UNIVERSITÀ CATTOLICA DEL SACRO CUORE
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Ph.D. in Agro-Food System

Cycle XXXV
S.S.D. AGR03

Vineyard soil management: new sustainable approaches

Coordinator:
Ch.mo Prof. Paolo Ajmone Marsan

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Capri Caterina
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Table of contents

Citation List of Included Publications

Capri, C., Gatti, M., Guadagna, P., Del Zozzo, F., Magnanini, E., & Poni, S. (2021). A low-cost portable chamber based on Arduino micro-controller for measuring cover crops water use. *Computers and Electronics in Agriculture*, *190*, 106361.

Gatti, M., Garavani, A., Squeri, C., Capri, C., Diti, I., D'Ambrosio, R., ... & Poni, S. (2022). Inter-row floor management is a powerful factor for optimising vine balance in a non-irrigated organic Barbera vineyard in northern Italy. *European Journal of Agronomy*, *136*, 126490.

Capri, C., Gatti, M., Fiorini, A., Ardenti, F., Tabaglio, V., & Poni, S. (2023). A comparative study of fifteen cover crop species for orchard soil management: water uptake, root density traits and soil aggregate stability. *Scientific Reports,* 13, 721. https://doi.org/10.1038/s41598-023-27915-7

Abstract

This project explored the key role of floor management and cover crop use in viticulture. First reported, a four-year study conducted in an organically managed cv. Barbera vineyard in North-West Italy where five inter-row floor management treatments were tested. While under-trellis was maintained as lightly tilled, inter-row management treatments were i) permanent grass, ii) tillage, iii) alternate tillage and permanent grass every second mid-row, iv) a variant of this last treatment, where the tilled mid-row was used for growing a temporary winter cover crop terminated in spring, and v) temporary grass where the grass was disked post-harvest (mid-October) until natural growth resumption in late winter (mid-February). During the trial, soil profile and physicochemical composition, floristic analyses, vegetative growth, yield components, grape maturity at harvest, single leaf gas exchange as well as midday and pre-dawn leaf water potential were performed. Treatments tested provided different responses, highlighting how the technique can be diversified according to specific environmental and production needs. Alternation of tillage and permanent grass every second mid-row resulted in a reduction of the competition proportional to the degree of soil cover, thus making it possible to adjust operational protocols that can moderate the effects of competition according to the proportion of the grassed and tilled area. Both the temporary grass and the alternate tillage-cover crop treatments were effective in favoring vegetative growth compared to permanent spontaneous grassing, achieving the highest production. In particular, the first one led to an increased yeast available nitrogen (being particularly interesting for certain wine types) while the latter, together with an adequate technological and phenolic maturity, registered a significant decrease in K^+ accumulation in the must. This is interesting as mitigating K uptake in the vine and thus lowering the risk of excessive must and wine pH is among the main challenges posed by climate change.

Moreover, a new, custom-built, low-cost closed chamber system for vineyard cover crop evapotranspiration measurements was described here. Details for setup, calibration, and operational data were provided. Chamber calibration was performed either as instantaneous evaporation rates under laboratory conditions and daytime cumulative evapotranspiration rates performed outside in small pots sown with different cover crops (i.e., *Lotus corniculatus* and *Festuca arundinacea*) or managed with light tillage. A very close linear relationship between gravimetric vs chamber values were found for lab and outdoor calibration runs, and, interestingly, running calibration under ambient conditions (as opposed to controlled) greatly reduced chamber biases and provided the best accuracy. Hence, the chamber proved to be a reliable, efficient, and accurate way to measure

evapotranspiration for a range of time scales (i.e., instantaneous and cumulated daily) under bare soil conditions and sown crops.

Aimed at identifying cover crops for vineyard floor management, the last trial characterized several species according to their evapotranspiration rates, root growth patterns, and soil aggregate stability potential. The study was performed in Northern Italy, on bare soil (i.e., control) and fifteen cover crop species grown in pots kept outdoor and classified as grasses, legumes, and creeping plants. Evapotranspiration was assessed through a gravimetric method and using the new, custom-built closed portable chamber. Measures were performed starting before mowing and then repeated 2, 8, 17, and 25 days thereafter. Above-ground dry biomass, root length density, root dry weight and root diameter class length were measured, and mean weight diameter was calculated within 0-20 cm depth. The selection of cover crop species to be used in the vineyard was here mainly based on water use rates (i.e., evapotranspiration measurements) as well as the dynamic and extent of root growth patterns. In particular, among grasses, *Festuca ovina* stood out as the one with the lowest water use, making it suitable for a permanent inter-row covering. While, creeping plants confirmed their potential for under-vine grassing, assuring rapid soil coverage, lowest evapotranspiration rates, and shallow root colonization.

