

UNIVERSITÀ DEGLI STUDI DI PADOVA

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Functional diversity of sown and spontaneous service crops and their impacts on the soil, the
vine and biological regulations in an agroecological vineyard

Supervisor

Dr. Davide Scaccini

Co-supervisors

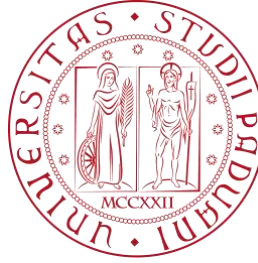
Dr. Léo Garcia

Dr. Elena Kazakou

Graduate student

Victória Dutra de Oliveira Tomás

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Relatore

Dr. Davide Scaccini

Co-relatori

Dr. Léo Garcia

Dr. Elena Kazakou

Laureanda

Victória Dutra de Oliveira Tomás

Anno Accademico 2023/2024

ABSTRACT

Viticulture is one of the oldest forms of agriculture, its global significance enhances the concerns arising from the impending climate changes, making the search for sustainable and ecologically sound management techniques crucial. Aligned with this necessity, the integration of service crops in vineyard management strategies has become a growing topic due to their capability to drive ecosystem resilience by promoting soil quality, facilitating biodiversity conservation, and hosting biological control agents. This study investigates the impact of agroecological management systems in a Mediterranean vineyard in two consecutive years. Plant community functional traits were measured, along with root markers and indicators for soil quality, vine vigor and biological regulation. The study demonstrates that vineyard management system significantly influences plant community functional traits. The employment of soil tillage reduces biomass production, taxonomic and functional diversity favoring more ruderal species. Agroecological systems while reducing vine vigor, selected plant communities with traits that contributed to the presence of natural enemies and to soil structure and stability. Our findings highlight the potential of agroecological practices to enhance vineyard sustainability by promoting biodiversity, supporting ecosystem services and ensuring the longevity of the sector

Key words: Agroecology, Vineyard, Service Crops, Ecosystem Services, Functional Traits

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1. INTRODUCTION

1.1 Wine and grape production

Wine and grape production has been a part of human history for millennia, making viticulture one of the oldest forms of agriculture. From ancient to modern populations, viticulture remained a pillar of the cultural heritage. With its wide adaptability, vineyards cover 7.2 million hectares worldwide (OIV, 2023) with the capability of thriving in various latitudes and climates. Products of this agricultural sector, such as wine, play a key role in numerous economies, promoting international trade and tourism, enriching the economy and cultural exchanges. France stands strong in this scenario, with 20% of the global production of wine (OIV, 2023).

Viticulture's global significance enhances the concerns arising from the impending climate changes. Temperature variance, precipitation irregularities and the increased frequency of extreme weather events raise concerns surrounding the income of many (Nabhan et al., 2020). This scenario is especially important in the Mediterranean region where rainfall and drought events are predicted to become more intense (IPCC, 2023), requiring adaptations from the agricultural production systems. Additionally, wine consumers have been favoring environmentally responsible products (Schäufele and Hamm, 2017). With the growing consciousness of the population about environmental issues, social implications, and the consequential prioritization of sustainable products, changes in the agricultural sector are expected to reflect the values of those who support it.

1.2 Limitations of the traditional management

The traditional management of vineyards is characterized by intensive use of pesticides and soil tillage; however, it has been increasingly questioned due to its detrimental effects on the environment, including soil microbiota, plant nutrition, wine quality and human health (Chou et al., 2018; Morozova et al., 2017; Zaller et al., 2018; Mailly et al., 2017). This has heightened the interest for sustainable management practices that can promote production while also protecting the environment, conserving soil, water, and energy.

Vineyards commonly employ tillage due to its effect on improving vine vigor and yield, with the promotion of better water availability and decreased competition for resources (Cruz et

al., 2012). However, tillage-induced soil erosion is a growing issue that leads to the removal of soil horizons and accumulation of sediments and nutrients, increasing spatial variability (Oost et al., 2006). It is particularly problematic in vineyards, due to its usual location in slope areas, with rows planted along the slope. High intensity of tillage operations in a less than desirable direction together with topographical characteristics facilitates soil erosion (Gristina et al., 2022).

Synthetic phytosanitary treatments in agriculture involve a broad range of products, including herbicides, insecticides, fungicides, seed treatments, and others. For a detailed map illustrating pesticide use across French agricultural land, refer to Annex A.

When considering pesticide use, vineyards are one of the most intensive agricultural sectors (Urruty et al., 2016). In 2016, French vineyards showed an average of 20 treatments (Simonovici, 2019), with approximately 80% involving fungicides, 3-26% insecticides, and minor proportion of herbicides (Mailly et al., 2017). These intensive treatments are due to the significant pest and disease pressure in vineyards, particularly from fungi. Fungal pathogens are the causal agents of downy and powdery mildew, which can lead to total grape loss in years with high disease pressure (Fermaud et al., 2016). These challenges highlight the importance of the development and adoption of resistant varieties.

Although insecticide applications are not the most frequent, the use of broad-spectrum insecticides remains common in vineyards (Mailly et al., 2017). However, with the adoption of biological control agents increasingly rising, the use of such products puts at risk the populations of beneficial insects that would otherwise keep pest populations under control. This is particularly true in the case of predatory mites, such as Phytoseiidae mites, and the resistant pest spider mites, *Tetranychus urticae* (Wilson, 1998). Moreover, the use of pesticides is a serious health concern (Baldi et al., 2012; Rhaerison et al., 2019). Pesticides have been shown to negatively impact grapevine performance by limiting photosynthetic processes (Petit et al., 2008) and also contributing to soil erosion and biodiversity loss (Cerdeira et al., 2021; Keesstra et al., 2019).

Furthermore, strategies employed in the conventional management of vineyards, including intensive soil tillage and herbicide application, have been shown to affect biodiversity and environmental services (Winter et al., 2018; Guerra and Steenwerth, 2012). The drive for higher yield has promoted agricultural systems based on monoculture, landscape simplification, and diminished biodiversity (Grant, 2007). These practices show undesirable effects on parameters such as soil erosion, pest invasions, and soil fertility (Foley et al., 2005; Russo and Smith, 2013).

1.3 Agroecological management

The design of more resilient grapevine production systems is intricately connected to agroecology, which applies ecological principles with a focus on biodiversity, bringing a sustainable approach to production systems (Altieri, 2019). Biodiversity concept has developed into an ecological, social and economic topic, providing further knowledge on how people benefit from nature's services.

Ecosystem services (ES) are defined as “conditions and processes through which natural ecosystems, and the species that make them up, sustain and fulfill human life” (Daily, 1997). They can be seen as a consequence of biodiversity, playing an important role in regulating the environment, contributing to human well-being by providing recreation and possessing cultural and religious significance. ES are classified into provisioning, regulating, cultural and supporting services (Haines-Young and Potschin, 2010). Provisioning services refer to goods that can be harvested and consumed, regulating services encompasses modulators of conditions such as climate and soil, cultural services are recreational and supporting services are essential ecosystem processes like photosynthesis and nutrient cycling (Balvanera et al., 2017).

Agroecological principles are based on traditional methods. Through diversification, conservation, higher resilience, and stability, agroecological systems can support agrobiodiversity's longevity (Altieri and Nicholls, 2020; Ploeg et al., 2019). Systems that employ these principles have been recorded to present higher efficiency in supplying a wide variety of ES (Altieri et al., 2015).

Aligned with the growing consumer preference for environmentally conscious products (Pomarici et al., 2016) and European Union incentives for environmental sustainability (European Commission), intercropping has become an increasingly significant topic. Vegetation management, such as intercropping, can support sustainable production, positively impacting the ecosystem, soil, and grapevine production. With broad benefits to the environment and contributing to a balanced vineyard ecosystem, intercropping can play a key role in dealing with the adversities stemming from climate change.

Service crops are commonly referred to plants grown without the purpose of using their direct production, but for the ecosystem services they can provide (Garcia et al., 2018). Intercropping with service crops can supply a multifaceted approach to sustainability.

1.3.1 Benefits of service crops

Starting from the ground up, soil is the foundation upon which crops grow, a key component of the production system, particularly in viticulture where the quality of wines is closely associated with the interactions between climate, soil and vine (van Leeuwen and Seguin, 2006). Soil quality is defined by Doran and Parkin (1994) as “the ability of a soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health”. Therefore, soil is also a source of sustainability for the biosphere (Bastida et al., 2008).

Service crops can support the improvement of soil quality by enhancing soil biological activity (Ramos et al., 2010) and supporting carbon and nitrogen content (Abad et al., 2021; Ramos et al., 2010). Intercropping can also promote underground diversity that enhances nutrient availability, and crop productivity (Martin-Guay et al., 2018) improving nutrient uptake from the soil due to enhancements of the rhizospheric interactions among plant roots (De Conti et al., 2019). These plants play a vital role in soil erosion prevention (Prosdocimi et al., 2016), they reduce the risk of compaction (Polge de Combret-Champart et al., 2013), increase infiltration rates (Gaudin et al., 2010) and aggregate stability (Abad et al., 2021; Le Bissonnais et al., 2007). In alignment, with the reduction of surface water and run-off, they contribute to the prevention of potential pesticide flow and contamination of water sources (Andrieux et al., 2007; Alletto et al., 2010).

In addition to soil benefits, service crops drive ecosystem resilience by facilitating biodiversity conservation (Teasdale, 1996). The adoption of low-competitiveness service crops can be an alternative to chemical herbicides and tillage (Jordan et al., 2016; Karl et al., 2016), promoting weed suppression (Moonen and Barberi, 2008), reduction of greenhouse gas emissions (Abad et al., 2021) and agronomic resilience in climate extremes (Power, 2010).

Service crops also favor the presence of pollinators (Kehinde and Samways, 2014), and host biological control agents (Shields et al., 2016). They provide beneficial predators alternative food sources such as pollen and nectar, supporting their presence even in the absence of prey. It is the case of Phytoseiidae predatory mites, which are naturally present in agroecosystems and capable of controlling pest mites and other small arthropods (McMurtry and Croft, 1997; Gerson et al., 2003). Additionally, service crops can reduce the need for chemical interventions, which follows production and consumption trends, helping the maintenance of naturally occurring pest

enemies (Tixier, 2018; Moonen and Barberi, 2008). Predatory mites' dispersal is also influenced by factors, such as temperature, humidity, food availability (Sabelis and Dicke, 1985) and canopy connectedness (Tixier, 2018), which are enhanced by intercropping.

In a production aspect, grapevine is positively affected when experiencing moderate water stress after the flowering stage. Service crops can therefore support the production, both yield and quality, by inducing this favorable competition for water and limiting the grapevine vegetative development (Gaudin et al., 2014; Pellegrino et al., 2006).

1.3.2 Challenges

Agriculture occupies a unique position, balancing between providing ES and generating ecosystem disservices (EDS). Service crops, while beneficial in many ways, can also enhance certain EDS, and potentially their inclusion in an agricultural system can affect the commercial crop.

Many vineyards located in rainy climates, or employing irrigation, have already included intercropping strategies in their management techniques (Monteiro and Lopes, 2007). However, in areas where the water supply is not abundant, there is still resistance from growers to adopt these plants in their vineyards. Summer with low precipitation combined with a semi-arid climate can affect grapevine yield in the year of occurrence and the next one (Guilpart et al., 2014). Concerns can also stem from their coexistence with the commercial crop and the possibility of competition for soil resources (Celette et al., 2008) affecting the grapevine performance (Monteiro and Lopes, 2007; Winter et al. 2018). The presence of service crops can also affect the humidity in the field and facilitate the risk of frost at the beginning of spring (Sánchez et al., 2007) and serve as shelter for grapevine pests (Hanna et al., 2003).

