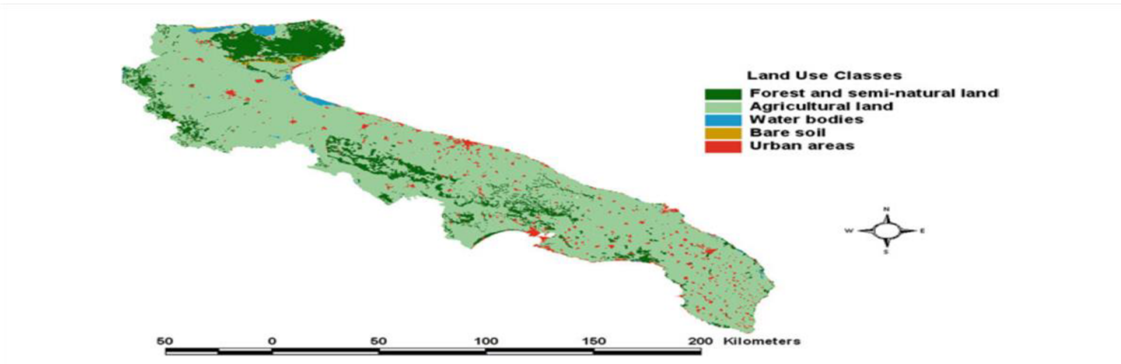

Figure 6. Apulia region: main land use classes according to the CORINE Land Cover data-catalogue (in Ladisa, 2007).

2.2.3 Olive agroecosystems landscape

Another important characteristic is the high landscape fragmentation. This is due to the land division into private properties but is also result of the huge work of stone removal done during the centuries. If the fragmentation may have detrimental effects on the mobility of species across the landscape, reducing meta-population connectivity, on the other hand it provides an important habitat for rare species. Perrino et al. (2011) argue that stony walls host more biodiversity than open fields, probably because of the "margin effect". Moreover, whether therophytes life-forms results dominant in the centre of plantations, perennials forms are prevalent on the edge. In a highly disturbed landscape, these "ecological infrastructures" are an important element for biodiversity conservation. In addition, creating a physical barrier, they could have a role in reducing soil erosion (and stop fire expansion), a very concerning issue, especially in hills (Caliandro et al., 2005).

Figure 7, taken by the thesis' author in March 2023. On the left, vegetation growing after spring ploughing. Much more flowering plants are visible. On the right, vegetation growing probably in a organic olive orchards. Studies about aridity have revealed that onset flowering is a typical behaviour of herbaceous plants strategy to escape drought (Nunes et al., 2017).

Figure 8 shows which olive plantations are more prone to desertification. The area of the study approximately overlaps with the red spots at the right of the sub-region. According to the index presented by Caliandro et al. (2005) these plantations are more prone to desertification risk. Interestingly, in the southern area (Salento and Brindisi) which is affected by the *Xylella fastidiosa* pandemic since 2013, olive plantations are considered less prone to desertification[6].

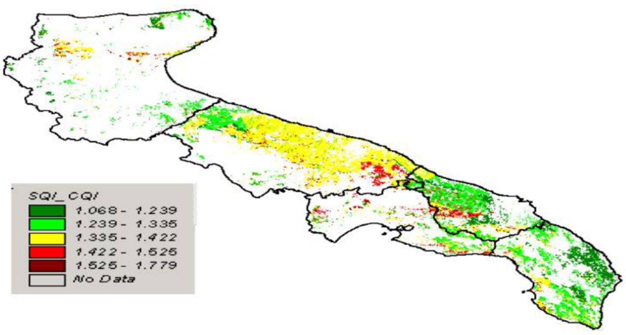

Figure 8 shows olive plantations prone to desertification according to Soil Quality Index by Caliandro et al. (2005).

[6] CO.DI.RO. or "complex of rapid disseccation of olives" have started in 2013 in Salento sub-region of Apulia and was attributed to Xylella fastidiosa sub.pauca (Saponari et al. 2013). Millions of trees have disseccated. Many have been eradicated in order to try to stop pandemy to advance toward northern regions.

2.3 Management of the olive agroecosystems

2.3.1 Traditional management

Apulia has undergone a long history of human impact (Caracuta, 2019). The landscape has been shaped since the Neolithic period into an agrarian landscape. Apulian flora and vegetation have been degraded during the last forty years due to intensification of this impact. Today, 80% of land is used for agriculture in the region (Marini et al. 2019; Ladisa et al. 2007). However, traditional farming may have been a source for biodiversity before the emergence of modern agriculture. Rather than be detrimental for species, it could provide a heterogeneous landscape increasing edge effect and boosting productivity (Vera, 2000). Olive orchards were historically managed through 1) agro-sylvo-pasture or 2) ploughing. In the first case, the management type was a traditional and sustainable way of farming akin to Montado agroecosystems in Spain and Portugal. The herbaceous vegetation was left to grow as understory in the inter-rows, mowed or left to the cattle. The animal stock per hectare was probably often in balance with the carrying capacity of the environment. In a low-energy agriculture such as the pre-Anthropocene epoch, the energy to provide feed to animals and increase the herd stock, was often insufficient to reach overgrazing. With the emergence of the modern agriculture the domesticated herbivores have mostly disappeared from the Apulian landscape, or they are concentrated into dairy farms. They do not more provide ecological services, such as spreading and mobilizing nutrients across the landscape (Doughty, 2017). The result is that there is not more natural disturbance to the vegetation and reduced nutrient cycling (Butterfield et al., 2019). Somebody sustains agro-sylvo-pasture as the most efficient form of carbon sequestration (Toensmeier, 2016). The second type of management is traditional as well. Ploughing was provided by animal labour and was therefore less intense and deep than nowadays.

2.3.2 Conventional management

Apulia is the primary Italian oil producer accounting for 51% of total oil production (data from ISMEA, 2023). After the second world war, with the arising of the Green Revolution, Olive orchards and plantations were almost all managed in conventional management. Olive yields are higher in average managed in conventional agriculture. Today it accounts for 70% of regional production. Herbaceous vegetation is left grow for half of the year (autumn-winter) and then ploughed at the beginning of spring or sprayed with herbicides (generally glyphosate). Chemical insecticides (e.g., dimetoate) are used to combat olive pests (especially the olive fly *Bactrocera oleae*). Chemical fertilizers are used to increase olive yield, however, are probably less diffuse compared to other techniques. Plantations are often organized into monocultures to maxims yield per hectare. It is not rare to see plantations managed as “tennis fields” (because of the compaction operated by machines on the “mediterranean red earths”) to facilitate harvesting operations. In the past soil fumigation was also considered normal to “disinfect” soil. Apulian institution have chosen to adopt conventional as mandatory practices to combat Xylella pandemic expansion[7].

2.3.3 Organic management

Organic olive production is increasing during last decade accounting for 30% of Apulian production (ISMEA, 2023). Organic production does not necessarily avoid ploughing, neither monoculture. Grasslands may be left grow in spring although this is not always the case. It is more common to see grasslands understory of olive plantation in spring in organic agriculture compared to conventional. There is consensus behind the idea that there is a “yield gap” between organic and conventional agriculture (Reganold, 2016). One reason may be the lower amount of nutrients that organic fertilizers provide. However, some authors argue that this result is biased by the way organic seeds are produced. Because the majority of commercial seeds is produced by multinational corporations in experimental stations with high nutrients inputs, once cultivated in “organic conditions” (lower nutrient addition) cannot provide comparable yields.

2.4 Ecological issues of agricultural management

2.4.1 effects of nutrient addiction in conventional agriculture

According to Tilman (1998) conventional agriculture account for 60% of nitrate leaching. Nitrogen and phosphorous leaching are well known to be responsible for lakes and sea eutrophication (Odum, 2004; Rockstrom et al. 2009; Schlesinger & Bernhardt 2020). Nitrates are a source of pollution for acquirers affecting freshwater use. When chemical fertilizers are spread onto bare soils, as in the case for most of the apulian olive plantations, they find their way into the deep horizons and eventually to the aquifer. Use of these fertilizers impede the methanogenesis reaction in soil, which is an important planetary mechanism of methane sequestration. Therefore, fertilizers might contribute to GHG emissions (Brady & Weil, 2017). Moreover, nitrates are not directly assimilated by the plant, but are rather utilized by the soil microbiota to build organic matter (Brady & Weil, 2017). Nitrogen is known to alter vegetation structure favouring dominance by certain species (Odum, 2004; Tilman, 1987). This is known to reduce biodiversity. Nitrates are known to boost olive yield. However, excess nitrogen may alter the plant physiology increasing palatability for herbivores (Altieri, 2012). Excess nitrogen is also considered detrimental for crop quality. It may favour N-pathway rather than shikimate C-pathway. This is particularly important in the case of olive oil, especially for the extra-virgin oil, which is harvested in early stages to maximize

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[7] Action plan to combat Xyella fastidiosa diffusion (Well et al) in Apulia (D.G.R. n.1866 12/12/2022) includes, among mandatory phytosanitary measures useful to reduce populations of vector (Philaenus spumarius) of Xylella fastidiosa, superficial soil tillage (ploughing, milling, mulching, harrowing) and use of pesticides.

polyphenols contents. Excess of nitrate impedes the plant capacity to self-defence and produce phytochemicals (Altieri, 2012). Regarding the case of olive plants, nitrates (compared to ammonium release by organic mulches) appeared to be linked with Xylella-induced pathologies (Johal & Huber, 2009).

2.4.2 effects of nutrient addiction in organic agriculture

Nitrate and phosphorous losses have been found lower when expressed per unit production area. However, given the lower land-use efficiency the positive effect in some cases is reversed when expressed per unit product (Reganold et al., 2016; Clark & Tilman 2017). Nitrate leaching is reduced, and denitrifying activity seems to be enhanced (Kramer et al. 2006).

2.4.3 effects of chemicals on the environment in conventional agriculture

Chemical pesticides are xenobiotics, therefore sources of environmental pollution (Brady & Weil, 2017). They may be toxic for living organisms both in soil and water organisms. Some forms of insecticides (e.g., neonicotinoids) are detrimental for pollinators (Brady & Weil, 2017). Chemical herbicides, especially glyphosate, may affect the plant capacity to withstand fungal pathogens. Glyphosate is a strong systemic herbicide which act disrupting EPSP enzyme empeding synthesis of aromatic aminoacids compounds in plants. It may affect also olive trees altering their metabolism making them more predisposed to soil pathogens (Johal & Huber, 2009). Glyphosate seems to be linked with increased Xylella-induced pathologies. Moreover, glyphosate is of concern when it reaches its way to the acquifer, even though its human health toxicity is debated (Matthias, 2016). Herbicides and insecticides influence the plant and the environment of the plant. From an evolutionary perspective, these chemicals should feedback on the plant altering its phenotype (phenotype*environment; de Bello et al., 2021). Considering the trait-based ecology perspective, insecticides may be interpreted as stress factor, affecting photosynthesis, plant growth rates and eventually fitness. Herbicides should be considered as a disturbance factor as well as grazing and mowing. According to certain studies, chemical herbicides have founded to significantly change the herbaceous vegetation structure of the olive plantation (for apulian olive plantations see Terzi et al., 2021). Herbicides might significantly affect persistence of rare plants in olive plantations (Tarifa et al., 2021).