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Chapter 1. Introduction

1.1 General Introduction

Vineyards are frequently established on inherently poor soils (Coll et al., 2011) and subjected to intensive management practices, threatening soil functions and associated ecosystem services (Diti et al., 2020; Garcia et al., 2018; Salomé et al., 2016). Moreover, the Mediterranean climate is often characterized by severe summer droughts associated with short, yet heavy rainstorms in autumn-spring, favouring the run-off of surface waters (Rodrigo-Comino et al., 2018; Salomé et al., 2016), soil degradation and erosion (González-Hidalgo et al., 2007; Ruiz-Colmenero et al., 2011).

Vineyard floor management has proven to be potentially a very efficient practice to reach multiple goals such as improved weed management, soil health, and conservation, reducing soil resource availability to control vigour (hence with less stringent needs of summer pruning), concurrently achieving better indirect control of some diseases and positively influencing vine balance, grape composition and final wine appreciation (Steenwerth and Guerra, 2012). In general, while tillage and herbicide-induced bare soil are increasingly regarded as traditional and quite harmful techniques for the environment, end-users and consumers, cover crops (here referred to as either native resident or seeded vegetation) and mulching have gained a lot of credit as more sustainable practices (Diti et al., 2020; Ferreira et al., 2020; Novara et al., 2021).

Floor management solutions deriving from different combinations of the four basic practices (i.e., tillage, cover cropping, herbicides, and mulching) are many. In fact, in the large majority of cases, different alleyways and under-the-row practices are chosen. Moreover, there might be variability in space (i.e., varying width of a weed-free strip under the row, each row vs. alternate row patterns), time (i.e., permanent or temporary grassing), and type (i.e., grass and cover crop floristic composition, grass species undergoing a summer dormancy, winter cover crops suppressed with different termination methods, etc.). Furthermore, the aims of floor management might totally differ depending on climate conditions.

Even though the adoption of cover crops is suggested as a more sustainable practice as it allows for achieving many ecosystem services (ES), some ecosystem disservices (EDS) may be generated. Competition for soil resources (e.g., water and nutrients) is a good example of cover crop EDS (Celette and Gary, 2013; Klodd et al., 2016). This happens to be even more important in a viticultural context of climate change where, along with quite certain global warming, a higher frequency of hot spells and slightly reduced total precipitations are expected over most land areas on daily and seasonal timescales (Pachauri et al., 2014). In viticultural areas, these changes would lead to a reduction of the water available to plants and, as a likely consequence, the occurrence of significant summer drought will increase especially in traditionally non-irrigated districts with negative influences on both grape and wine quality (Mirás-Avalos and Intrigliolo, 2017; Pagay et al., 2016). Within such a scenario, the demand for irrigation will probably rise. To schedule irrigation events, a more comprehensive knowledge of the dynamic and magnitude of water used by all the vineyard ecosystem components (i.e., vines, grass, and soil) is needed (Centinari et al., 2013). Previous studies have mainly focused on quantifying the whole vine's transpiration rate (Dragoni et al., 2006; Poni et al., 2014) however, data available regarding direct measurements of the amount of water used by a grass cover in a vineyard are still quite limited (Centinari et al., 2012; Lopes et al., 2004; Uliarte et al., 2013).

Soil and grass cover water loss can be measured with different methods, each with advantages and limitations. Cover crop evapotranspiration can be gravimetrically determined by using mini-lysimeters, through micrometeorological techniques (e.g., the eddy covariance), or using chamber enclosures (either closed or open chambers). Even though micrometeorological techniques (e.g., eddy covariance) allow continuous measurements without disturbing the field micro-environment, they do not apply to small-scale experiments (Baldocchi, 2014). Conversely, chamber enclosure still holds as a non-invasive method for small-scale reading (Steduto et al., 2002). However, complex systems are required in order to maintain the micro-climate inside an open chamber reasonably close to ambient (Corelli-Grappadelli and Magnanini, 2019) and although cases, where an open chamber system was successfully used, are reported in the literature (Centinari et al., 2009), its portability is usually limited, and the airflow fed to the chamber needs to be carefully measured. Closed chamber systems were reported to better suit the need for portability (Luo et al., 2018) allowing several sampling locations in the field although there is some controversy concerning the accuracy of the closed chamber method when comparisons have been made with other evapotranspiration measurement techniques (Luo et al., 2018; McLeod et al., 2004; Steduto et al., 2002).