Regardless of the documented benefits outweighing the possible cons (Guerra and Steenwerth, 2012), there is still a struggle to promote the entrance of service crops in vineyards, especially in drier climates (LaRose and Myers, 2019) and scenarios with water scarcity during spring and summer (Delpuech and Metay, 2018).

1.4 Taxonomic and functional diversity of service crops

Worldwide we experience a loss in biodiversity, bringing its conservation to the front line of agri-environmental policies and measures (European Commission, 2005). Whilst biodiversity is mainly associated with taxonomic diversity, including individual species in a natural or semi-natural environment, it is also important to consider its role in regulating ecosystem functions. Functional biodiversity is defined as the biotic components that stimulate the ecological processes driving the agroecosystem and providing services (Altieri and Nicholls, 2018). Functional biodiversity considers morphological, physiological and phenological features measurable at the individual level (Violle et al., 2007). These features are closely connected to functions related to ES (Garnier et al., 2016, De Bello et al., 2008), and can be extended to a community level.

Above ground traits of plant communities such as specific leaf area (SLA) and leaf dry matter content (LDMC) can help reflect plant strategies for resource acquisition and adaptation to environmental stress (Díaz and Cabido, 1997; Cunningham et al., 1999; Kazakou et al., 2009; Cortez and Pérez-Harguindeguy, 2007). Below ground structures are essential for plant development and function, with a direct effect on the ecosystem services provided (Gregory, 2006; Freschet et al., 2021). Root markers are capable of shedding light into plant communities' strategies for resource acquisition and their contribution to the environment. Traits such as density and length can reflect the roots' influence on soil biotic and chemical properties (Lange et al., 2015).

A combined approach of taxonomy and functional traits can promote a deeper understanding and representation of the biodiversity present in plant communities and how they interact providing services or disservices to the ecosystem.

1.5 Purpose and research goals

As the climate conditions continue to require higher resilience from the vineyard, the agricultural management must change to achieve their products' longevity and commercial viability. Nevertheless, the interaction and competition for resources between service crops and grapevine is very complex and not fully understood. Growers must adapt their management strategies according to a multitude of factors. Consequently, there is a pressing need for further

comprehension of these interactions to determine the optimal scenario that enhances the vineyard ecosystem.

The present study aims to highlight how different vineyard management strategies can affect plant diversity, community dynamics, ecosystem regulations and grapevine vigor. With three different management strategies in a diversification gradient, this study explores the taxonomic and functional diversity of the vegetative cover, measures indicators for grapevine vigor and for ecosystem services between two consecutive years. It hypothesized that: (i) the taxonomic and functional structure of plant communities reflect their abilities to use available resources; (ii) soil management practices shape functional traits of plant communities best suited to the soil conditions; (iii) the plant community competes with grapevine for resources, impacting its vigor; (iv) systems that are less disturbed and with more diverse plant communities have positive effect on soil quality and biologic regulation indicators.

This study incorporates research data obtained in 2023 by former intern Laure Martin-Lefevre. It also contributes to the ongoing research of PhD candidate Lou Tabary.

2. MATERIAL AND METHODS

2.1 Experimental site and design

The experimental vineyard is located within the Domaine du Chapitre, in Villeneuve-Lès-Maguelone (43°31'50.46"N 3°52'05.95"E) in the vicinity of Montpellier, in the Occitanie region of France. The region is located within the Mediterranean climate, classified as “Csa” according to Köppen and Geiger, with average annual temperature of 15.9°C and precipitation of 526 mm (Delannoy et al., 2022). The soil texture is classified as silty-clay-loam, with pH of 8.2.

The experiment was established in 2018 with a split-plot-like design with three management systems. Each system has three repetitions referred to as “blocks”, within each block 5 plots are allocated for sampling and measurements (Figure 1). All the systems have double cordon trained vines.

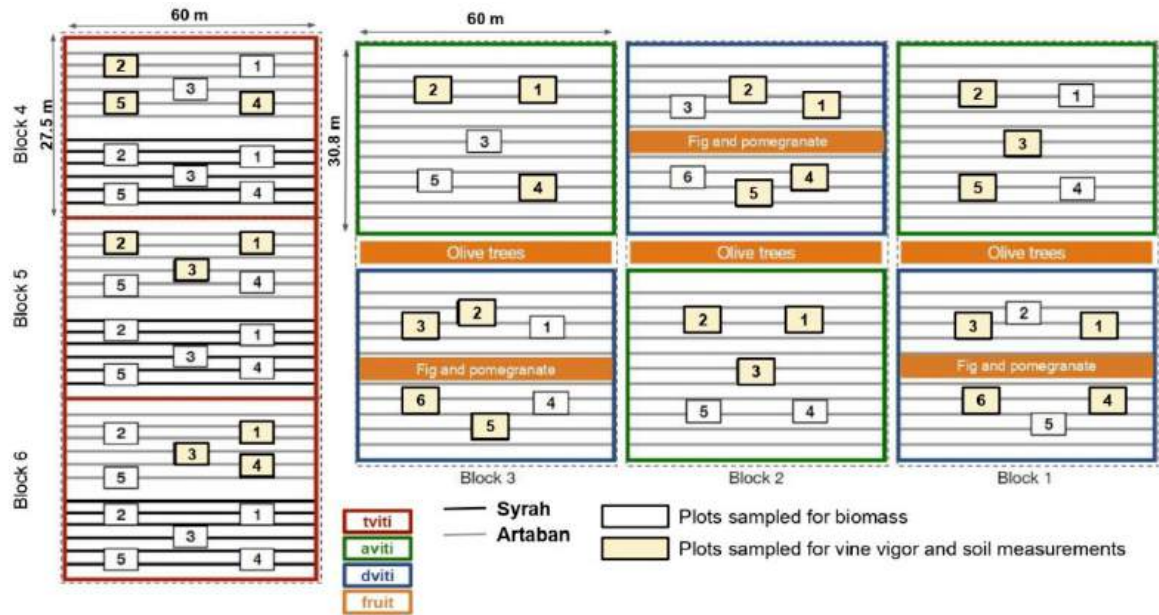


Figure 1. Experimental design with study plots and systems

The control system (TVITI) includes soil tillage within and between rows and no soil cover. The TVITI system has two grape varieties present: Syrah and Artaban (a resistant variety to downy and powdery mildew), only the latter was considered for this study.

The other two systems are referred to as the agroecological systems (AE), differentiated based on the sowing of service crops and the presence of trees. In the first agroecological system, AVITI, no tillage is employed and the inter-rows (2.8m) were sown with different service crops mixes in autumn of each year. The species of service crops were chosen to provide diversity of botanical families, growing cycles and behavior (Table 1). Each AVITI block has 12 rows of 60 Artaban variety vines, following a density of 3.600 vines hectare⁻¹.

The third system, DVITI, is integrated with agroforestry principles. In this system, the four central grapevine rows are replaced with two rows of fruit trees (fig and pomegranate) planted 2.8m apart, and 5.8m away from the grapevine, with a density of 890 trees ha⁻¹. Soil tillage is done within the rows but not on the inter-rows. No cover crop has been sown in this system since autumn of 2021, the vegetation present on the inter-rows is result of spontaneously occurring species and possible regrowth of previously sown species. Each DVITI block has 8 rows of 60 Artaban variety vines. The technical management activities conducted in each system are as described in Annex B.

Table 1. Sown service crops for each studied year

Year of sowing	Species	Family	Sowing density
2022	<i>Trigonella foenum-graecum</i>	Fabaceae	15 kg ha ⁻¹
	<i>Raphanus sativus</i>	Brassicaceae	8 kg ha ⁻¹
	<i>Sinapsis alba</i>	Brassicaceae	5 kg ha ⁻¹
2023	<i>Avena sativa</i>	Poaceae	60 kg ha ⁻¹
	<i>Lathyrus sativus</i>	Poaceae	50 kg ha ⁻¹
	<i>Pisum sativum</i>	Fabaceae	50 kg ha ⁻¹
	<i>Vicia faba</i>	Fabaceae	100 kg ha ⁻¹
	<i>Raphanus sativus</i>	Brassicaceae	8 kg ha ⁻¹
	<i>Sinapsis alba</i>	Brassicaceae	5 kg ha ⁻¹

This study encompasses measurements of various components (Figure 2). The following sections details the methods applied at each stage.

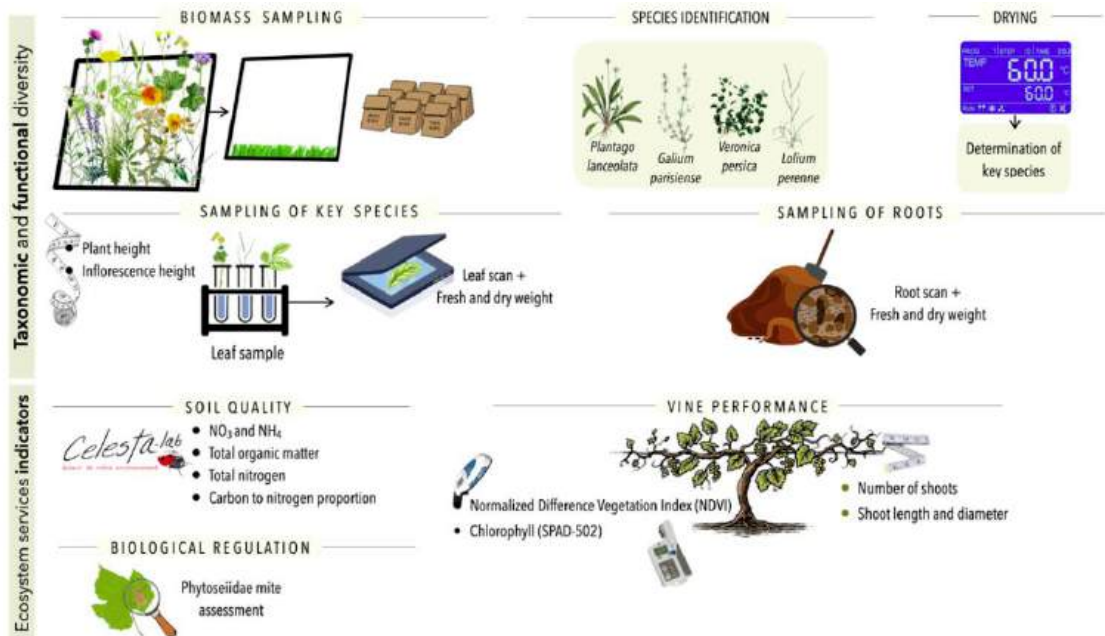


Figure 2. Activity chart with graphic illustration of the assessments carried out

2.2 Service crops measurements

2.2.1 Biomass measurements and taxonomic diversity

In both years studied, 2023 and 2024, the assessments to characterize taxonomic diversity of the cover vegetation were conducted in March and May. The data chosen to be represented in this study is the latter, due to its coincidence with the sampling of mites. In all study plots, a quadrat (50 x 50 cm) was placed in the inter-row and the above ground biomass sampled. The individuals obtained were separated into species and oven-dried at 60°C for 72 hours for dry weight determination.

To characterize the plant community structure, taxonomic diversity parameters including species richness and Shannon's diversity index were calculated for each quadrat (i.e., plant community). Species richness shows a straightforward measure of biodiversity, calculated as the number of distinct species present in each quadrat sampled. Shannon's diversity index (H') incorporates species richness and evenness, calculated as the following equation:

$$H' = - \sum_{i=1}^S (p_i \ln(p_i))$$

with S being the total number of species in the community (species richness), p_i the proportion of species relative to the total number of species in the sampled quadrat.