The “package” (seeds, fertilizers and pesticides) have found several critics by environmentalists (Shiva, 1991). GMOs-herbicides package are new technologies the environmental consequences of which arise new questions, for example the increasing herbicide resistance developing by some weeds. Analogous phenomena occur for pesticide-resistance. Biological populations exposed to the same active principle for enough amount of time, tend to become resistant and co-evolve. Is this a well-known ecological and biological principle: when attacked by herbivors plants tend to evolve defence system to escape pressure (Erlich & Raven, 1964). Insects respond as well, and the cycle continue.

2.4.4 effects of chemicals on the environment in organic agriculture

Tilman (1998) argue that organic agriculture uses -53% energy and -97% pesticides. Plant-derived pesticides generally do not have the same power to stop pests and have solely a preventive use.

2.4.5 effects of ploughing

Ploughing is in general no more considered a sustainable agricultural practice (Birkas, 2008). However, it may depend on the environment, frequency, and intensity. Ploughing determines soil exposure to atmospheric agents (wind, sun, rain, etc) which tends to degrade it on the long run. It decreases water infiltration and increase run-off and sediment loss (Kairis, 2013). Moreover, it exposes soil to organic matter oxidation and mineralization, which tend to reduce soil carbon levels and impede carbon sequestration (Kairis, 2013).

There are ecological reasons to justify but also to reject this management in Apulian landscape. The main argument is probably the reduced fire risk induced by the destruction of the vegetation which could provide fuel for fire becoming dry during summer. Somebody has argued that ploughing may provide more biodiversity (Hernandez Plaza, 2011) and rare species conservation (Pereira, 2024) because arable field communities have evolved through secular adaptation to regular soil disturbance. Other studies have not seen differences in terms of species richness between mown vegetation and ploughing (Terzi et al., 2021). Eliminating the herbaceous vegetation reduce competition for water, because plants transpire soil available water to the atmosphere. However, this practice has contrasting effects. It is questionable whether undisturbed vegetation transpire more water than bare soils during summer (Brady & Weil, 2017). Moreover, ploughing alters the physic-chemical structure of the soil reversing horizons; it alters the soil environment, destroying micro and mesofauna; increase dramatically soil erosion, especially in steep slopes (>10%; Calabrese et al., 2015); it reverses ecological succession to pioneers' stage. From the trait-based ecology perspective ploughing is considered a disturbance factor as it destroys vegetation (ch. 1.3). Considering the brittleness of the mediterranean environment (ch. 2.1) and the challenge for water conservation in semi-arid landscapes, it is hard to consider as a sustainable practice. Ploughing is associated with increased desertification risk in olive groves (Kairis, 2013).

3. Materials and Methods

3.1 Location of the Study

The study site is in Castellana Grotte, Bari province, in Apulia region [40°53′01.58N 17°10′04.29E]. This town is located at 300 m above sea level in the south-eastern part of "Le Murge". Looking at geology and physical constitution of the land it is very similar to Alta Murgia's geomorphological features (same stony and rocky forms of reliefs and same superficial and deep karsic phenomena) from which the name "grotte" = caves. Soils have high clay contents[8]. The average annual temperature is 16°C. Average rainfall is 596 mm/year. The elevation is 293m above sea level.

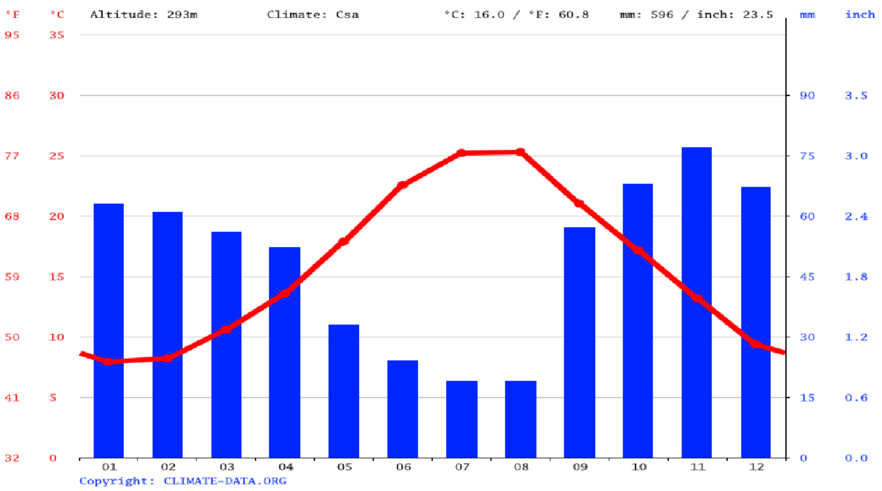

Figure 9. Source: climate-data.org

"Torre dei Mastro" organic farm was used as control plot. Given the predominance of conventional managed plantations it was not possible to find other organic farms. "Torre dei Mastro" is made up mainly of olive plantations (more details in Appendix 1). The property of the farm is scattered around Castellana Grotte's municipality. This condition is typical of the high fragmentation of the agrarian landscape in Italy (see picture) where farmers historically own small pieces of land.

Vegetation is mown sporadically, not more than once or twice a year. Residues from olive pruning is chopped/mulched within the field but pruning do not occur every year. Perennial herbaceous understory cover is maintained all year round. Soils are not ploughed since at least ten years; twenty

[8]Information gained by owner. Soil analysis the lab provided for the present study do not also included texture. Heavy clay soils are considered soils with more than 25% clay of the USDA texture triangle. It is difficult to rely only on this kind of experienced-based information, however farmers know their land and the "strength" or "tenacity" of land to be ploughed; the slowness of warming during spring; the long time for the land to dry up gives good proxy to consider this a "heavy soil".

years in some fields. This is an unusual condition, both for traditional landscape management and because ploughing is mandatory by laws to prevent *Xylella fastidiosa* further expansion. For these reasons and according to the indices explained in previous chapters, this site can be considered a good spot to study desertification.

3.2 Study Design

As the conventional agricultural practice is widespread in the region, to each organic plot were selected paired plots with conventional practice. This design reduces heterogeneity of soil conditions or other microclimatic conditions (Smilauer & Leps, 2014). Information about management applied on the landscape were provided only by the organic farmer owner of Torre dei Mastro. Available plots were not sufficient to assess a factorial design studying the specific contribution of fertilization, herbicides application, pesticides and ploughing. At least one of these management practices was used as criteria to define non-organic management (called conventional hereafter; note on ploughing, ch. 1.2; 2.3.2).

Figure 10. Map developed with QGIS (version 3.26.3) showing study design. Six pairs of plots are scattered around the municipality.

3.3 Sampling

The study was conducted from 4th March 2023 to 4th April 2023. Sampling was conducted during early morning or late afternoon in most cases. In some cases, due to the short window and available time, this rule was not performed. Sampling was done not in the middle of the plot neither close to the border to avoid the “edge effect” (Smilauer & Leps, 2014).

3.3.1 Vegetation Sampling

Phytosociological releves were initially performed to investigate flora and species composition of all plots. Releves were conducted by visual estimation of percentage cover of individual species and of total vegetation cover of 5m x 5m. This was also a preliminary step for the selection of suitable species (dominant individuals present in the landscape) for functional traits measurements. The species present in site belong to Stellarietae mediae (Class), likely to Caucalidion platycarpi (Alliance). This alliance includes annual invasive communities, often rich in species, which grows in wheat fields or vineyards, and develop on clay-silt soils, in correspondence of temperate or warm Mediterranean climate (Ubaldi, 2019).

3.3.2 Plant functional traits sampling for ITV

Five species were selected for functional traits measurements. One grass (*Avena fatua*) and four forbs (*Calendula arvensis, Chrysanthemum segetum, Erodium malacoides, Medicago orbicularis).*

Figure 11. *Avena fatua* (common wild oat) Monocot, Poaceae family. Euri-asiatic distribution. Native to Eurasia, was introduced and naturalized in other temperate regions of the world. Therophyte, erect floral axis with none or few leaves.

Figure 12. *Medicago orbicularis.* Dicots, Fabaceae family. Euri-Mediterranean distribution. Annual plant with elonged floral axis. It forms symbiotic relationship with N-fixing *Sinorhizobium medicae* bacteria.

Figure 13. *Chrysantemum segetum.* Dicots, Asteraceae family. Euri-mediterranean distribution. Native to east Mediterranean, diffuse in central-south Italy. Therophyte with erect floral axis.

Figure 14. *Calendula arvensis.* Dicots, Asteraceae family. Euri-mediterranean distribution. It can occur as Therophyte or biennial plant.

Figure 15. *Erodium malacoides*. Dicots, Geraniaceae family. Steno-mediterranean distribution. Therophyte.

These species were selected because dominant in the region and present in all plots. Plants were extirpated rather than cut above collar to reduce water loss by transpiration. In each plot I collected eight best developed individuals per species per plot which resulted in a total of 440 individuals of plants sampled. Individuals were water-saturated by closing into plastic bags containing water and injected with breath. This procedure is suggested to avoid transpiration (Perez-Harguindeguy et al., 2013). Samples were then stored into fridge at 4°C no later than one hour from their sampling. The entire procedure was standardized. All sequences of sampling and measurements were repeated in the same way to reduce standard error (SE) at minimum. Measurements of traits were carried out after 12 hours (Perez-Harguindeguy et al., 2013)[9].

For leaves trait measurements I selected only healthy undamaged leaves (two leaves per *Avena fatua*; five leaves for all other species). Wet weight was measured with a portable KERN-TAB balance (with accuracy 0.001g). Before every session it was calibrated. Leaves were dried at 20°C for at least 36h and stored. Dry weight was measured during last week of March for all samples. Scanning (for SLA and LDMC measurements) was conducted with a portable IRIScan Book scanner. Area calculations were conducted with ImageJ software in 8-bit type and binary mode.

3.3.3 Community-weighted mean

To assess functional trait composition of whole community Plant Height, SLA, and LDMC were excerpted from TRY database (Kattge et al., 2020). Based on species composition of the community I have computed Community Weighted Mean (CWM) of those traits. Leaf area and Leaf mass were not available for the whole community and were excluded for community level analysis.

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[9] After gaining raw field data (wet mass in grams, dry mass in grams) I transformed dry weight into mg. Through leaf dry mass (mg) / leaf saturated mass (g) formula, Leaf Dry Matter Content (LDMC) was computed. SLA was then computed dividing Leaf area (LA) / (dry mass * 1000). LA and Leaf mass were recounted to single leaf. The sequence for Traits measurements was: Plant Height > cutting leaves > wet weight > area scanned > leaves essication > dry weight > surface area calculation. After the scanning, leaves were stored into paper bags.