Being able to determine soil grass cover water consumption rates can be useful for more wisely scheduling vineyard irrigation events, but it can also be helpful in identifying herbaceous species with lower competitive potential (i.e., competition for water with vines) to be used in the vineyard, and more efficient cover crop management practices. Typically, the most common technique of cover cropping involves the management of native species as readily available and inexpensive (Diti et al., 2020; Pardini et al., 2002) yet, usually being the most competitive for both water and nutrients (Celette and Gary, 2013; Porqueddu' et al., 2000). To mitigate or remove competition, cover crop is often terminated in spring with tillage (Diti et al., 2020) although losing several benefits bound to the permanent cover of the vineyard soil (Biddoccu et al., 2020; Diti et al., 2020). Therefore, identifying appropriate strategies (i.e., cover crop species and adoption of the best cultural practices) to maintain the permanent soil cover benefits, while reducing cover crop competition in vineyards, is still necessary. According to the literature, mowing can be used as a useful short-term water preservation strategy (Celette and Gary, 2013; Centinari et al., 2013). After mowing, sward residual mass left *in situ* further protects the soil from erosion and runoff (Baumhardt and Blanco-Canqui, 2014; Prosdocimi et al., 2016), and improves soil health in the short term (Warren Raffa et al., 2021), while reducing water competition and soil evaporation (Centinari et al., 2013; Lopes, 2018). To exploit as many positive externalities as possible and to reduce the potential problems associated with the presence of CC in a vineyard, it is advisable to switch from the use of native species to sown (i.e., selected) ones (Pardini et al., 2002). Unfortunately, to date, the winegrowers' demand for low-competitive species is still largely unmet (Delpuech, 2013).

1.2 Research Objectives

Based on what was said above, it follows that a lack of knowledge exists about the fraction of soil cover crop coverage that might represent the best compromise between ES and EDS, and different cover crops' water use patterns. Moreover, as data available regarding direct measurements of the amount of water used by grass cover in a vineyard are still quite limited, we considered the need to develop a low-cost yet reliable and fast-enough instrument for water use assessments. Hence, the objectives of this project were to:

- Determine effective inter-row tillage/grassing combinations to regulate vine balance, maintain yield, and improve grape composition in a non-irrigated vineyard.
- Describe a new, custom-built, and low-cost closed chamber system for vineyard cover crop evapotranspiration measurements.
- Perform the closed chamber system proper calibration and provide examples of the kind of datasets and degree of accuracy that the system can achieve.
- Assess different cover crops' water use before and after mowing.
- Characterize selected cover crops root traits and clarify their effects on soil aggregation.
- Identify the most recommended species for vineyard cover cropping.

1.3 Linking Statement

The research presented in this thesis is ordered into chapters and includes three published manuscripts.

Chapter 1 is an introduction to the project and its aims.

Chapter 2 is a published manuscript presenting a four-year study (2017–2020) that was conducted in an organically managed cv. Barbera/420 A vineyard in the North-West of Italy, comparing five inter-row floor management treatments. Soil profile and physicochemical composition assessment and floristic analysis were also performed.

Chapter 3 is a published manuscript providing details for setup, calibration and operational data of a self-constructed, low-cost, small, closed-type chamber for measuring cover crops water use. The proposed device represents an effective Internet of Things (IoT) application, ideal for fast multipoint readings of grass (and soil) water losses in the field.

Chapter 4 is a published manuscript reporting the results of a comparative study of fifteen cover crops for orchard (e.g., vineyard) soil management. With the aim of identifying the most recommended species for vineyard cover cropping, characterization was based on their evapotranspiration rates, root growth patterns, and soil aggregate stability potential. The mowing effect on cover crops' water use dynamics was also assessed.

Chapter 5 is a brief concluding discussion. It considers the significance of the results reported in the different sections of this thesis and highlights their key findings and implications. It also considers the remaining questions and the possible direction that future research could take to achieve further understanding in this area.

Chapter 2. Published Manuscript 1: Inter-row floor management is a powerful factor for optimizing vine balance in a non-irrigated organic Barbera vineyard in northern Italy