Due to different management techniques and plant community structure, study plots located in the tree inter-row of the DVITI system are not included in the general analysis but have been studied separately.

2.2.2 Functional traits

To select the plant species for trait measurements, those whose cumulative dry biomass reached 80% of the total dry biomass of the sampled quadrat were determined as the key species and used to assess functional diversity (Pakeman and Quested, 2007) (Table 2).

The determined key species for each year were then resampled for foliar trait analysis with 12 replicates each. From a healthy adult plant, the total height and height of inflorescence was measured with a measuring tape, and a leaf sampled with immediate placement in contact with Milli-Q water. The sampled leaves were then located in a cold chamber at 4°C overnight (Pérez-

Harguindeguy et al., 2013). Each leaf was weighed for fresh biomass with a precision scale, scanned with Epson Perfection 12000 and the image was processed by the software WinFOLIA to obtain the leaf area. The material was then oven-dried at 60°C to obtain the dry weight.

Table 2. Key species for functional trait assessments for each studied year

Year	Species	Family
2023	<i>Carduus pycnocephalus</i>	Asteraceae
	<i>Convolvulus arvensis</i>	Convolvulaceae
	<i>Crepis foetida</i>	Asteraceae
	<i>Dactylis glomerata</i>	Poaceae
	<i>Erigeron sumatrensis</i>	Asteraceae
	<i>Festuca arundinacea</i>	Poaceae
	<i>Heminthotheca echioides</i>	Asteraceae
	<i>Malva sylvestris</i>	Malvaceae
	<i>Medicago sativa</i>	Fabaceae
	<i>Onobrychis viciifolia</i>	Fabaceae
	<i>Picris hieracioides</i>	Asteraceae
	<i>Plantago lanceolata</i>	Plantaginaceae
	<i>Raphanus sativus</i>	Brassicaceae
<i>Rumex crispus</i>	Polygonaceae	
	<i>Scabiosa atropurpurea</i>	Caprifoliaceae
2024	<i>Avena sativa</i>	Poaceae
	<i>Bromus sterilis</i>	Poaceae
	<i>Convolvulus arvensis</i>	Convolvulaceae
	<i>Erigeron sumatrensis</i>	Asteraceae
	<i>Erodium malacoides</i>	Geraniaceae
	<i>Fumaria parviflora</i>	Papaveraceae
	<i>Galium parisiense</i>	Rubiaceae
	<i>Helminthotheca echioides</i>	Asteraceae
	<i>Hordeum murinum</i>	Poaceae
	<i>Lamium amplexicum</i>	Lamiaceae
<i>Lathyrus sativus</i>	Poaceae	

<i>Lolium perenne</i>	Poaceae
<i>Malva sylvestris</i>	Malvaceae
<i>Medicago sativa</i>	Fabaceae
<i>Onobrychis viciifolia</i>	Fabaceae
<i>Papaver rhoeas</i>	Papaveraceae
<i>Pisum sativum</i>	Fabaceae
<i>Plantago lanceolata</i>	Plantaginaceae
<i>Raphanus sativus</i>	Brassicaceae
<i>Veronica persica</i>	Plantaginaceae
<i>Vicia faba</i>	Fabaceae

Specific leaf area (SLA) and leaf dry matter content (LDMC) were chosen as key functional traits in this study due to their strong ecological significance in plant strategies for resource acquisition and adaptation to environmental stress. SLA reflects the leaf area produced per unit of dry mass and was calculated by dividing the leaf area by its dry biomass. LDMC, measures the ratio of leaf dry mass to fresh mass and was calculated by dividing the leaf dry biomass by its fresh biomass.

To characterize the functional diversity of plant communities and the ecosystem service they provide, measurements done at an individual section are transferred to a community scale by calculating the community weighted means (CWM), which produces results with a tighter relationship between traits and the environment by considering the species abundance (Garnier et al., 2004; Garnier and Navas, 2012).

Therefore, traits such as specific leaf area (SLA), leaf dry matter content (LDMC), height of individuals and height of the inflorescence were calculated as community weighted mean values, as the equation below:

$$CWM = \sum_{i=1}^n p_i \text{trait}_i$$

where, p_i the proportion of species i , trait_i the trait value for species i , and n the total number of species in the community.

For the below ground functional traits, soil cylinders with 10 cm of diameter and 20 cm of depth, were taken for root analysis at the selected plots (yellow plots in Figure 1), with the

exception of those located in blocks 5 and 6 of TVITI system, due to the lack of vegetation present. The cylinders were stored at -18°C before being thawed for analysis.

The service crop roots were separated from the soil and the grapevine roots. For each sampled plot, three subsamples were scanned with Epson Perfection 12000 and the image processed by the software WinRHIZO to obtain the roots length, surface area and the average diameter (avr_diam). Five other root markers were calculated to represent the community. Specific root length (SRL) was calculated by dividing the total root length by the dry root matter. Root dry matter content (RDMC) was obtained by the dividing the dry mass by the fresh mass. Root length density (RLD) was done by the extrapolation of total root length based on the weight of scanned and not scanned roots, divided by the soil volume. Root mass density (RMD) was calculated dividing the dry root mass by the soil volume and the very fine root fraction (VFRf) was obtained by the division of the total length of the roots with a diameter below 0.1mm by the total root length.

Table 3. Functional traits studied

Functional traits	Unit	Formula
Height	cm	-
Height of inflorescence	cm	-
Specific leaf area (SLA)	m ² kg ⁻¹	<i>leaf area ÷ dry leaf mass</i>
Leaf dry matter content (LDMC)	mg g ⁻¹	<i>dry leaf mass ÷ fresh leaf mass</i>
Specific root length (SRL)	m g ⁻¹	<i>total root length ÷ dry root mass</i>
Root dry matter content (RDMC)	-	<i>dry root mass ÷ fresh root mass</i>
Root length density (RLD)	cm cm ⁻³	<i>total root length ÷ soil volume</i>
Root mass density (RMD)	kg m ⁻³	<i>dry root mass ÷ soil volume</i>
Very fine root fraction (VFRf)	-	<i>total length of roots with diameter < 0.1 mm ÷ total root length</i>
Average root diameter (avr_diam)	mm	-

2.3 Soil measurements

In May of each year, soil samples were taken from a subset of 30 plots within the experimental site (yellow plots in Figure 1) and analyzed by Celesta Laboratory for the content of moisture, NO₃ and NH₄. Nitrogen was extracted with KCL and quantified using a colorimetric method.

Since 2024 represents the final year of data collection for Lou Tabary's PhD, the samples were additionally analyzed for total organic matter, total nitrogen, and carbon to nitrogen proportion. Organic matter was determined with dry combustion method. From the data provided the content of total carbon and inorganic nitrogen were calculated (Table 4).

2.4 Grapevine measurements

To determine grapevine vigor, measurements were conducted at the flowering stage. The Normalized Difference Vegetation Index (NDVI) was measured on both sides of the row along 10 individuals with the "Greenseeker" equipment (Trimble). On the subset of 30 plots (yellow plots in Figure 1), a healthy leaf from three different individuals was selected and five measurements of the chlorophyll index were made on each leaf with the "SPAD-502" (Minolta). On 6 individuals of the plot, the number of shoots was counted and the length and diameter of one shoot per grapevine was measured.

2.5 Phytoseiidae mite density

As a proxy of natural regulation, Phytoseiidae quantification was done at the Center for Biology and Management of Populations in Montferrier-sur-Lez, France. In both years, in the month of May, 10 young but fully developed grapevine leaves, were sampled from all plots. The underside of the leaves was photographed, and the total leaf area evaluated with ImageJ software. The fauna was recovered using the "soaking-washing-filtering" technique (Boller, 1984): the samples soaked in water with soap for 24 hours, then rinsed and filtered through a 90 µm sieve. Recovered Phytoseiidae mites were counted under a stereoscopic microscope and the density was calculated dividing the abundance by the leaf area.

Table 4. Grapevine vigor, soil quality and predatory mite indicators

Indicators	Unit	Formula
Normalized difference vegetation index (NDVI)	-	$(NIR - RED) \div (NIR + RED)$
Chlorophyll index (SPAD)	-	-
Shoot length	cm	$(\sum_1^6 \text{ shoot length}) \div 6$
Shoot diameter	mm	$(\sum_1^6 \text{ shoot diameter}) \div 6$
Shoot number	-	$(\sum_1^6 \text{ number of shoots}) \div 6$
Total carbon	kg ha ⁻¹	$(\text{total organic matter} \div 1.72)$
Total nitrogen	kg ha ⁻¹	-
C/N	-	$\text{total carbon} \div \text{total nitrogen}$
Inorganic nitrogen (inorgN_kg_ha)	kg ha ⁻¹	$NO_3 + NH_4$
Soil water content (h_mm)	mm	$(H\% \div 100) \times 1.4 \times 0.2 \times 1000$
Phytoseiidae density (phyt_dens_1000_cm2)	individuals 1000 cm ⁻²	$(\text{Phytoseiidae abundance} \div \text{sample area}) \div 1000$

2.6 Data analyses

All analyses were performed using R Statistical Software version 4.3.3 (R Core Team, 2024).

2.6.1 Modeling the effect of cropping system on plant communities between years

The present study compares the above-mentioned functional markers (Table 3) and ecosystem indicators (Table 4) between the systems and between the years of 2023 and 2024. The variability between systems and years was assessed with a generalized linear mixed model, with the R package “glmmTMB” (Brooks et al., 2017). The model design included the fixed effect of system interaction with year and a random effect of blocks nested within systems, in order to take into account the spatial structure and soil heterogeneity associated with each block. The R formula is as follows:

$$\text{glmmTMB}(\text{trait} \sim \text{system} * \text{year} + (1 | \text{block} : \text{system}))$$

The model residuals were verified with the “DHARMA” package (Hartig, 2022) which simulates the standardized residuals from fitted models and assess the model goodness-of-fit. When appropriate results were obtained, post-hoc analysis was conducted with the “emmeans”

package (Lenth, 2024). In the case of grapevine vigor measurements, conditions for linear model were not met and therefore data was analyzed with Kruskal-Wallis test, with package “agricolae” (Mendiburu, 2023).

2.6.2 Principal component analysis (PCA)

To identify correlations among the variables studied a principal component analysis (PCA) was conducted with R package “FactoMineR” (Husson et al., 2008) for each year. Principal components with the highest eigenvalues were selected for further analysis, and only variables with the square cosine (\cos^2) greater than 0.3 were retained for the visualization of biplot.

2.6.3 Partial least squares-path modeling (PLS-PM)

To further the understanding of the multivariate relationships among observed and latent variables, the partial least squares-path modeling method was used with the R package “plspm” (Sanchez, 2015). The model was constructed based on hypotheses present in published papers (Table 5).

Table 5. Hypothesis used to construct the PLS-PM model

Hypothesis	Reference
The functional structure of the plant communities reflects their abilities to use available resources, which impacts biomass production	Garnier et al., 2004
Soil management practices shape plant communities to display the functional traits best suited to the soil conditions.	Fried et al., 2012
The plant community competes with the grapevine for resources, impacting its vigor.	Cruz et al., 2012 and Abad et al., 2021

A latent variable (LV) is an unobserved variable which can’t be directly measured but is described by one or more manifested variables (MV) (Sanchez, 2015) (Table 6). The correlation between the latent variables and its indicators were estimated using the coefficient “loading” (λ) and the strength and direction of the relationship between LVs were estimated with “path coefficient” (β) (Sanchez, 2015). The mean and standard error values were obtained from bootstrap analysis.