3.3.4 Soil sampling

Soil samples were taken in each plot always at the end of plant sampling. One sample was obtained mixing 5 sub-samples, collected at random spots (within the same 5m x 5m quadrat). Every sub-sample was taken at 0-10 cm depth. There is no consensus about sampling depth, depending on ecosystem types and type of research is carried on (Brady & Weil, 2017). Soil samples were then stored into paper bags and then dried at 20°C for 24h. Then, samples have been shipped to Analytical Lab of Institute of Botany in Trebon.

Table 5 shows parameters analysed in this study, type of analysis used in Lab and units.

Parameter	Type of analysis	Unit
pH	In H20	0-14 scale
Electrical Conductivity	EC meter	uS/cm
P-PO4	Mehlich III	mg/Kg
Ca	Ammonium Acetate	mg/Kg
K	Ammonium Acetate	mg/Kg
Mg	Ammonium Acetate	mg/Kg
N	FLASH Elemental Analyzer	%
C	FLASH Elemental Analyzer	%

4. Statistical Analysis

Multivariate data on plant species community composition were initially assessed by Detrended Correspondence Analysis which showed relatively short gradient (1.6 SD). Plant species community composition was therefore analysed by linear ordination method of Redundancy Analysis (RDA), as recommended by Smilauer and Leps (2014). I did not log-transformed the data set. The Management type (organic vs conventional) was used as explanatory variable. Identity of pair of plots was used as covariate. Significance was tested by Monte Carlo permutation test with 999 permutations. Only plots within the pairs were permuted. To visualize the differences in community species composition without direct testing was performed also the Principal Component Analysis (PCA). All multivariate analyses were conducted in Canoco 5 software (ter Braak and Smilauer, 2012).

I used R 4.1.1 version for all univariate analysis (R core team, 2022). Measured functional traits were analysed by analysis of variance (ANOVA) where management type, species identity and their interaction were used as fixed effect explanatory variable. Identity of pair plots was used as random effect variable. To disentangle the species-specific responses to management, five species measured for functional traits were analysed separately with similar ANOVA, where management type was used as fixed effect explanatory variable and identity of pair of the plots as random effect variable. Accordingly, CWM of database traits and soil properties were analysed by ANOVA, where management type was used as fixed effect explanatory variable, while identity of pair of the plots was used as random effect variable. All ANOVAs were conducted using function *aov.*

5. Results

5.1 Phytosociological Releves

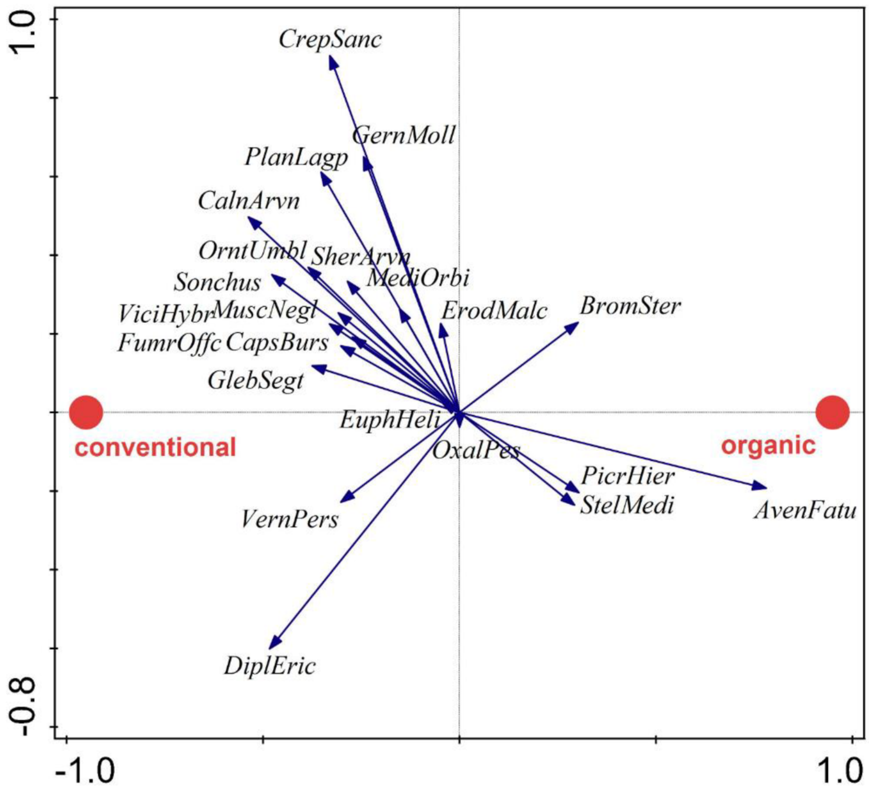

Figure 16 Ordination diagram of RDA testing the effect of management on the community composition. Focus was scailed on interspecies correlations. Abbreviations are: AvenFatu - *Avena fatua*, CalnArvn - *Calendula arvensis*, GlebSegt - *Chrysantemum segetum*, MediOrbi - *Medicago orbicularis*, ErodMalc - *Erodium malacoides*, MuscNegl - *Muscari neglectum*, GernMoll - *Geranium molle*, SherArvn - *Sherardia arvensis*, CrepSanc - *Crepis sancta*, DiplEric - *Diplotaxis erucoides*, ViciHybr - *Vicia hybrida*, EuphHeli - *Euphorbia helioscopia*, StelMedi - *Stellaria media*, OrntUmbl - *Ornithogalum umbellatum*, Sonchus - *Sonchus sp.*, CapsBurs - *Capsella bursa-pastoris*, FumrOffc - *Fumaria officinalis*, PicrHier - *Picris hieracioides*, OxalPes - *Oxalis pes-caprae*, PlanLagp - *Plantago lagopus*, VernPers - *Veronica persica*, BromSter - *Bromus sterilis.*

Plant species composition significantly differed between organic and conventional plots (F= 4.2, *p*= 0.042, explained variability was 29.33%). Most of the species were associated with conventional management, however the second (non-constrained) axis explain almost the same degree of variation (28%), revealing that another factor should explain this pattern. *Veronica persica* and *Diplotaxis erucoides* preferred conventional as well but deviated from that group. Among perennial species *Muscari neglectum* and *Ornithogalum umbellatum,* appeared more abundant in conventional management (*Chrysantemum segetum* and *Calendula arvensis* showed high preference). Poaceae species appeared more correlated with organic management. *Avena fatua* showed high preference for this management. *Bromus sterilis* was more represented in organic management, however its abundance in all plots was too low to be relevant.

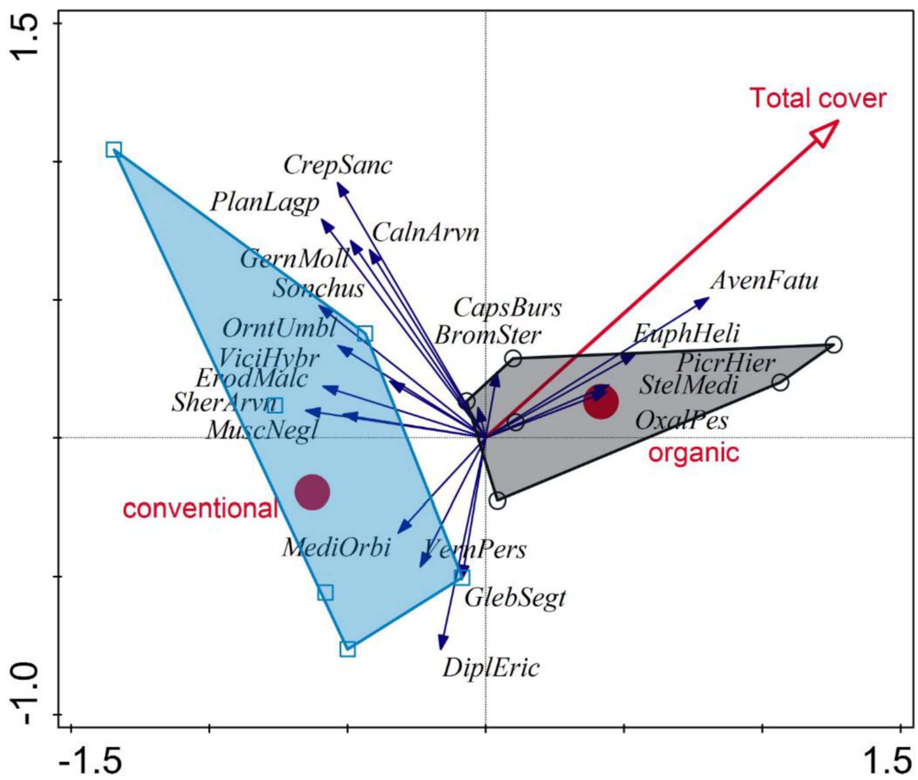

Figure 17 PCA ordination diagram of plant species composition in organic and non-organic plots. Envelopes encloses the plots of individual managements. Total cover and symbols of conventional and organic management are shown as passive variables. Focus was scaled on inter-sample distances. For abbreviation of species see Figure 16.

PCA in Figure 17 showing that species composition of both managements well differentiated in two groups. Organic Management and Total Cover appeared highly correlated (red arrow length and direction).

5.2 Functional Traits

5.2.1 Overview of ANOVA results of infraspecific trait variability

All traits significantly differed between species and were significantly affected also by the management. Changes in traits induced by management were species specific for all traits, appart the Leaf area. ANOVA results were highly significant, except for the interaction of management and species on Leaf area (Table 1).

Table 1 Results of ANOVA testing the effect of management and species identity on functional traits. Displayed are F and p-values.

	Management		Species		Management * Species	
	F	p	F	p	F	p
Height	106	**$<10^{-3}$**	123	**$<10^{-3}$**	4.3	**0.001**
LDMC	9.7	**0.001**	220	**$<10^{-3}$**	5.6	**$<10^{-3}$**
SLA	11.1	**$<10^{-3}$**	11.2	**$<10^{-3}$**	5.5	**$<10^{-3}$**
Leaf area	10.6	**0.001**	506	**$<10^{-3}$**	2.0	0.099
Leaf mass	15.7	**$<10^{-3}$**	419	**$<10^{-3}$**	4.2	**0.002**

ANOVA for each species separately showed that Plant Height significantly differed with management in all species (Table 2). The other traits did not differ between managements for all species. Solely *Medicago orbicularis* revealed significantly results for all traits. However, all traits responded in *Chrisanthemum segetum,* except for LDMC.

Table 2 Results of ANOVA testing the effect of management on functional traits for each species separately.

	Height		LDMC		SLA		Leaf area		Leaf mass	
	F	p	F	p	F	p	F	p	F	p
A. fatua	12.2	**$<10^{-3}$**	32.3	**$<10^{-3}$**	0.15	0.701	0.05	0.829	0.012	0.914
C. arvensis	44.4	**$<10^{-3}$**	13.5	**$<10^{-3}$**	0.97	0.329	0.37	0.543	0.027	0.871
G. segetum	52.5	**$<10^{-3}$**	0.82	0.37	4.5	**0.038**	11.7	**0.001**	15.61	**$<10^{-3}$**
M. orbicularis	13.7	**$<10^{-3}$**	10.0	**0.002**	18.7	**$<10^{-3}$**	7.3	**0.008**	21.52	**$<10^{-3}$**
E. malacoides	22.4	**$<10^{-3}$**	0.65	0.423	1.6	0.214	2.1	0.149	3.192	**0.051**

5.2.2 Plant Height

Organic plots had taller plants in average, with few outliers. ANOVA test was significant for Management, Species. The interaction was significant.