The inner model was built to quantify the influence of management strategies on the vegetation traits, soil resources, grapevine vigor and fauna. The validity check of the model was carried in three steps. Firstly, the unidimensionality of the indicators is checked through the indices of Cronbach's alpha (α), Dillon-Goldstein's rho (ρ), and the eigenvalue. In sequence, the loadings were examined and values over 0.7 were kept. Cross-loadings, the loading of an indicator with the other LVs, were also checked. Thirdly, a bootstrap validation was conducted to obtain confidence intervals of the PLS estimates. An additional step was carried to determine the quality of the structural model with the R2 coefficient, the redundancy index and the Goodness-of-Fit (GoF).

Table 6. Descriptions of latent (LV) and manifest (MV) variables used in the partial least squares path model (PLS-PM model)

Latent variables (LV)	Manifest variables (MV)	Meaning	Unit
Management	Service crops	Presence or absence of service crops	1= yes; 0= no
	Trees	Presence or absence of trees	1= yes; 0= no
	Green pruning	Presence or absence of green pruning	1= yes; 0= no
	Row tillage	Presence or absence of row tillage	1= yes; 0= no
Plant community	RLD	Root length density	cm cm ⁻³
	RMD	Root mass density	kg m ⁻³
	CWM_SLA	Community weighted mean specific leaf area	m ² kg ⁻¹
	CWM_LDMC	Community weighted mean leaf dry matter content	mg g ⁻¹
	CWM_height	Community weighted mean plant height	cm
	CWM_inflorescence	Community weighted mean inflorescence height	cm
	Total biomass	Dry biomass of plant community	t ha ⁻¹
Soil	Total C	Soil total carbon content	kg ha ⁻¹
	Total N	Soil total nitrogen content	kg ha ⁻¹
	C/N	Soil carbon to nitrogen proportion	-
	H_mm	Soil moisture content	mm

	Inorganic N	Soil inorganic nitrogen content	kg ha ⁻¹
Grapevine	NDVI	Normalized difference vegetation index	–
	SPAD	Chlorophyll index	–
	Shoot number	Number of shoots per study plot	–
	Shoot length	Length of shoot	cm
	Shoot diameter	Diameter of shoot	mm
Fauna	Phytoseiidae density	Phytoseiidae mite density in 1.000 cm ²	-

3. RESULTS

3.1 Variations of service crops biomass and community taxonomic composition according to management and across years

For an overview of the vegetation results see Box 1. Dry biomass in the grapevine inter-rows indicated significant differences between the systems and between the years (Figure 3). In both years, TVITI showed lower biomass than the agroecological systems (1.11 ± 0.93 for 2023 and 0.15 ± 0.3 for 2024). Between the agroecological (AE) systems, DVITI showed slightly higher biomass in both years (4.16 ± 1.01 for 2023 and 5.15 ± 0.93 for 2024). In both years in the DVITI system, sample plots located in the grapevine inter-rows showed higher production of dry biomass than the plots located between the trees (Figure 4).

Taxonomic diversity was compared between the systems by species richness and Shannon's diversity index (Figure 5). In both years, TVITI showed lower values for taxonomic diversity, while between the AE systems, AVITI presented higher values.

Throw-out all sample plots, in 2023, both AE systems had 24 distinct species, while in 2024 this number increased for 40 and 42, in AVITI and DVITI respectively. TVITI also showed an increased number of species going from 4 in 2023 to 6 in 2024. For a detailed list of the most significant species present and their cumulative dry biomass in each system and year refer to Annex C.

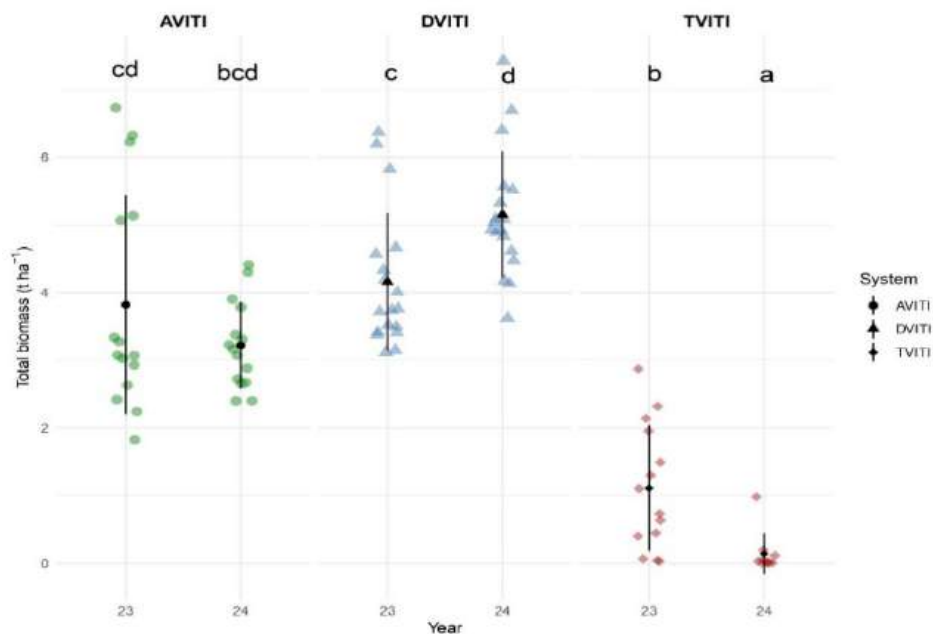


Figure 3. Total dry biomass of communities in tons per hectare by cropping systems in different years. Black dots represent the mean and black bars represent the standard deviation. Different letters indicate significant differences among the interaction of system and year (Tukey test) with significance level at 0.05.

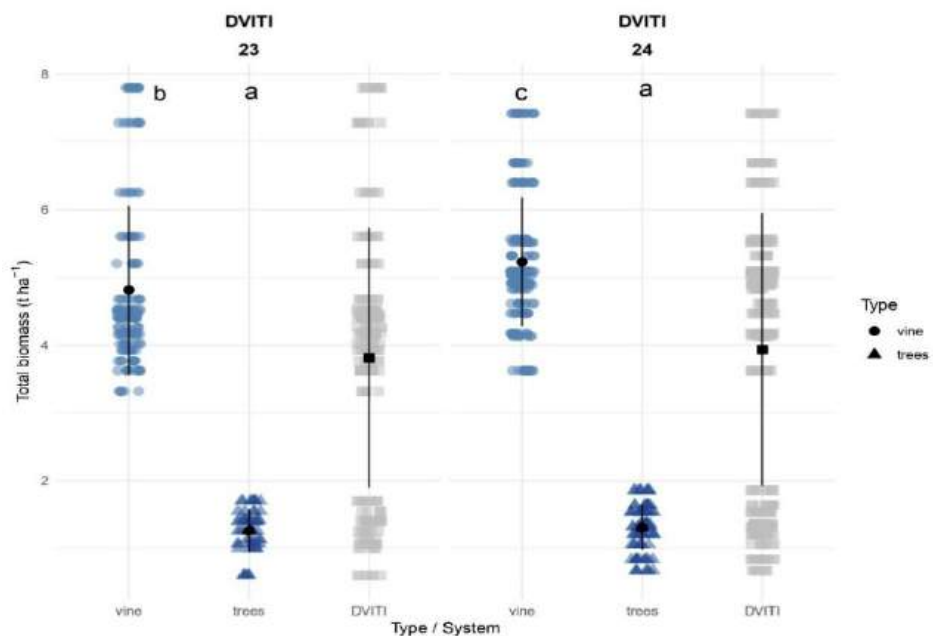


Figure 4. Total dry biomass of communities in the DVITI system in tons per hectare by plot type (■: vine and trees; ▲: between tree rows; ●: between vine rows) in different years. Black dots represent the mean and black bars represent the standard deviation. Different letters

indicate significant differences among the interaction of type of plot and year (Tukey test) with significance level at 0.05.

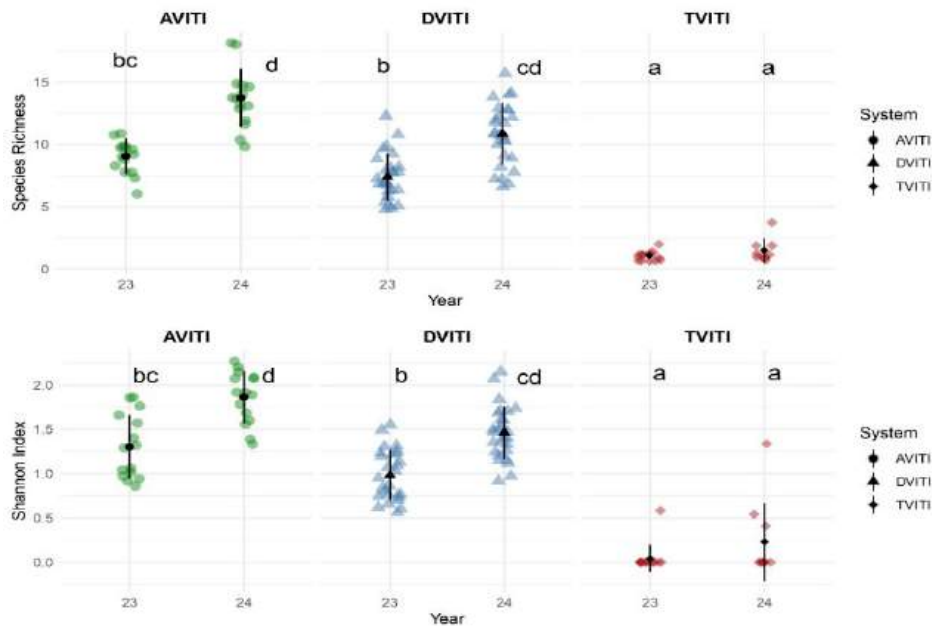


Figure 5. Taxonomic diversity of communities by cropping systems in different years. Black dots represent the mean and black bars represent the standard deviation. Different letters indicate significant differences among the interaction of system and year (Tukey test) with significance level at 0.05.

3.2 Variations of functional traits according to management and across years

Leaf dry matter content (CWM_LDMC) results were higher in the DVITI system in both years (Figure 6). Specific leaf area (CWM_SLA) showed higher values for all systems in 2024, with TVITI higher than the AE systems. In both years, AE systems had higher values for height of plants (CWM_height) and height of inflorescence (CWM_inflor) compared to the TVITI system.

From the root markers (Figure 6), VFRf showed no statistically significant differences between the systems and years. In 2024, average root diameter showed no difference between systems. In 2023, SRL showed no difference between the systems. When compared to the AE systems, the TVITI system showed higher values of average root diameter in 2023 and RDMC in both years. In 2023 and 2024, AE systems showed higher values for RMD and RLD. Between the AE systems, there was no significant difference within the years for RDMC.