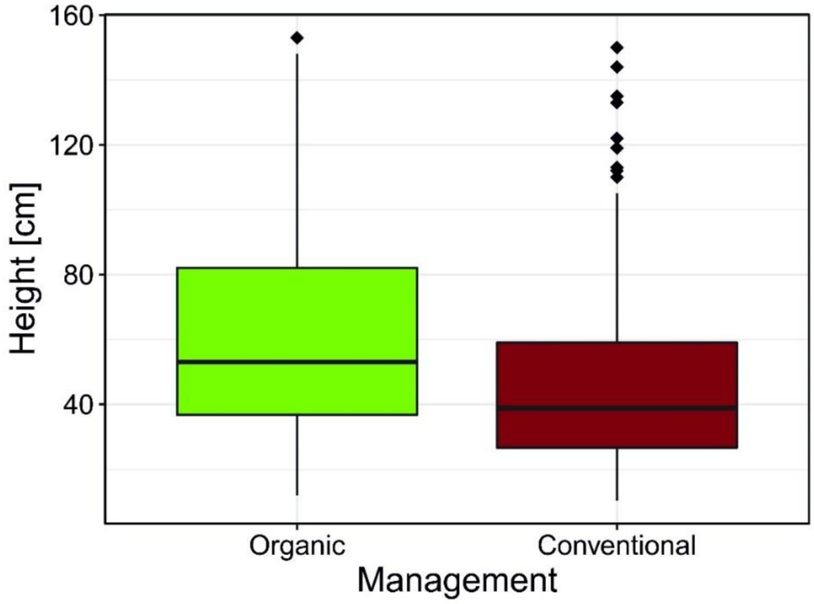

Figure 18. Boxplots showing organic and conventional management effect on Plant height.

ANOVA test for individual species gave significant results for all. All Species were taller in organic plots, as expected by initial hypothesis (1b). Also, *Medicago orbicularis* had higher value, although increment was lower compared to other species.

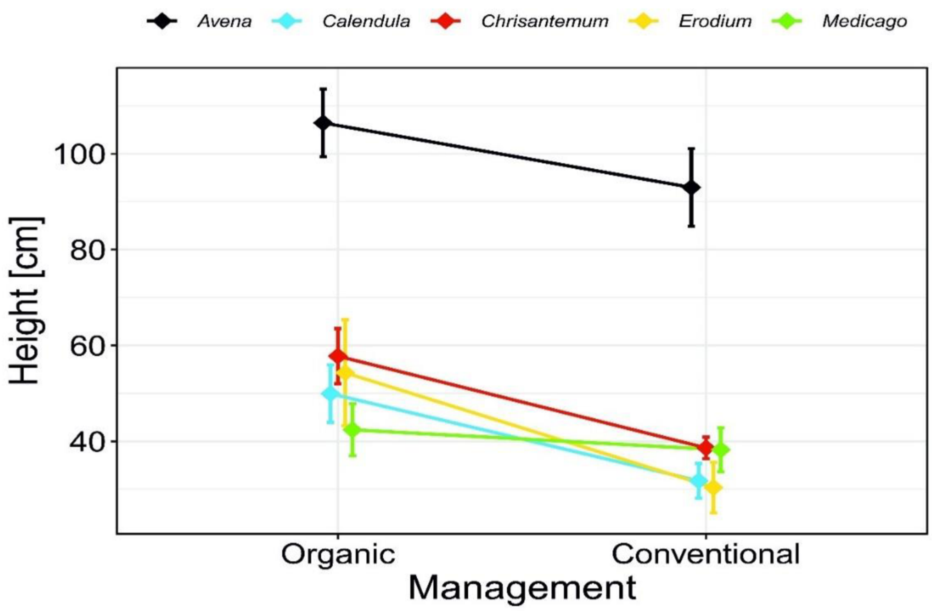

Figure 19 showing differences in Plant Height between species and management.

5.2.3 Leaf Dry Matter Content (LDMC)

ANOVA test gave high significant results for Species, Management and interaction. On organic plots species had higher LDMC on average. This result was unexpected and apparently contradict our initial hypothesis (1b).

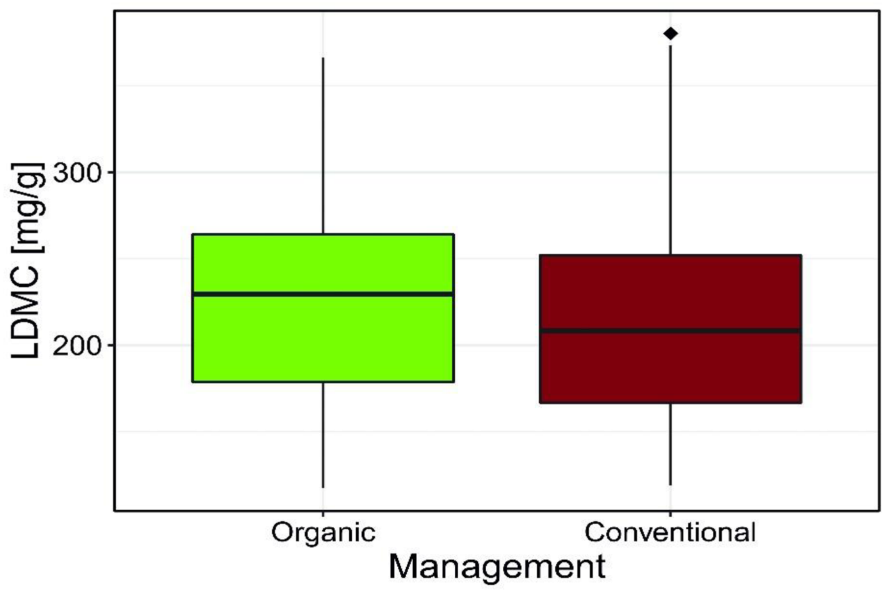

Figure 20. Boxplots showing organic and conventional management effect on LDMC.

However, looking at ANOVA test for individual species, the response is not univocal. *Avena*, *Calendula* and *Medicago* gave highly significant results, however *Medicago* showed an opposite trend. Whereas *Avena* and *Calendula* had higher values on organic, *Medicago* had lower values on organic plots as expected by initial hypothesis (ch. 1.3). *Erodium* and *Chrysanthemum* gave not significant results.

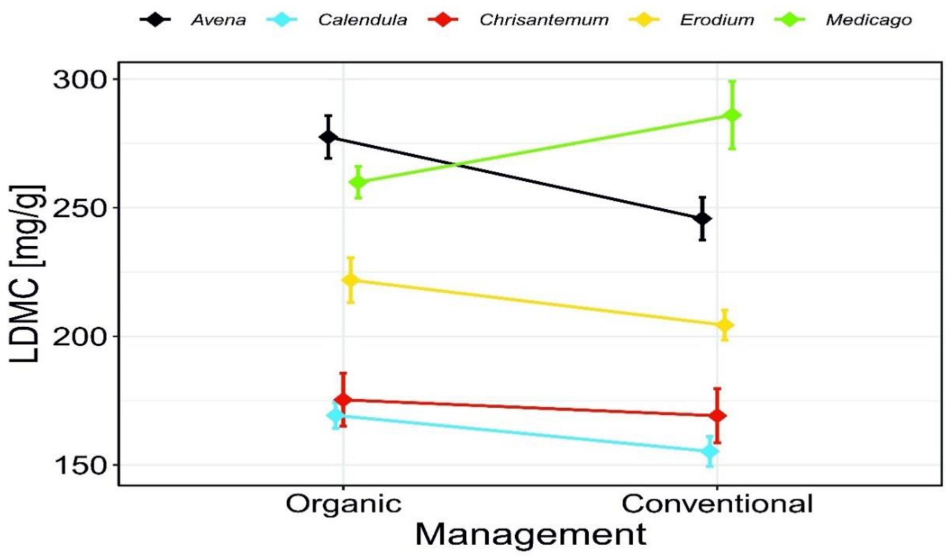

Figure 21 showing differences in LDMC values between species and management.

5.2.4 Specific Leaf Area (SLA)

ANOVA test gave high significant results for Species, Management and interaction. Overall, SLA was higher on organic plots for most of the species, as expected by hypothesis (1b).

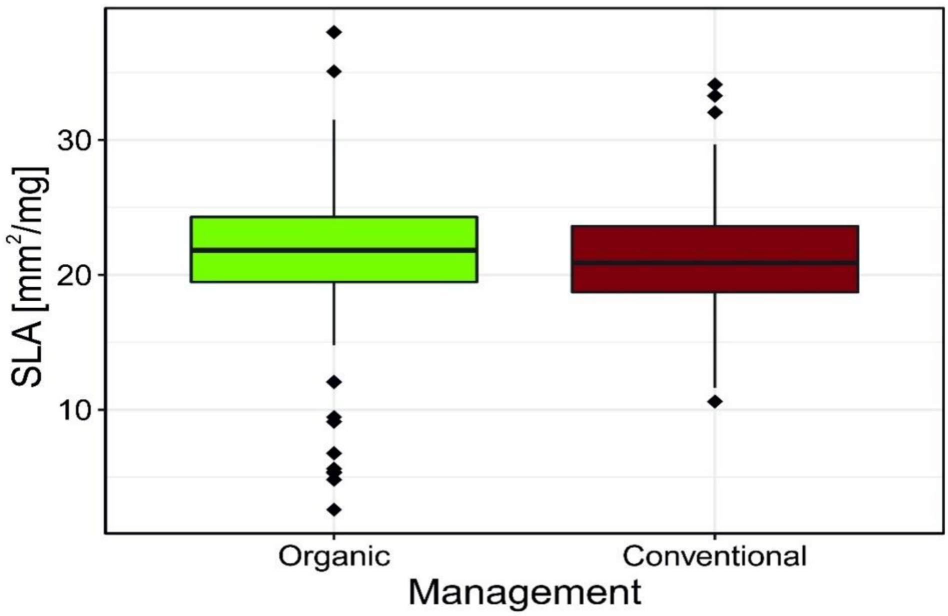

Figure 22. Boxplots showing organic and conventional management effect on SLA.

Looking at individual Species, ANOVA test was not significant for *Avena*, *Calendula* and *Erodium*. Results were significant for *Medicago* and for *Chrysantemum*. Whereas *Calendula* and *Avena* had lower SLA values on organic plots on average (although non-significant), values were higher on organic plots for the other species.

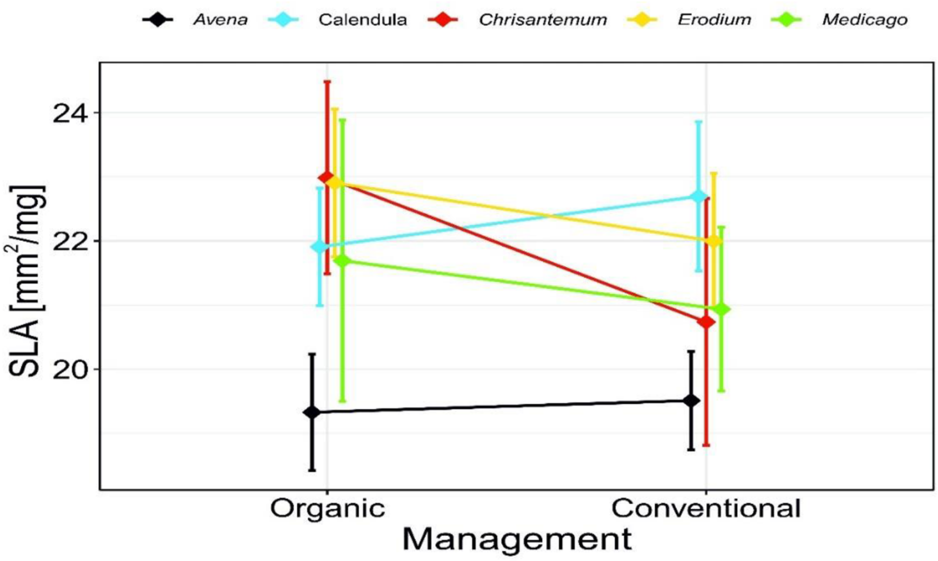

Figure 23 showing differences in SLA values between species and management.

5.2.5 Leaf area (LA)

Leaf area on organic plots appeared lower, however ANOVA test for Leaf area (LA) gave not significant results for Species and Management interaction (p=0.09).

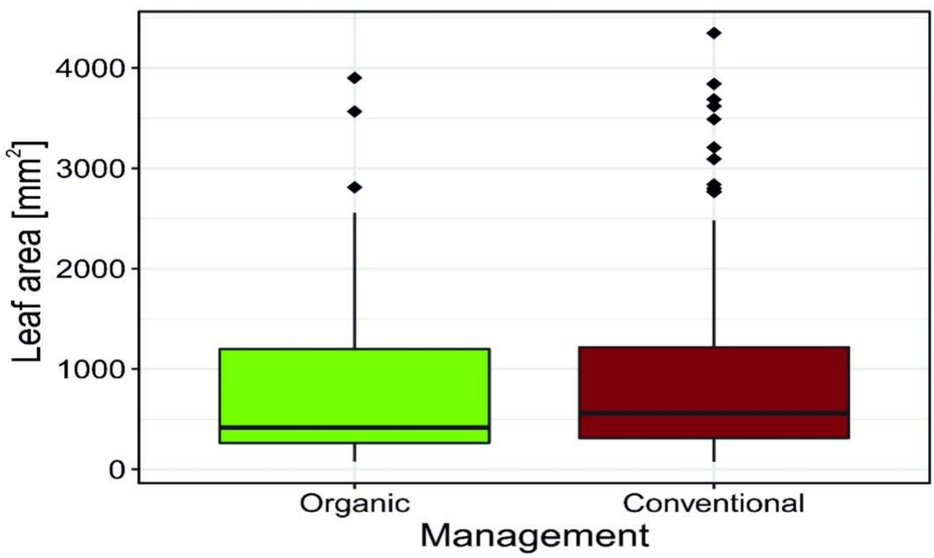

Figure 24. Boxplots showing organic and conventional management effect on Leaf area.

ANOVA results for individual species gave not significant results for *Avena*, *Calendula* and *Erodium* (p=0.1) but significant results for *Chrysantemum* and for *Medicago*.

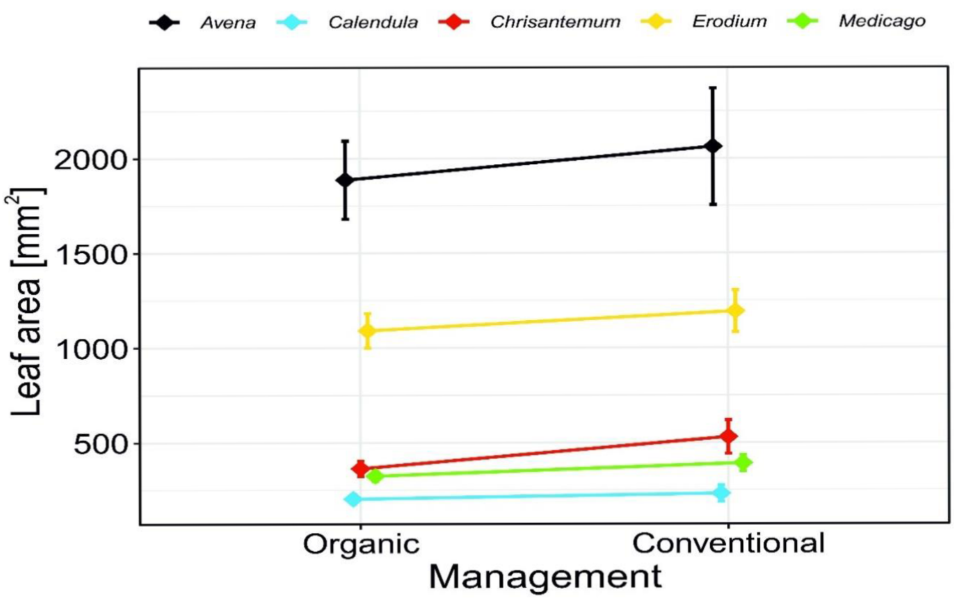

Figure 25 showing differences of Leaf area values between species and management.

5.2.6 Leaf mass (LM)

ANOVA Test of Leaf mass gave significant results for Species and Management interaction. Leaf mass was higher on conventional plots.

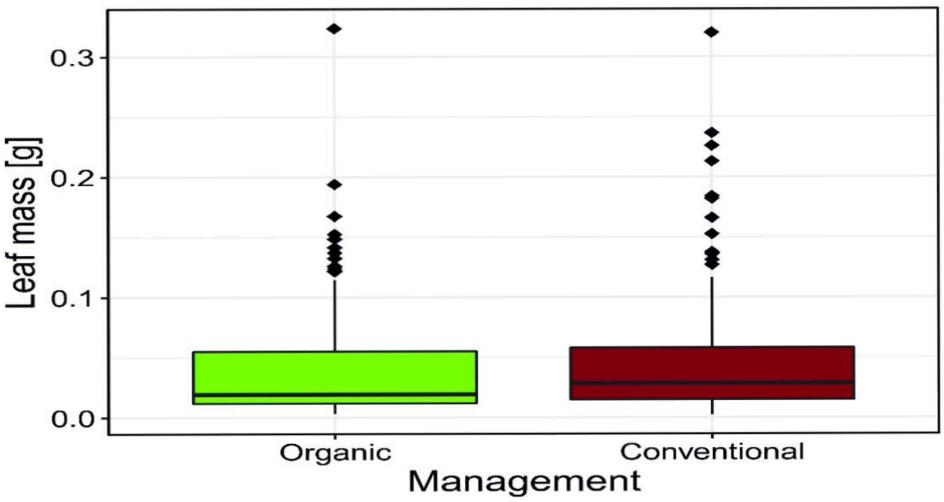

Figure 26. Boxplots showing organic and conventional management effect on Leaf mass.

Individual species analysis gave not significant results for *Avena*, not significant results for *Calendula*, but significant results for *Chrysantemum* , *Medicago* and marginally significant for *Erodium*.

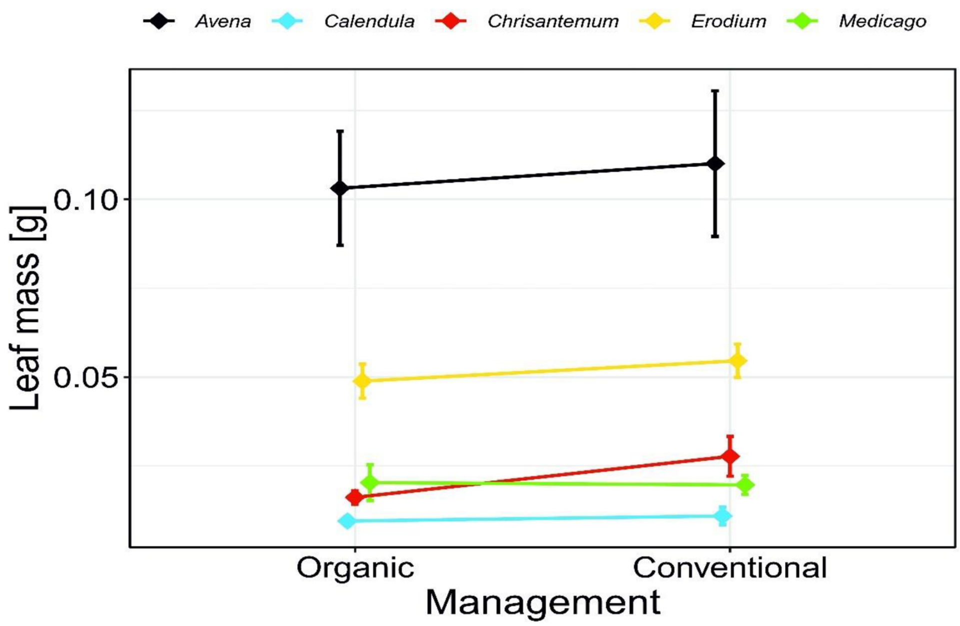

Figure 27 showing differences of Leaf mass values between species and management.

5.2.7 Correlation among functional traits

Plant Height and Leaf area resulted positively correlated, as well as Plant Height and Leaf mass. Leaf area and Leaf mass resulted highly positively correlated. Correlation among LDMC and SLA is negative, as well as that among SLA and Leaf mass, as expected by theory (ch. 1.3). It must be noticed that, despite this positive correlation, plants are taller, and leaves are smaller in organic plots compared to conventional.

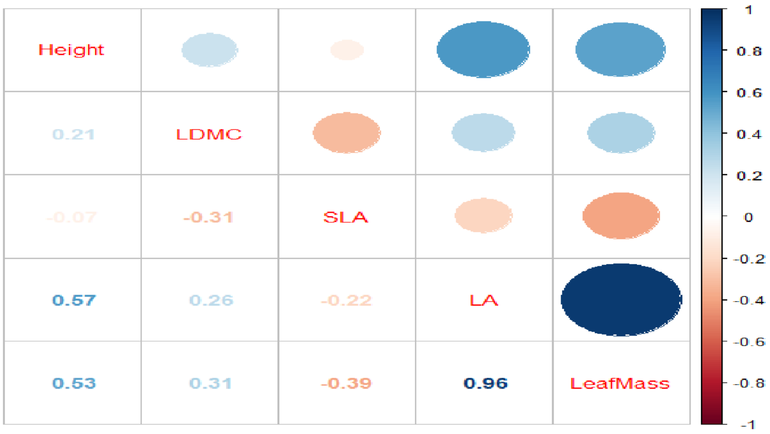

Figure 28. Correlation matrix of individual traits. Shown is correlation coefficient (R). Positive R is depicted with blue colours and negative R with red colours according to legend on the right.