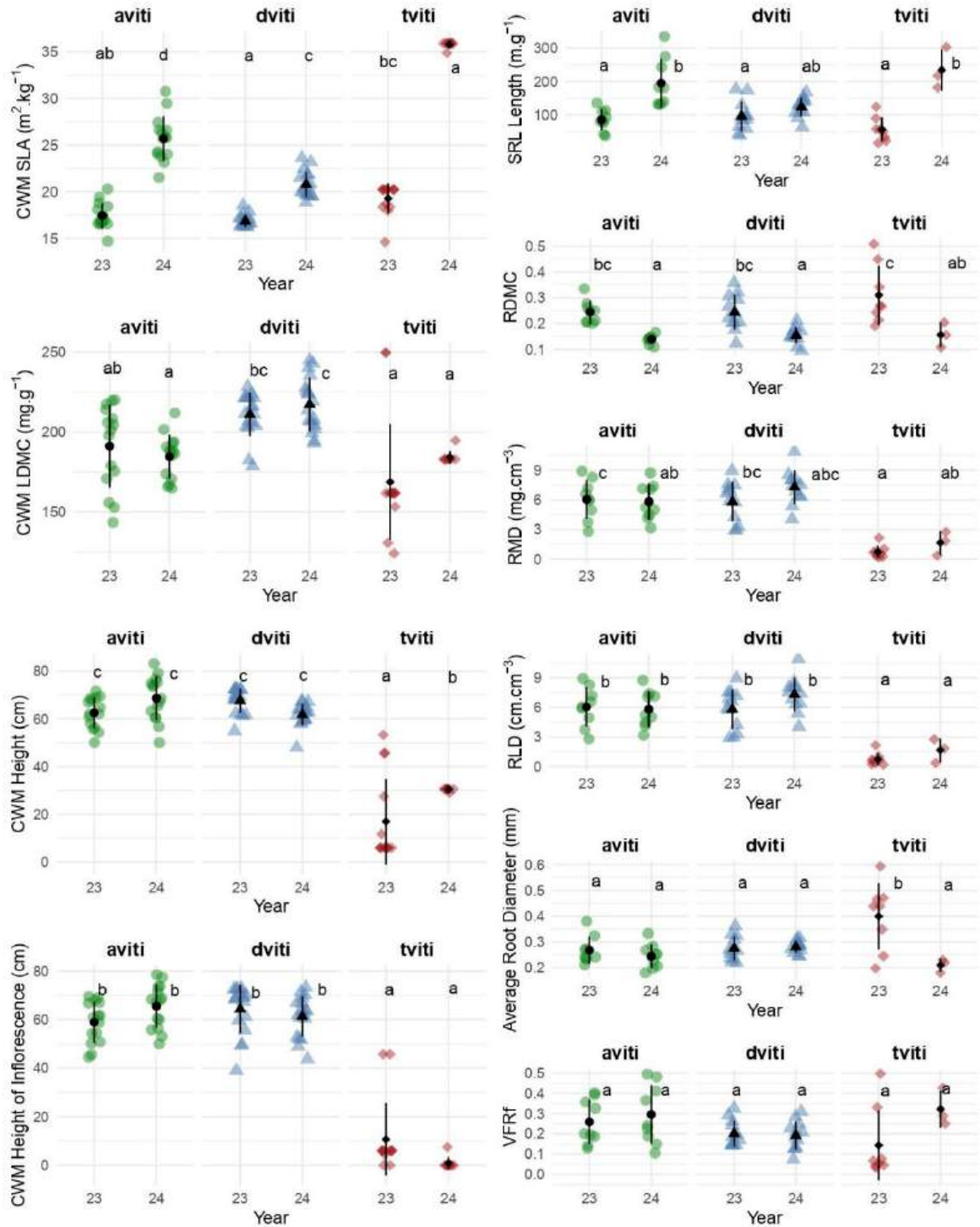
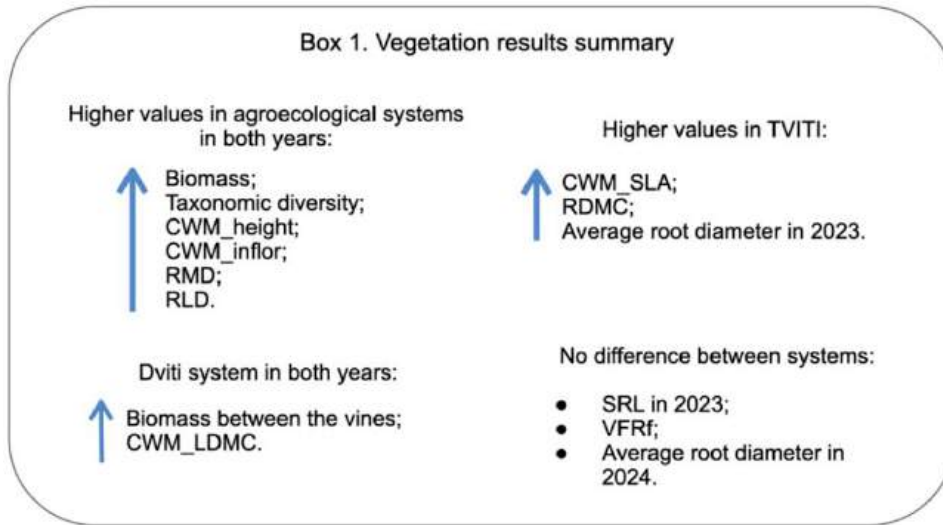


Figure 6. Functional traits of communities by cropping systems in different years. Black dots represent the mean and black bars represent the standard deviation. Different letters indicate significant differences among the interaction of system and year (Tukey test) with significance level at 0.05.



3.2 Soil indicators variation according to management and across years

Soil indicators measured strictly for the year 2024 (Total N, Total C and C/N) revealed no statistical differences between the systems (Table 7).

In 2023, soil moisture did not vary between systems (Figure 7). Comparing the years, soil moisture was lower in 2024. Inorganic nitrogen content was higher in TVITI, and remained consistent across years for AVITI and were higher in 2023 for DVITI and TVITI.

Table 7. Summary of soil indicators mean and standard deviation (SD) by system. Different letters indicate significant differences among the systems (Tukey test) with significance level at 0.05.

Variable	System (mean ± SD)		
	AVITI	DVITI	TVITI
Total N	3.28 ± 0.76 a	3.27 ± 0.54 a	2.61 ± 0.16 a
Total C	39.72 ± 6.6 a	40.81 ± 5.24 a	33.48 ± 1.84 a
C/N	12.24 ± 0.79 a	12.56 ± 0.6 a	12.84 ± 0.54 a

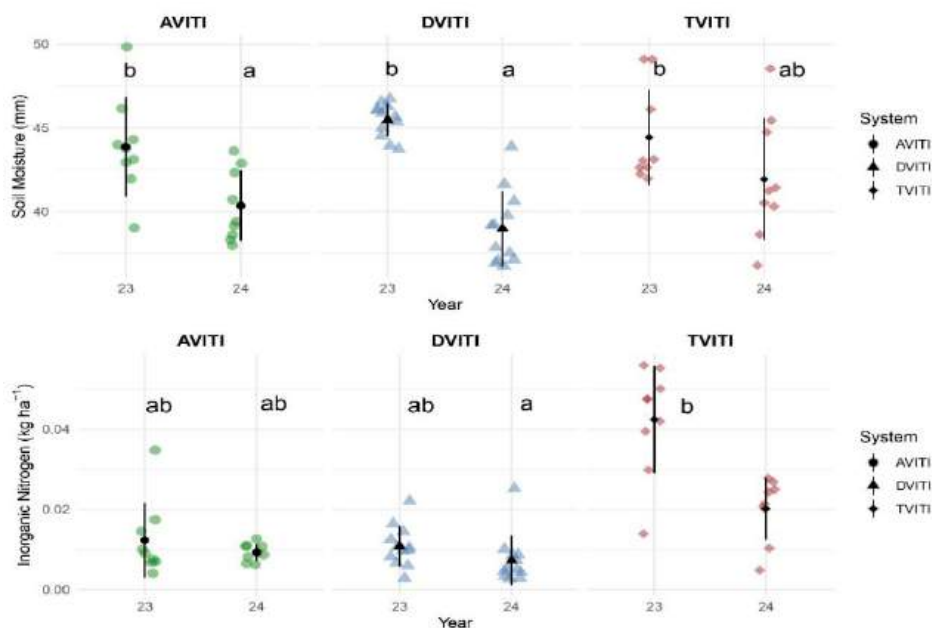


Figure 7. Soil indicators of communities by cropping systems and year. Black dots represent the mean and black bars represent the standard deviation. Different letters indicate significant differences among the interaction of system and year (Tukey test) with significance level at 0.05.

3.3 Grapevine vigor indicators

When comparing between the systems within each year, grapevine vigor indicators showed higher value in TVITI (Table 8). Between the years shoot number showed increasing values, while shoot diameter was the opposite. SPAD values were lower in 2024 for AVITI and TVITI, and higher for DVITI.

Table 8. Summary of grapevine vigor indicators of communities by cropping systems and year. Different letters indicate significant differences among the interaction of system and year (Kruskal-Wallis test) with significance level at 0.05.

Variable	System (mean ± SD)					
	AVITI		DVITI		TVITI	
year	2023	2024	2023	2024	2023	2024

NDVI	0.8 ± 0.02 e	0.8 ± 0.03 d	0.78 ± 0.03 f	0.82 ± 0.02 c	0.87 ± 0.01 b	0.88 ± 0.01 a
SPAD	36.04 ± 4.16 b	33.59 ± 6.15 e	31.57 ± 4.94 f	34.47 ± 5.41 d	40.79 ± 4.87 a	34.45 ± 2.8 c
Shoot number	8.96 ± 0.55 e	9.7 ± 1.31 d	12.49 ± 1.44 c	14.68 ± 1.86 b	15.28 ± 1.33 b	18.2 ± 1.95 a
Shoot length	98.04 ± 20.63 c	72.43 ± 18.43 d	71.4 ± 14.04 e	70.94 ± 14.67 de	110.28 ± 14.93 b	121.57 ± 10.94 a
Shoot diameter	7.63 ± 0.94 b	6.79 ± 0.84 c	6.84 ± 0.8 c	6.51 ± 0.66 d	8.72 ± 1.08 a	7.42 ± 0.67 b

3.4 Phytoseiidae density

Phytoseiidae density showed higher values in 2023 for both AE systems. TVITI system had no statistical difference between both years (Figure 8). In 2024, there was overall no statistical difference between the systems.

For an overview of the ecosystem indicators results see Box 2.

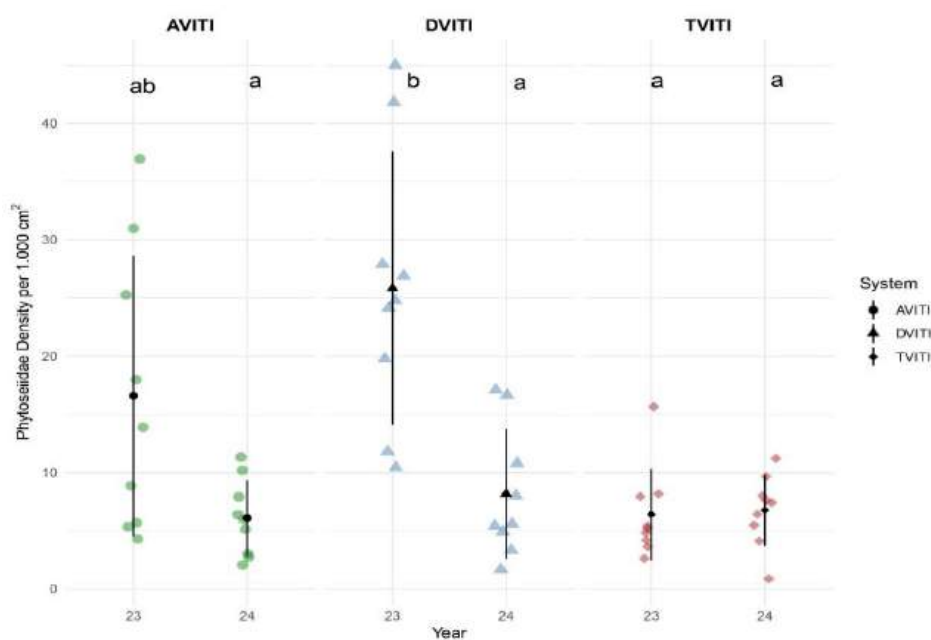
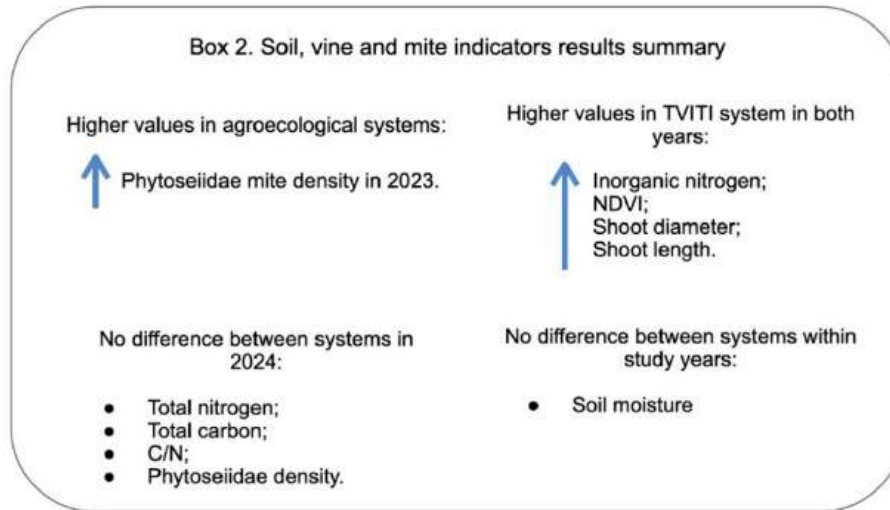


Figure 8. Phytoseiidae density on grapevine leaves by cropping systems and year. Black dots represent the mean and black bars represent the standard deviation. Different letters indicate significant differences among the interaction of system and year (Tukey test) with significance level at 0.05.