5.2.8 ANOVA results of the Community-weighted-mean

Table 3 Results of ANOVA testing the effect of management on CWM of database traits.

	F	p
SLA	8.98	**0.03**
LDMC	3.16	0.10
Plant Height	5.47	0.06

Analysis of CWM of database traits partially confirmed the results on ITV (ch. 3.3.1 for species list) with higher plant height, LDMC and SLA in organic plots. However, ANOVA test gave significant results for SLA, marginally significant for Plant Height, not significant for LDMC (Table 3).

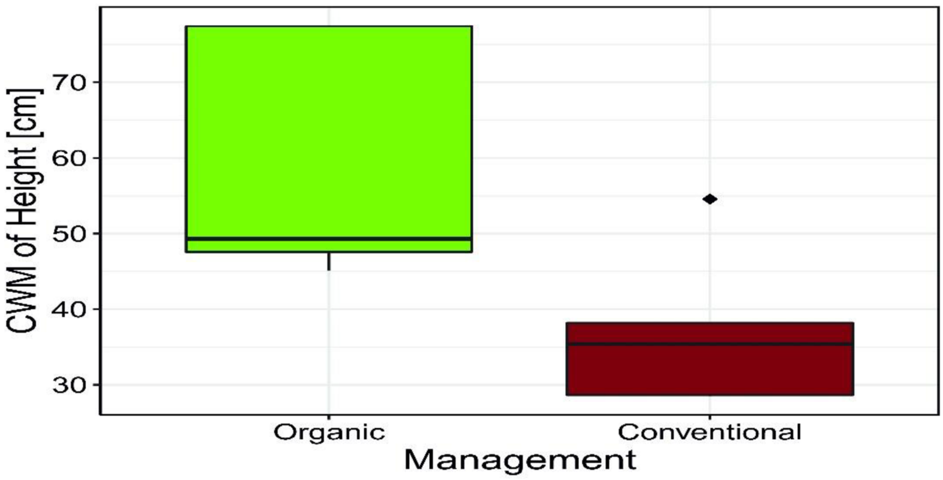

Figure 29. Boxplots showing organic and conventional management effect on CWM for height.

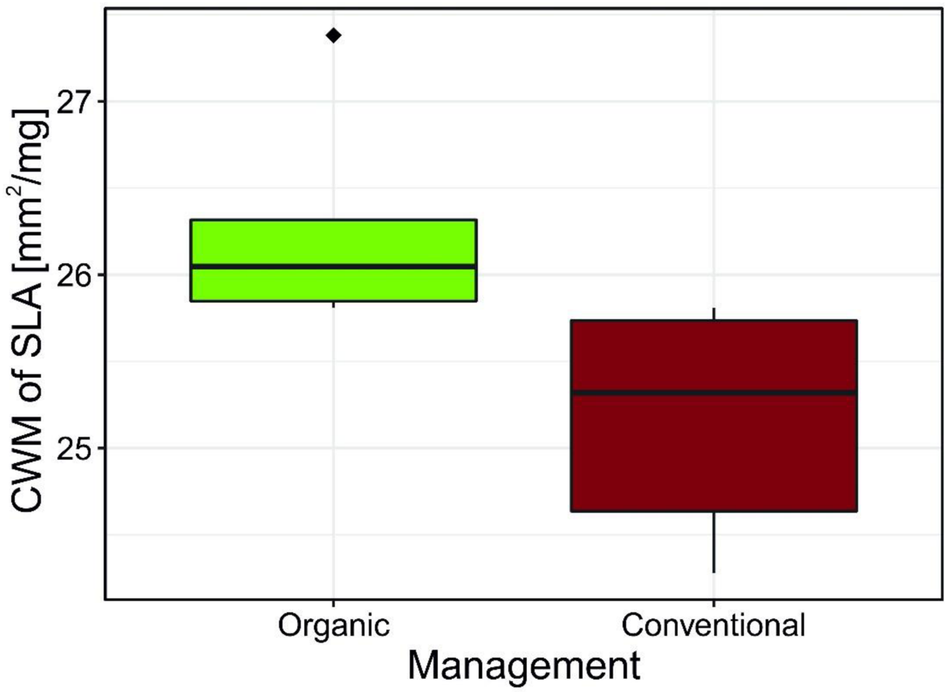

Figure 30. Boxplots showing organic and conventional management effect on CWM for SLA.

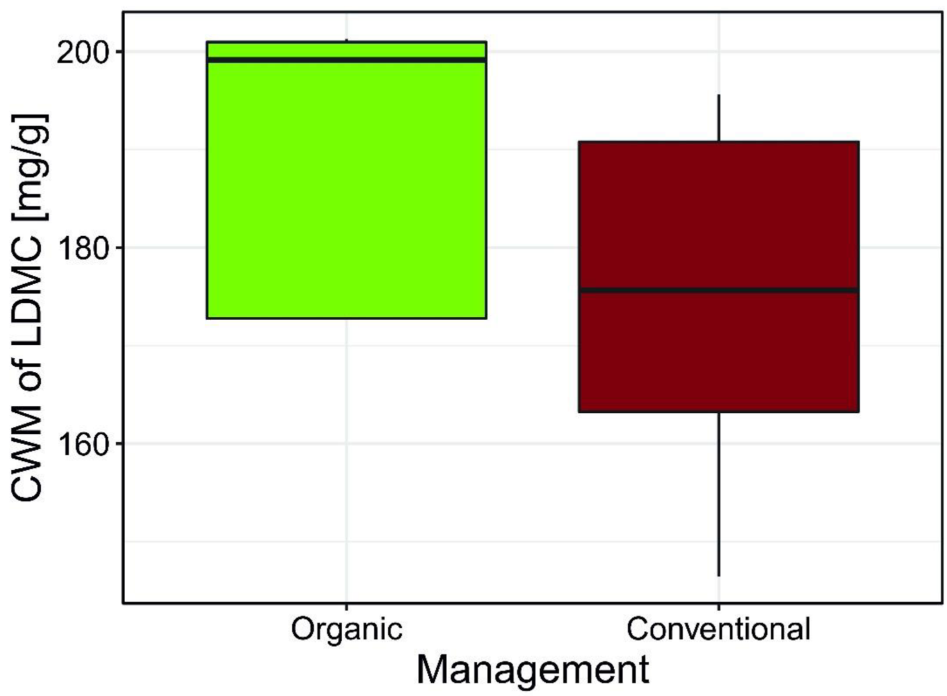

Figure 31. Boxplots showing organic and conventional management effect on CWM for LDMC.

5.3 Soil results

Table 4 Results of ANOVA testing the effect of management on soil parameters.

	F	p
pH	0.574	0.483
Electric conductivity	4.854	0.078
P	1.126	0.337
N	11.89	**0.018**
C	28.94	**0.002**

Levels of SOC were higher on organic plots, as expected by initial hypothesis (ch. 1.3).

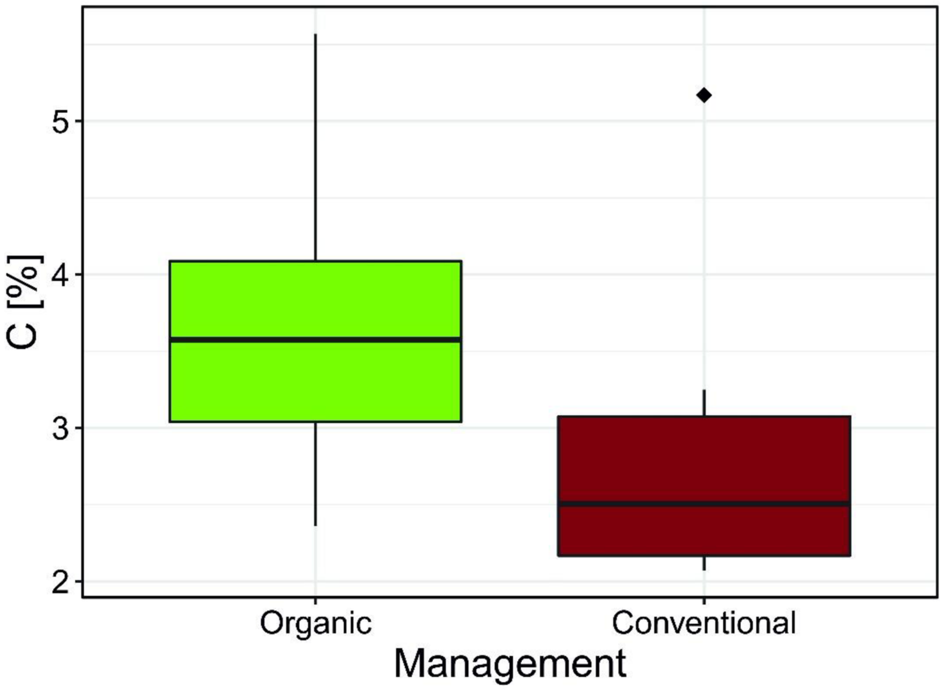

Figure 32. Boxplots showing effect of organic and conventional management on soil organic carbon (C, values in %).

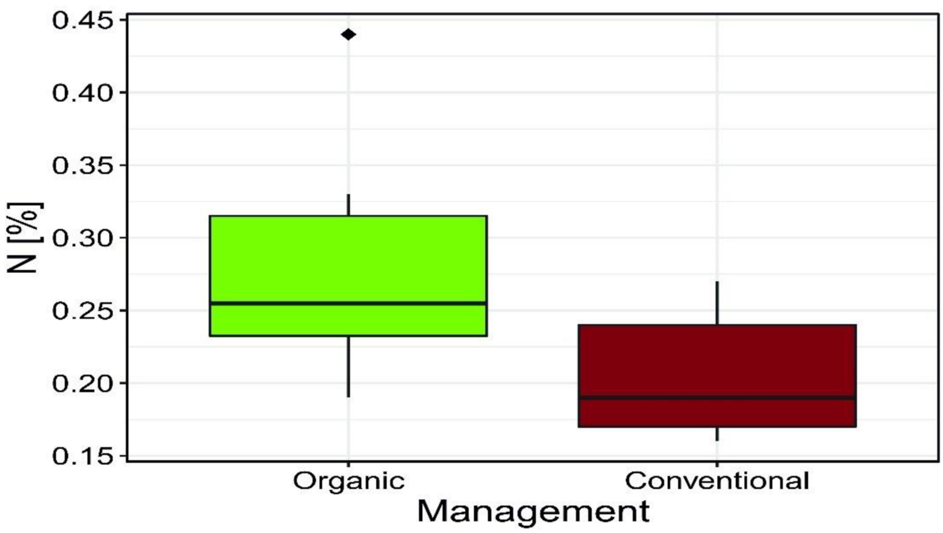

Figure 33. Boxplots show effect of organic and conventional management on total nitrogen (N, values in %).