3.5 Component-based data analysis

PCA was conducted for both years of the study to provide a visual representation of the variable interactions. Axes 1 and 2 of the PCA explain 65.4% of the data variance in 2024 and 59.8% in 2023 (Figure 9). Axis 1 represented more of the community traits analyzed. In both years, data obtained from the AE systems were overlapped and separated from those in the TVITI system, and community weighted SLA was negatively correlated with biomass, LDMC, plant height, inflorescence height and root mass density. In both years, grapevine vigor indicators were positively correlated between themselves and negatively with service crops height, biomass and RMD. In 2023, the PCA showed positive correlation between Phytoseiidae density, height, RLD and biomass, while in 2024 Phytoseiidae density was not representative to the analysis.

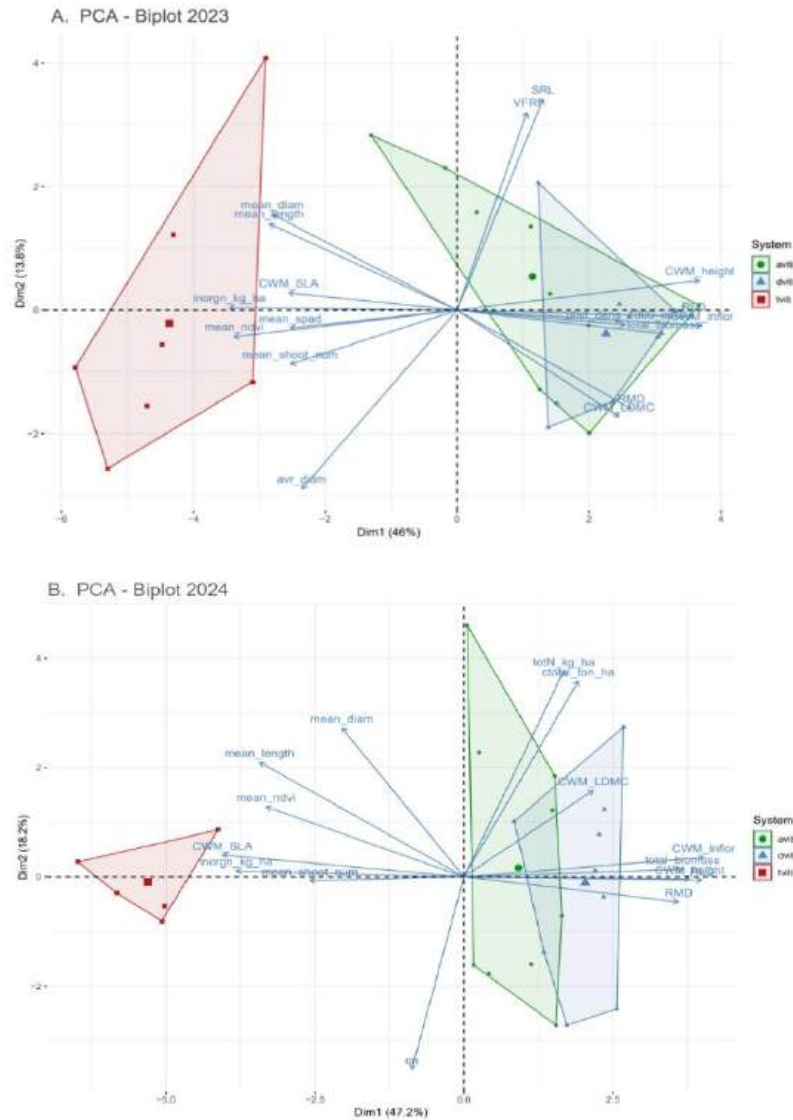


Figure 9. A. Principal Component Analysis (PCA) biplot for 2023; B. Principal Component Analysis (PCA) biplot for 2024. Total_biomass: dry biomass per quadrat; CWM_height: community weighted mean height of plants; CWM_inflor: community weighted mean height of inflorescence; CWM_SLA; community weighted mean specific leaf area; CWM_LDMC: community weighted mean leaf dry matter content; SLR: specific root length; RDMC: root dry matter content; RLD: root length density; RMD: root mass density; VFRf: very fine root fraction; Avr_diam: average root diameter; Mean_ndvi: Mean of NDVI readings; Mean_spad: mean of SPAD readings; Mean_shoot_num: mean number of shoots; Inorgn_kg_ha: inorganic nitrogen content; TotN_kg_ha: total nitrogen content; Ctotal_ton_ha: total carbon content; Cn: carbon to nitrogen proportion; phyt_dens_1000_cm2: Phytoseiidae mite density.

3.6 Pathway-based data analysis

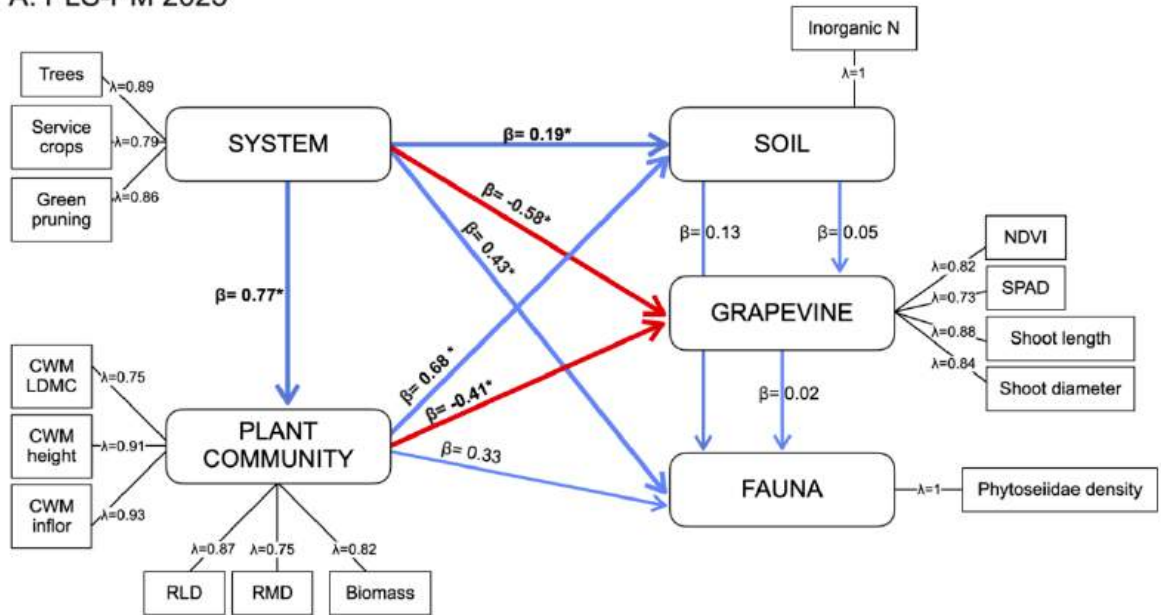
For both years, the system latent variable was explained by the inclusion of green pruning, and the presence of service crops and trees (Figure 10). In 2023, the presence of these practices had a significant positive effect on soil parameters ($\beta = 0.19$). The soil latent variable in 2023 was explained by the negative of inorganic nitrogen content. Management practices and plant community in 2023, showed a positive effect on soil latent variable, which reflects as a reduction of inorganic nitrogen content.

In 2023, management practices also had a positive effect on the plant community traits ($\beta = 0.77$) and on the fauna ($\beta = 0.43$), and a negative effect on grapevine ($\beta = -0.58$). In 2024, green pruning and the presence of service crops and trees had a significant positive effect on the plant community ($\beta = 0.31$) and grapevine vigor indicators ($\beta = 0.57$).

Plant community latent variable was explained in 2023 by RLD, RMD, CWM_LDMC, CWM_height, CWM_inflorescence and biomass, while in 2024 it differed by the absence of CWM_LDMC and the presence of negative CWM_SLA. In 2023, plant community traits had a positive effect on soil ($\beta = 0.68$) and a negative effect on grapevine ($\beta = -0.41$). In 2024, plant community variable had a negative effect on grapevine vigor ($\beta = -1.52$) and on the soil variable ($\beta = -0.75$). Soil latent variable in 2024 was explained by inorganic nitrogen content, the negative of total nitrogen and the negative of total carbon. Therefore, the plant community negative effect on the soil latent variable in 2024 reflects a decrease in inorganic nitrogen content and an increase in total nitrogen and total carbon.

In 2023, no significant link was shown between the soil, grapevine, and fauna. In 2024, the soil quality latent variable had a negative effect on grapevine vigor ($\beta = -0.36$) and positive with the fauna variable ($\beta = 0.43$) which is explained by the density of Phytoseiidae mites.

A. PLS-PM 2023



B. PLS-PM 2024

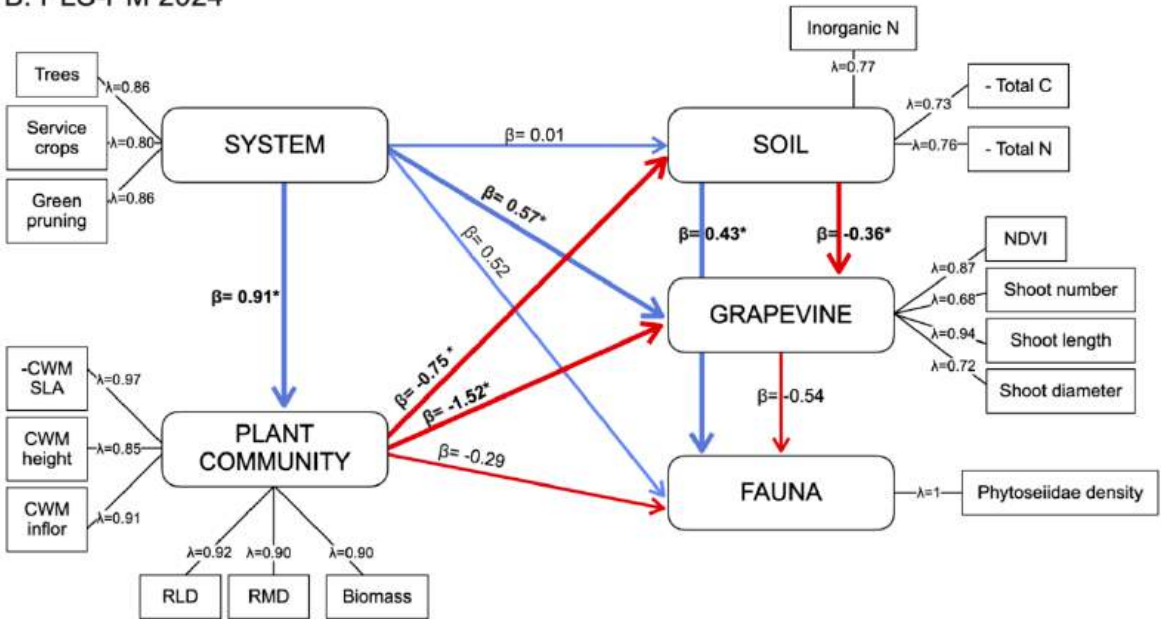


Figure 10. A. Partial least squares-path modeling (PLS-PM) for 2023; B. Partial least squares-path modeling (PLS-PM) for 2024. Values in bold and with asterisk indicate that the path coefficients (β) were significantly different from 0 based on 95% percentile confidence intervals calculated using 200 bootstrap samples

4. DISCUSSION

The present study evaluated the effects of vineyard management systems on the functional traits of inter-row plant communities, and its relation to three ecosystem services: soil quality, grapevine vigor and biological regulation.