Levels of nitrogen (N) were higher on organic plots, as expected by initial hypothesis (ch. 1.3).

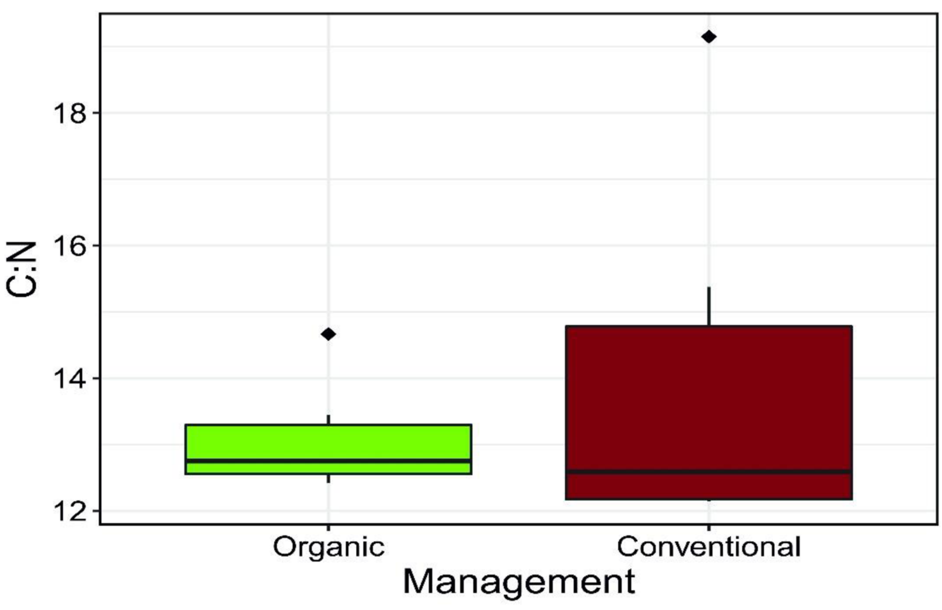

Figure 34. Boxplots showing effect of organic and conventional management on carbon to nitrogen ratio.

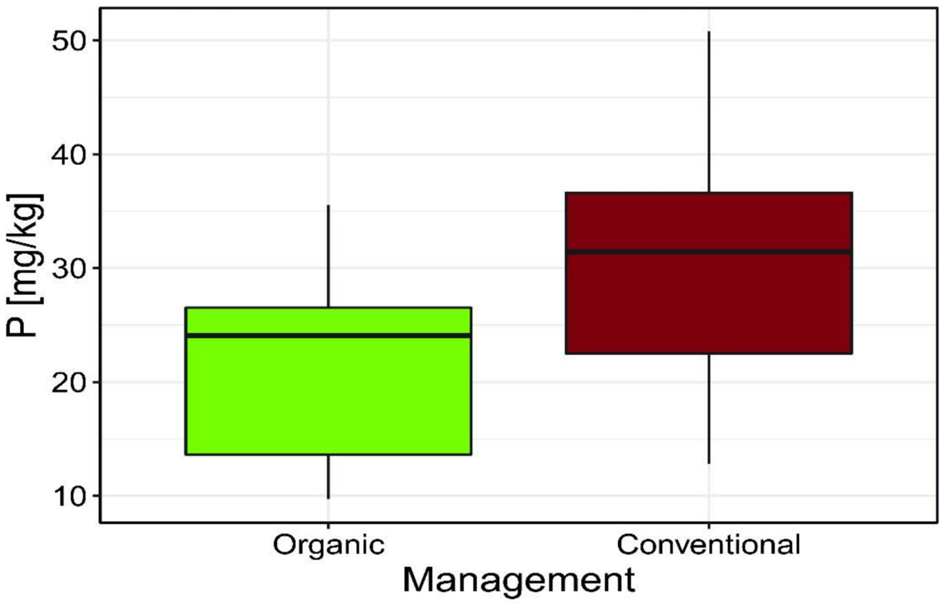

Figure 35. Boxplots show effect of organic and conventional management on available Phosphorous (P-P04, values in mg/kg).

Levels of phosphorous (PO_4) were higher on conventional management. However, Management was not significant (*p*=0.33).

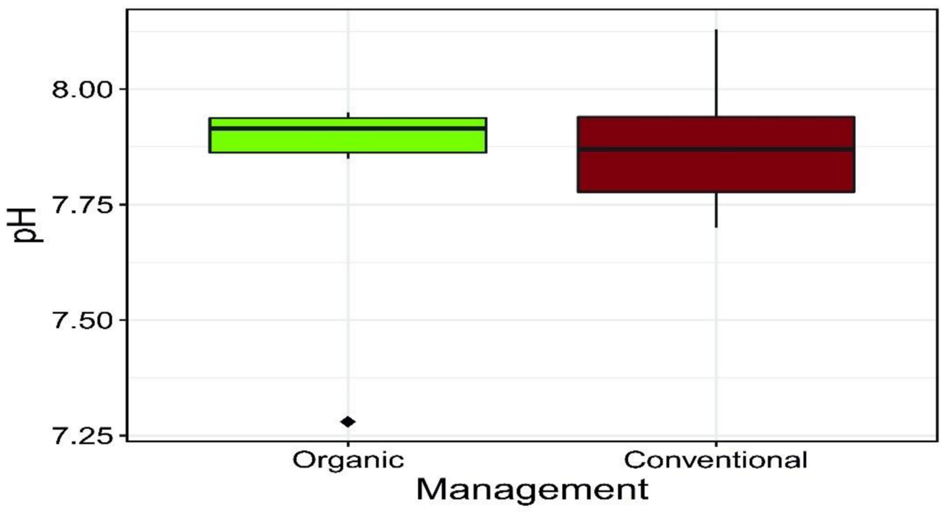

Figure 36. Boxplots show effect of organic and conventional management on pH.

pH was higher in organic plots, on average. However, management had no significant effect (*p*=0.48).

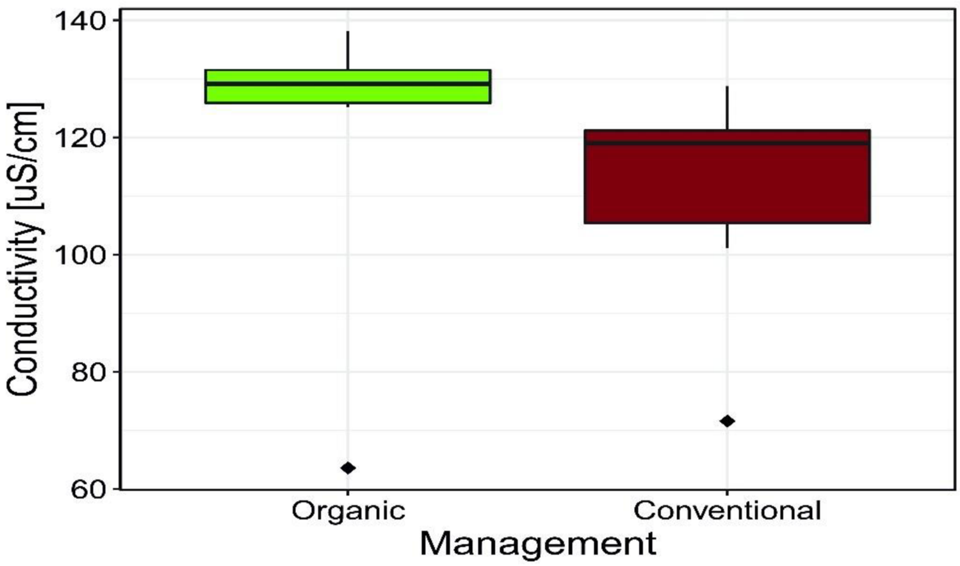

Figure 37. Boxplots depicts effect of organic and conventional management on electrical conductivity (values in uS/cm).

Electrical conductivity was higher in organic plots, on average, but management had marginally significant effect (p=0.07).

6. Discussion

To my best knowledge, very few studies have assessed the role of infraspecific trait variability in mediterranean olive plantations. A synthesis carried out until 2018 list 164 studies carried out in Italy which have used functional traits methodology. There were not found studies assessing ITV nor CWM in olive plantations. Some other studies have assessed role of ITV in grasslands which can be used to interpret results of the present thesis. Besides Italian territory, some studies have assessed ITV and CWM on different types of mediterranean environment and management and may be used to interpret present thesis results.

Species composition was found to significantly differ between organic and conventional plots (confirming hypothesis 3). Most of the selected functional traits and soil parameters have confirmed initial hypothesis (1a; 1b; 2a) but some results were unexpected. LDMC, in average, resulted significantly higher in organic plots, as well as SLA. Usually, these two traits are considered to respond in opposite ways. Apparently, this have not happened, however trait correlation (ch. 5.2.7) have shown a negative relationship among these two traits, as expected by general theory. This might be interpreted looking at specific responses of species. Some species responded to SLA; some other to LDMC and this trait have responded in a contrasting way. *Medicago orbicularis* was the only species responding to all traits and the only species which responded as expected, confirming initial hypothesis (1b). *Avena fatua* and *Calendula arvensis* have shown increased values of LDMC in organic plots, contradicting 1b hypothesis. SLA values for these species were lower in average on organic plots (a negative relationship would be expected), however results were not statistically significant and cannot be attributed to management effect. At the same time all species have grown taller in organic plots. This was expected. We may notice that *Medicago* was still higher on organic, but have grown on a less extent, compared to other species. This suggests a specific behaviour of this species.

Leaf area and Leaf mass are additional traits which have provided useful insights to put light on others. Taller plants with smaller leaves likely indicate that plants are investing in height to compete for light, rather than in maintenance of photosynthesis. Analysis of traits correlations reveal that plant height and leaf area (and leaf mass) are positively correlated indicating that plant tend to grow higher and increase leaf area simultaneously. However, the magnitude of change is higher on conventional plots, where leaves have grown larger and denser.

Taller plants are associated with augmented competition in plant communities whereas higher SLA is associated with faster growth due to enhanced photosynthesis (Hodgson et al., 1999; Westoby, 1998; ch. 1.3.2). This is generally linked with better soil (nutrients) and habitat conditions which do not limit plants' interspecific competition for nutrients but favour competition for light. The denser cover found in organic plots corroborate this idea, because higher abundance of individuals determines more competition for light. On the contrary, smaller plants are often associated with increased aridity (Nogueira & Nunes, 2018). Decreased precipitation act as a strong stress for plants, especially in the Mediterranean climate. Smaller size decrease risk for cavitation and embolism (Nunes, 2017), through reduced surface for water transpiration. In the present thesis, plants have been found smaller on conventional plots, confirming the "aridity hypothesis" (1c). Ploughing has been found associated with smaller plant size (height) by several authors (Fernandez, 1993; Nunes, 2017). Even though this seems obvious, some authors have found contradictory results. Garnier et al. (2019) have found that some life-forms (chamaephythes and hemicriptophytes) grow higher on more aridic conditions. Studying ITV of two common annuals on a steep precipitation gradient, Bergholz et al. (2017) have found that one species decreased height with decreased precipitation, whereas the other did not responded. However, these seems to be minor results, at least for what concern plant height.

SLA is considered a key-trait to study drought response, since it is linked to plant water strategy. SLA tend to decrease with reduced water availability (Poorter et al., 2009). In mediterranean environment plants tend to reduce SLA to increase water use efficiency (Wright, 2001; Ackerly, 2004). Some CWM studies have found reduced SLA with increased aridity (Ackerly, 2002; Costa Saura, 2016). Nogueira et al. (2018) have found no significant CWM changes for SLA. However, it was found to augment during a very dry year suggesting a shift in physiological strategy occurring in extreme conditions. In the present thesis, two out of five species have increased their SLA in organic plots (others have not significantly responded) which should indicate increased soil water availability. Soil analysis have provided further validation of this idea. Water-holding capacity was not directly measured but it was argued (ch. 1.3) that higher SOC levels are linked with augmented capacity of storing water in soil, which is clearly linked with reduced drought stress. Soil organic carbon was found almost one percent higher in average on organic plots (confirming 1c “aridity hypothesis”).

Some authors have revealed differences among grasses and forbs in responding to drought. In a study assessing ITV in temperate and mediterranean environment, have shown that they respond in opposite ways to extreme drought manipulation (Wellstein et al., 2017). Whereas grasses have shown decreased SLA in temperate region, they have reacted increasing SLA in sub-mediterranean region. Forbs have not responded to drought stress in the temperate region but have decreased SLA in mediterranean drought plots. Forbs have reacted in a similar manner in the present thesis. Of the species responding to SLA, only two forbs have decreased SLA in conventional plots. This may be interpreted as faster growth occurring in organic plots. Grasses (*Avena fatua*) have not responded significantly to SLA. However, *Avena fatua* has responded increasing LDMC in organic plots. Higher LDMC is generally interpreted as plant response to increased drought stress (ch. 1.3; see also Majekova et al., 2021). This result is unexpected and deserve attention since this species was dominant in organic plots (see RDA, fig.16, ch. 5.1). Moreover, it was the tallest and most abundant plant in both management. This means that this plant is responsible for high above-ground biomass production, but since leaves had higher LDMC content in organic plots, decomposition rate (K) is slow, due to low herbivores palatability (Garnier et al., 2007). This has further consequences on soil, because low K rates determines high carbon accumulation (Kardle & Wardle, 2013).

These results are akin to what has been found by Garnier et al. (2007) in a study investigating grassland species along a gradient of decreased management intensity. The study, carried out in four European sites and one Israelian site, have considered different climate, from oceanic to temperate to mediterranean arid. Plants were found to be taller and with higher LDMC in lower intensity sites. Soils were found higher in SOC and total nitrogen. Authors suggest that plants have adopted resource-conservation strategy. Interestingly, Nunes et al. (2017), have found on mediterranean increased aridity gradient that plants augment LDMC but reduce plant height, suggesting these traits are not necessarily correlated. Similar to Garnier and present thesis results have been found also by Helm et al. (2018), where after agricultural abandonment grasslands species responded with higher LDMC, higher plant height, even though lower SLA compared to fallows. Studying ITV in mediterranean grasslands in italian Appennini mountains, Targetti et al. (2018) had lower LDMC levels of three grass species with increased management intensity (grazing). This confirm link among fertilization (manure by animals) and reduced LDMC, although LDMC have been found higher than expected in some high-intensity plots. Investigating ITV in mediterranean grasslands under extreme drought manipulation, Jung et al. (2014) have found a general tendency for increased LDMC with drought, but five out of thirteen species responding in opposite direction. He suggested species-specific response to drought.

Considering the type of management carried out on organic plots, results suggest a very low disturbance to vegetation. Probably vegetation is mown too sporadically to allows fast nutrient cycling of residues. Moreover, chopping of pruning residues (although sporadically practiced) on one hand may increase plant nutrients on the long run but may generate nitrate depression on the short-term (Brady & Weil, 2017). The absence of animal grazing and low rate of mowing slow down nutrient cycling resulting in organic carbon accumulation (Targetti et al., 2018).

A study carried out in a mediterranean dry vineyard confirm tendency of increased LDMC with mowing (Guerra et al., 2021). Interestingly, this study suggest that ploughing may provide better nutrient availability, since higher SLA was associated with this management. Ploughing is considered as a severe disturbance (ch. 2.3.2; 2.4.5) but may have secondary effects of increased SOM mineralization due to augmented oxygen levels in soil. This may provide enhanced plant nutrients. Ploughing should be associated with stress-avoidance plants (ruderals) with high SLA (Fernandez et al., 1993; Nunes, 2017).

Higher LDMC and higher SLA in organic plots contrast with expectations but may be interpreted considering specific response of each species. *Chrysantemum* and *Medicago* appeared to grow faster on organic plots. *Medicago* response can be interpreted as "independent", on a certain extent, from the community since it is a legume and may be self-sufficient for nutrients. Legumes are phosphorous-efficient plants (Sulieman, 2015; Ghosh et al., 2005) and its *synorhizobium* symbiosis (ch. 3.2.2) may provide enough nitrogen despite slow nitrogen mineralization to organic pool.

There is consensus over the idea that fertilization is associated with increased SLA (Nunes, 2018). Studies carried out in mediterranean annual grasslands confirm this hypothesis (Carmona et al., 2019). For present thesis results, there are no indications that sustain conventional management to provide a nutrient-richer habitat, but rather the opposite (since SLA values were found higher on organic plots).

The most important result concern SOC levels, which strongly allows to support the idea that organic management provide much higher carbon sequestration than conventional management. Moreover, LDMC values allow to argue for reduced soil carbon loss (Kardle & Wardle, 2013). Nevertheless, we must consider that SOC levels on conventional plots were above desertification threshold. Therefore, it would be hazardous to conclude that this management predisposes

agroecosystems to desertification risk. Higher total nitrogen levels were found on organic plots. This is not itself a guaranty of improved fertility since carbon to nitrogen ratio is not assessed (ch. 1.3). C/N ratio have been found slightly higher on organic plots (although test was not significant for management). Moreover, we measured total N, which might not be directly related to N available to plants (Brady & Weil, 2017). Phosphorous levels found higher in conventional plots, although non-significant. This result is typical in conventional agriculture since it has been a tendency to over fertilize fields to build up phosphorous levels in soils (ch. 1.3). pH levels were slightly more alkaline on organic plots, although non-significant. This do not suggest a role of management in increased pH, even though Garnier et al. (2006) have found a similar pattern of increased pH and increased C and total nitrogen with reduced agricultural intensity. Electrical conductivity should be associated with higher cation concentrations in organic plots; however, results were unsignificant, therefore difficult to interpret as signs of higher fertility.
On a general consideration, may not be surprising to see species-specific response in studies assessing ITV role in ecosystems. It must be noticed that CWM results (ch. 5.2.8) highlighted the same trend of ITV. Higher SLA in organic plots for the whole community additionally confirm the idea of better habitat conditions in organic management. Organic plots had higher LDMC for the whole community, however results were not statistically significant. It can be concluded that intraspecific analysis has revealed a species-specific response, suggesting an important role of plantations' understory grass in shaping soil conditions. Although not always significant, CWM trend shows that organic plots should provide higher soil fertility and reduced drought stress.

Conclusion

Results shown in the previous sections have revealed that initial hypothesis have been generally confirmed. However, some unexpected results have raised questions which demand further investigations. Organic management appears to provide more carbon sequestration and water availability, which are crucial factors in regions at strong desertification risk. On the other hand, it is not entirely clear whether this management provide a better plant nutrition. Nevertheless, organic management is just a wide category; how it is carried out on specific environmental conditions depends on many factors, among which owner's knowledge and ability plays a central role. The degree of disturbance to vegetation exerted by farmers should determine different outputs.

To my best knowledge, this is the first study assessing how agricultural management affect infraspecific trait variability of grasslands in olive plantations. Therefore, it will be necessary to provide replications of this methodology in different soil and climate conditions to validate whether organic management is more sustainable. Further studies might take into account a) factorial design to disentangle specific contribution of different agronomic practices (herbicides, ploughing, fertilizers, pesticides); b) study of MAOC and POM fraction of organic matter might be considered to verify correlation among higher LDMC and carbon accumulation in soil (Grilli et al., 2021); c) sampling 10-30 cm (in addition to 0-10 cm) might be considered to avoid carbon sequestration bias (induced by canonical sampling; Plaza-Bonilla et al., 2010); d) assessing sodium (Na) levels in semi-arid agroecosystems might provide useful insights on structure, fertility and water-storing capacity of soils (Brady & Weil, 2017).

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Appendix. Description of the farm and Management of the agroecosystem

I choose “Torre dei Mastro farm” as centre for this study. This is an organic farm (not certified) which also adopt regenerative agriculture (RA) techniques[10]. The farm is spread on about 10 hectares of land. The main income is “extra-virgin” olive oil which comes by their secular olive

[10]Regenerative agriculture has been proposed in recent years following good experiences in conservation agriculture (CA) in US

plantations[11]. Secondary income comes by almonds and cherries. These kinds of crops are not divided into monocultures but as a mixed polyculture of which olive constitute the dominant tree. About 30 olive cultivars (varieties or sub-species) are grown in the farm. Cultivars were grafted onto “rootstock” obtained by seeds collected by farmers as tradition. Most of the olive trees are old secular trees with a not-defined age which can be also more than 500 years up to 1000 years for some individuals[12]. The harvest is done manually with “olive harvesters” but not with heavy machineries like tractors which shake trees to make olive fall on the ground. The farm owns two TPR horses which are used for heavy work in addition to machinery.

The undercover of the plantation is an herbaceous layer. It is kept growing all the year around, generally without manure addition by horses. This grassland is mowed once or twice across the year depending on rainfall. It is done for several reasons: 1) horses’ fodder; 2) facilitate farming works like pruning, harvesting, etc; 3) avoid spontaneous succession and maintenance of the agroecosystem (without some form of disturbance of the grassland it would evolve into a secondary forest).

Pruning is a fundamental practice for the maintenance of the olive plantation. Several types of pruning exist depending on the region and olive cultivar. The one used in the farm is called “inverted cone”. It is done not necessarily every year and requires the pruning of 30-50% of the canopy, generally from January to June. Pruning leftovers are shredded in site and not removed. This results in a source of carbon which is added to soil and might contribute to plant nutrients as well as soil structure amelioration. In the past agrochemicals were part of the management of the farm. Since about twenty years, with the new generation (son), no pesticides, no herbicides and no synthetic fertilizers are used. The owner makes himself some “bio-fertilizers” to assist olive nutrition during growing period. This is done with in-site organic materials fermented anaerobically into sealed bins.

[11] Extra-virgin oil is obtained by olives harvested in young stages. This provides a higher level of polyphenols which are important antioxidant substances. It is usually a lesser sweet oil compared to the virgin one, which was more typical of Salento some decades ago (southern sub-region of Apulia, the heal).

[12] I got this information by landowner.