4.1 Contrasted service crop development between the agroecological and control systems

4.1.1 Inter-row biomass influenced by mowing and tillage practices

In both years, 2023 and 2024, DVITI had higher biomass of inter-row vegetation than the other systems. Mowing is known to impact vegetation composition (Simoës et al., 2013), and in many cases used as a vegetation management strategy reducing the regrowth of ground cover (Alcántara et al., 2011; Humanes and Pastor, 1995). Considering the differences between the agroecological systems, AVITI with sown species and DVITI with spontaneous species, we can interpret that mowing was more effective in reducing the vegetation in AVITI, perhaps due to limited regrowth abilities of the sown species (Humanes and Pastor, 1995; Brandsæter and Netland, 1999), while in DVITI plant communities were capable of re-establishing themselves with higher biomass production after the disturbance.

TVITI, a system that employed the reoccurring use of soil tillage, showed lower biomass production. This is according to expected, since soil tillage is an efficient termination strategy for the species present on the inter-rows, even more than mowing (Garcia et al., 2024). Given that soil tillage is a commonly used strategy among farmers for the removal of ground cover, TVITI is a valid reference point for comparisons.

4.1.2 Agroecological systems increased taxonomic diversity

Taxonomic diversity showed variance between the agroecological systems, with higher values in AVITI. This can be attributed to the deliberate addition of sown species in the system while still allowing for the presence of spontaneous vegetation, enhancing the diversity in these communities.

The TVITI system presented lower taxonomic diversity, highlighting the findings that soil disturbance selects species from the seed bank that can respond to the conditions (Czerwiński et al., 2018). Additionally, tillage practices have been reported to favor annual species (Fried et al.,

2019), leading to a plant community with reduced diversity, as only more ruderal species that can tolerate such levels of disturbance are selected (Guerra et al., 2021). In both years of our study TVITI presented fewer species, selecting those with high vegetative reproduction through cuttings such as *Convolvulus arvensis* (Fried et al., 2019).

4.1.3 Functional diversity differed according to vineyard management strategy

Considering the functional traits measured, the TVITI system showed lower plant height in comparison with the agroecological systems. The height of the plant reflects the investments dedicated to accessing light, but it also comes at a cost for the plant; therefore, the benefits of allocating resources on height development depend on the abundance of other strategies present (Falster and Westoby, 2003). In TVIVI, the low biomass in the inter-rows due to soil tillage, led to reduced competition for light, making the investment on height not justified for this plant community.

In TVITI, community values for SLA were higher and LDMC values were lower. This is in accordance with findings that species with such characteristics are more competitive and employ acquisitive strategies (Tribouillois et al., 2015), further confirming that the employment of tillage promotes plant communities that function with more ruderal survival strategy (Grime, 1977).

Community weighted values for LDMC were higher in the agroecological systems. High values of LDMC have been seen in communities that experience low frequency and intensity of disturbance (Pontes et al., 2007). LDMC is also related to litter decomposition (Kazakou et al., 2009; Bumb et al., 2018), and to the trade-off between growth and physical or chemical protection of the plant. LDMC is also negatively correlated to nutrient availability (Kazakou et al., 2022). The agroecological systems selected plant species with conservative strategies for resource acquisition (low SLA and high LDMC) and produced litter that tends to have slower decomposition and therefore delayed nutrient releases (Tribouillois et al., 2015).

Specific root length (SRL) showed small variation between the systems, while root mass density (RMD) and root length density (RLD) were higher in the agroecological systems. SRL is commonly used as an indicator for soil resource uptake efficiency (Ostonen et al., 2007), however it is not only the surface of roots that influence such factors, it is also required a comprehensive understanding of the volume of soil being influenced by the roots. RMD and RLD are a key component of soil carbon stocks feeding the organic matter content and contributing to important

soil functions (De Deyn et al., 2008). Additionally, higher values of RLD have been related to reduced nutrient leaching, soil erosion and enhanced structural stability and water infiltration (Thorup-Kristensen et al., 2003; Gyssels et al., 2005; Berendse et al., 2015; Fischer et al., 2015). These differences bring to the conclusion that the plant communities differed on how they impact and influence soil parameters, with agroecological systems providing higher benefits.

4.1.4 Taxonomic and functional traits variation between years

Taxonomic diversity showed significant difference across the years, with higher number of species in 2024. While in AVITI this difference can be attributed to the number of sown species (three in 2023 and six in 2024), DVITI and TVITI also showed difference even without the addition of sown species. The germination of seeds from the soil seed bank has been related to soil and climatic conditions, especially rainfall (Figueroa et al., 2022). Indeed, climate conditions differed between the years studied, with cumulative rainfall from January to June of 164 mm in 2023 and 339 mm in 2024.

With a wider number of species, their complementarity also provided a broader variability of functional traits, exemplified by the higher values of CWM_SLA and SRL in 2024 for all systems. Higher diversity of species has also been linked with a wider range of functional traits and potential improvements in the ecosystem functions (Isbell et al., 2015).

4.2 Ecosystem service indicators differences between the agroecological s and control systems

4.2.1 Soil indicators

Soil measurements done only for the present year showed no differences between systems. In both years content of inorganic nitrogen was higher in TVITI. Nitrogen availability in the soil varies according to many variables, including seasonal changes (Steenwerth and Belina, 2008). Our results are consistent with previous findings that showed lower content of inorganic nitrogen in systems that adopt intercropping strategies (Celette et al., 2009). This is due to the direct effect of vegetation taking up inorganic nitrogen and the indirect effect of their water consumption, which stops the nitrogen mineralization process (Celette et al., 2009). Therefore, at the timing of sampling, TVITI system, due to the lack of vegetation present on the inter-rows, showed greater

values of inorganic nitrogen content. On the other hand, nitrogen losses have been shown to be lowered in intercropping systems (Celette et al., 2009) and can switch this scenario in rainy seasonal periods.

4.2.2 Agroecological practices reduced grapevine vigor

Considering the parameters analyzed to reflect the grapevine vigor, the TVITI system showed overall higher values. Our results showed that the presence of ground cover vegetation lowered grapevine vigor, which is in accordance with past studies conducted in European vineyards (Griesser et al., 2022; Muscas et al., 2017; Gontier et al., 2011). In conclusion, the higher values in the TVITI system can be due to absence of ground cover, which promoted lower competition for nutrient and water resources therefore not limiting or negatively affecting grapevine vigor.

4.2.3 Biological regulation indicator

The presence of predatory mites promotes ecosystem services due to their efficient predation strategies. In 2023, Phytoseiidae mite density was significantly higher in DVITI. Even though the mite's densities were measured on grapevine leaves, service crops can constitute a reservoir for predatory mites by provisioning of pollen and prey (Aucejo et al., 2003; Mailloux et al., 2010), explaining the higher density in the agroecological systems. Furthermore, the presence of service crops can facilitate the dispersal of predatory mites due to canopy connectedness and the colonization potential of these mites on the commercial crop have been associated with the proximity of natural vegetation (Tixier, 1998; Möth et al., 2021).

In 2024 the density of Phytoseiidae mites showed no statistical difference between the systems, which contradicts the hypothesis that plant diversity affects predator density in vineyards (Tixier, 1998; Möth et al., 2021). Studies have shown that Phytoseiidae populations are affected by factors such as temperature and relative humidity (Duso and Pasqualetto, 1993). This indicates that interactions within a vineyard system can suffer yearly changes and climate factors not considered in this study could explain the observed variation.

4.2.4 Ecosystem service indicators variation between years

When considering the variation of the ecosystem services indicators between the years, multiple factors must be analyzed. Firstly, in the time scale of viticulture's longevity, the

experiment is considered new, being established in 2018, and diversification effects take long to show visible differences. Secondly, the experimental design is done in close quarters, with systems close to each other the results could be masked.

Additionally, ecosystem service indicators can vary according to climatic conditions not analyzed in the present study. Another variable to consider is the timing of sampling each year. For example, soil moisture showed higher values in 2023, which is inconsistent with recorded cumulative rainfall (from January to June, 164 mm in 2023 vs. 339 mm in 2024). This difference in the soil moisture can be attributed to the fact that soil sampling in 2023 was conducted after rainfall and influenced the results obtained.

4.3 Interactions between variables and consequences on the multifunctionality of the systems

The principal component analysis (PCA) showed greater overlap between agroecological systems in 2023, meaning that the plant communities in that year shared more similarities than in 2024. This highlights that the increased number of sown and spontaneous species in 2024 drove the plant communities to have more differences. The separation of agroecological systems from TVITI, was present in both years, suggesting that differences in community composition are influenced by the management practices. Our finding showed that management practices with reduced disturbance and higher diversity promote a more varied plant community compared to more intensively managed systems like TVITI.

A difference that stood out between the years was the relationship between Phytoseiidae density and the other traits. In 2023, Phytoseiidae density was positively correlated with height, RLD and biomass, which suggest that these predatory mites thrive in conditions where inter-row vegetation was more robust. This has been previously reported, where better habitat conditions, prey, and alternative food sources resulted in higher Phytoseiidae population (Bianchi et al., 2013; Möth et al., 2021). Yet, in 2024, Phytoseiidae density was not represented in the PCA, highlighting the importance of considering yearly environmental changes when assessing biological control agents and their interactions with plant traits. Further research into the specific mechanisms driving these patterns, including the role of climate variability would be important for optimizing agroecological practices and enhancing ecosystem services in viticulture.

The results from the partial least squares path modeling (PLS-PM) also provided insights in the interactions between management practices, plant communities, soil, grapevine, and fauna. In 2023, PLS-PM indicated that management practices, such as the inclusion of service crops, trees and green pruning, had a positive effect the plant community; however, it also had an effect that resulted in lower inorganic nitrogen, and competitive pressure that negatively impacted grapevine vigor. This is consistent with other findings where the inclusion of service crops generates competition with the main crop for resources and therefore affecting the grapevine performance (Griesser et al., 2022; Muscas et al., 2017; Gontier et al., 2011).

In 2024, the impact of management shifted, showing a positive effect on both the plant community and grapevine vigor. This suggests that the relations within a vineyard system can vary from year to year and the additional consideration about local climate conditions can contribute to understanding this variation. It is possible that the higher precipitation in 2024 may have reduced competition for water between the grapevine and the inter-row vegetation, leading to the positive effect shown by the PLS-PM analysis.

Overall, our findings emphasize the importance of considering the dynamic interactions between management, plant communities, soil quality, grapevine vigor and fauna, accentuating the need for a nuanced approach to vineyard management, where long-term sustainability and resilience can be balanced with agricultural productivity.

4.4 Limitations of the present study

While the experiment outlined in the present study provides important insights, it has limitations that must be considered. To begin with the spatial structure, the experimental design of the study field shows great proximity between the systems, this can particularly influence data of fauna assessments due to faunal movements, spillover, edge effect and shared microclimate. Additionally, the scale of the experiment was not able to capture the full variability and complexity of agricultural systems. In the topic of spatial distribution, the separation of TVITI blocks from the agroecological blocks can add variability that influences the obtained data.

Another point of reflection relates to the management techniques employed on each system. In the agroecological systems, we have differences in management practices which add variability and can complicate the attribution of observed effects to specific variables. Also, a

detailed climate study would greatly enhance the discussion of the results seeing that it can influence both soil and plant conditions. Furthermore, as vineyards are perennial crops and agroecological regulations develop over an extended period of time, the continuation of the experiment is important in order to provide a better understanding of long-term effects and comparisons.

4.5 Perspectives

Agroecological systems provide a sustainable approach to vineyards, balancing production, and environmental health. Looking ahead, the experiment could benefit from a more comprehensive investigation into soil health assessments. With chemical, physical, and microbial aspects, the influence of vineyard management on the soil activity could be further understood. Additionally, a more detailed fauna quantification and classification along with assessments of food sources for natural enemies would deepen the understanding of ecological dynamics. Moreover, the inclusion of information on production, yield and quality, would contribute to better distinguish the systems. Besides that, the young age of the vines accentuates the need for continued research in order to gather the stability and capture the true variability between the systems as the vineyard matures.

5. CONCLUSION

The present study offers valuable insights into the impact of different vineyard management systems on functional plant traits and their connection to ecosystem services such as soil quality, grapevine vigor, and natural pest control. Over the two years studied, the differences between agroecological and conventional management strategies were highlighted. Production systems with reduced soil disturbance and higher diversity, fostered plant communities with higher biomass production, greater complementarity of functional traits, and enhanced indicators for soil quality and biological regulation.

To ensure long-term resilience in the face of environmental challenges, it is key to integrate ecological principles with agricultural productivity. While conventional systems may boost grapevine vigor, agroecological practices offer a more balanced approach, with higher biodiversity supporting ecosystem functions. In conclusion, although the benefits of service crops are complex

and not easily demonstrated, agroecological systems have potential to create sustainable balance between vineyard productivity and environmental health.

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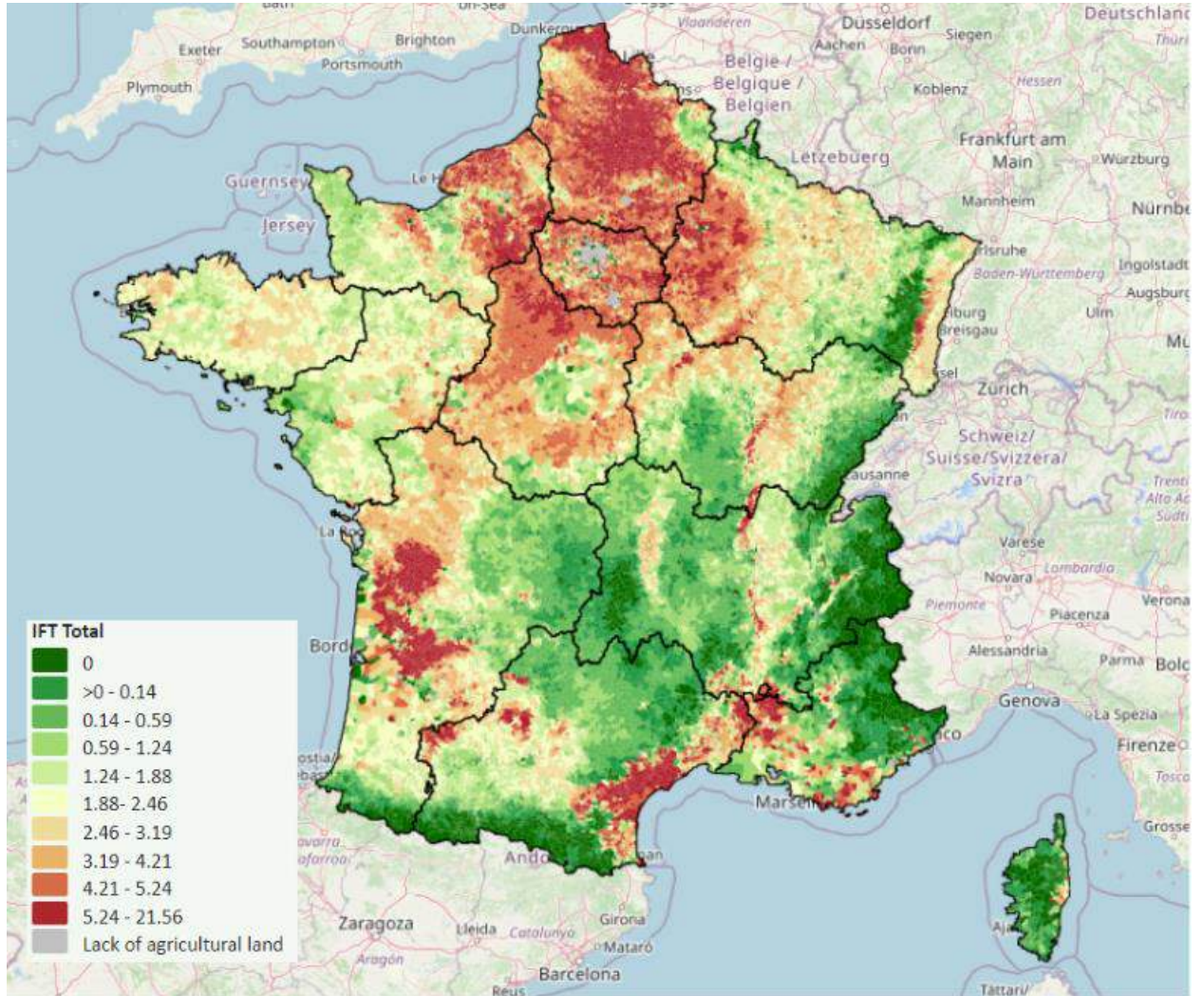
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7. Annexes

Annex A: Map of pesticide use in France. Source: Solagro (2021).



Annex B: Calendar of activities between January 2023 and May 2024.

Date	System	Activity	Observation
	AVITI, DVITI, TVITI	Pruning	-
January, 2023	TVITI	Soil tillage inside row and inter-row + mulching inside row	-
	DVITI, TVITI	Soil tillage inside row	-
February, 2023	AVITI, DVITI, TVITI	Trellising	Attaching branches to the trellis system
	AVITI, DVITI	Lifting	Orientation of branches inside wires of the trellis system
March, 2023	AVITI, DVITI	Mowing of inter-row vegetation + mulching inside row	-
	TVITI	Soil tillage inside row	-
April, 2023	DVITI, TVITI	Soil tillage inside row	-
	AVITI	De-budding	Removal of the counter-bud
May, 2023	AVITI, DVITI	Phytosanitary treatments	Products: Flosul SC (2kg ha ⁻¹) Fytosave (11L ha ⁻¹) and Planverte (5l kg ha ⁻¹)
	TVITI	Pruning + lifting + topping	Orientation of branches inside wires and removal of branches that exceed the last wire of the trellis system
June, 2023	AVITI, DVITI	Mowing of inter-row vegetation + lifting + topping	Orientation of branches inside wires and removal of branches that exceed the last wire of the trellis system
	DVITI, TVITI	Soil tillage inside row	-
	DVITI	Green pruning	-

August, 2023	AVITI, DVITI, TVITI	Mechanical harvesting	-
	AVITI	Sowing of service crops	-
November, 2023	TVITI	Soil tillage inter-row and inside row	
December, 2023	AVITI, DVITI, TVITI	Pruning	-
	TVITI	Soil tillage inter-row	
January, 2024	AVITI, DVITI	Pruning	-
February, 2024	AVITI, DVITI	Lifting	Orientation of branches inside wires of the trellis system
	DVITI (trees)	Soil tillage + fertilization	NPK (07-04-07) 250 kg ha ⁻¹
March, 2024	AVITI, DVITI, TVITI	Soil fertilization	NPK (14-5-20) 260 kg ha ⁻¹
April, 2024	AVITI, DVITI	Mowing of inter-row vegetation	-
	AVITI, DVITI, TVITI	De-budding	Removal of the counter-bud
May, 2024	TVITI	Soil tillage	-

Annex C: Cumulative mean of dry biomass of species by year and system.

Year	System	Species	Cumulative mean of dry biomass
2023	AVITI	<i>Scabiosa atropurpurea</i>	0.85
		<i>Erigeron sumatrensis</i>	0.84
		<i>Festuca arundinacea</i>	0.75
		<i>Helminthotheca echioides</i>	0.70
		<i>Raphanus sativus</i>	0.66
		<i>Onobrychis viciifolia</i>	0.63
		<i>Rumex crispus</i>	0.60
		<i>Medicago sativa</i>	0.60
		<i>Plantago lanceolata</i>	0.55
	DVITI	<i>Dactylis glomerata</i>	0.83
		<i>Festuca arundinacea</i>	0.82
		<i>Helminthotheca echioides</i>	0.80
		<i>Malva sylvestris</i>	0.80
		<i>Crepis foetida</i>	0.79
		<i>Plantago lanceolata</i>	0.75
TVITI	<i>Erigeron sumatrensis</i>	0.71	
	<i>Onobrychis viciifolia</i>	0.68	
	<i>Carduus pycnocephalus</i>	1	
		<i>Malva sylvestris</i>	1
		<i>Rumex crispus</i>	1

	<i>Convolvulus arvensis</i>	0.97
	<hr/>	
	<i>Avena sativa</i>	0.62
	<i>Galium parisiense</i>	0.63
	<i>Helminthotheca echioides</i>	0.73
	<i>Lathyrus sativus</i>	0.65
	<i>Lolium perenne</i>	0.75
	<i>Malva sylvestris</i>	0.70
AVITI	<i>Medicago sativa</i>	0.62
	<i>Onobrychis viciifolia</i>	0.80
	<i>Papaver rhoeas</i>	0.52
	<i>Pisum sativum</i>	0.46
2024	<i>Plantago lanceolata</i>	0.47
	<i>Raphanus sativus</i>	0.77
	<i>Vicia faba</i>	0.56
	<hr/>	
	<i>Bromus sterilis</i>	0.75
	<i>Convolvulus arvensis</i>	0.80
	<i>Erodium malacoides</i>	0.57
DVITI	<i>Fumaria parviflora</i>	0.74
	<i>Galium parisiense</i>	0.79
	<i>Helminthotheca echioides</i>	0.69
	<i>Hordeum murinum</i>	0.80

	<i>Lamium amplexicum</i>	0.74
	<i>Lolium perenne</i>	0.70
	<i>Malva sylvestris</i>	0.65
	<i>Medicago sativa</i>	0.79
	<i>Onobrychis viciifolia</i>	0.52
	<i>Papaver rhoeas</i>	0.59
	<i>Plantago lanceolata</i>	0.77
	<i>Veronica persica</i>	0.76
	<hr/>	
	<i>Convolvulus arvensis</i>	0.88
TVITI	<i>Lamium amplexicum</i>	0.84
	<hr/>	