2.1 Abstract

Floor management in organic viticulture plays a key role as weed suppression and soil health must be warranted through practices that minimize the recourse to extensive tillage and herbicides, while any resident vegetation or sown cover crop should exert moderate competition for water and nutrients towards the consociated vines. Lack of knowledge exists about the fraction of soil cover crop coverage (S_{cc}) which might represent the best compromise between the above needs. A four-year study (2017–2020) was conducted in an organically managed cv. Barbera/420 A vineyard in the North-West of Italy, comparing five floor management treatments each having light tillage as the practice chosen to control the under trellis weed growth. Inter-row treatments were permanent grass (PG), tillage (T), alternate tillage and permanent grass every second mid-row (AGT), a variant of this last treatment, where the tilled mid-row was used for growing a temporary winter cover crop terminated in spring (AGC) and temporary grass (TG) where grass was disked post-harvest (mid-October) until natural growth resumption in late winter (mid-February). An assessment was made for soil profile and physicochemical composition, floristic analyses performed in T, PG, and TG treatments, vegetative growth, yield components, grape maturity at harvest, single leaf gas exchange as well as midday and pre-dawn leaf water potential. While overall scant, mostly season-related differences were found for leaf function and water status, soil management heavily impacted vine performance. Year-round soil cover crop coverage (S_{cc}) regressed towards total pruning weight/vine and yield/vine showed high linear correlation ($R^2 = 0.93$) for pruning weight/vine (to be reduced by 38% at 75% S_{cc} vs. 0% S_{cc} of the T treatment), whereas yield/vine was quite poorly correlated ($R^2 = 0.21$) showing a 15% decrease in PG vs. T. Regressing S_{cc} vs total soluble solids (TSS), titratable acidity (TA), total anthocyanins and phenolics concentration disclosed mild linear correlation ($R^2 = 0.52$) for the two technology ripening parameters and a much tighter fit for colour and phenolics ($R^2 = 0.79$ and 0.90, respectively). AGT had an intermediate behaviour between its two extremes (i.e., T and PG) without assuring any significant marginal gain. Conversely, modulating PG into TG through a temporary removal of the resident vegetation in the fall and AGT into AGC by growing a winter cover crop terminated in the spring as mulching, gave the highest yield at adequate technological and phenolic ripeness. PG assured maximum grapes total soluble solids and total anthocyanin

concentration at harvest; however, due to its low vigour, several shortcomings also followed, such as low yeast available nitrogen and malic acid concentration, as well as a tendency to accumulate high amounts of flavonols. Our work led to the conclusion that AGC and TG treatments are quite valuable choices under the specific environment for successful soil management in organic vineyards.

2.2 Introduction

Vineyard floor management has proven to be potentially a very efficient practice to reach multiple goals such as improved weed management, soil health, and conservation, reducing soil resource availability to control vigour (hence with less stringent needs of summer pruning), concurrently achieving better indirect control of some diseases and positively influencing vine balance, grape composition and final wine appreciation (Steenwerth and Guerra, 2012). The four main soil management practices available today, that is, cultivation (tillage), grassing (meant either as native resident vegetation or sown cover cops), herbicides, and mulching have been the object of numerous comparisons under different environments and varieties (Abad et al., 2021; Ferreira et al., 2020; Steenwerth and Guerra, 2012). On a general basis, regardless of the area of conduct of each specific study, it can be stated that while tillage and herbicide-induced bare soil are increasingly regarded as traditional and quite harmful techniques for the environment, end-users and consumers, cover crops and mulching have gained a lot of credit as more sustainable practices, especially in an organic farming scenario or under soil conservation protocols (Diti et al., 2020a; Ferreira et al., 2020; Novara et al., 2021).

However, floor management solutions deriving from different combinations of the four basic practices are many: not just because, in the large majority of cases, different alleyway and under-the-row practices are chosen, but because there might be variability in space (i.e. varying width of a weed-free strip under the row, each row vs. alternate row patterns), time (i.e. permanent or temporary grassing) and type (i.e. grass and cover crop floristic composition, grass species undergoing a summer dormancy, winter cover crops suppressed with different termination methods, etc.).

Moreover, the aims of floor management might totally differ depending on climate conditions: in humid districts, the recourse to alleyway native or cover crop vegetation is highly recommended to better preserve soil structure, limit erosion, allow machinery transit, reduce excessive vine vigour and, in turn, promote fruit ripening (Belmonte et al., 2018; Bogunovic et al., 2019; Pérez-Álvarez, 2017; Reeve et al., 2016). However, native vegetation and five different cover crop mixtures established in alleyways and managed by spring and summer mowing were rather ineffective at reducing vine vigour, compared to a clean cultivated control in Western Oregon (Sweet and Schreiner, 2010). More lately, interest is increasing in a more aggressive approach using under-trellis cover crops (UTCC) to limit vine and nutrient resources and reduce excessive vine growth. Coniberti et al. (2018) have shown for cv. Tannat grown in a humid climate in Uruguay that replacing 1-m wide free-row strip with red fescue (*Festuca rubra*) significantly reduced the vigour with large benefits such as higher TSS and anthocyanins and less cluster rot incidence. Ideal results were also shown in Cabernet Sauvignon (Giese et al., 2014), where UTCC established with tall fescue (*Festuca arundinacea,* var. Elite II) replacing herbicides spraying resulted in greatly reduced vigour with no changes in yield and quality. However, results for the UTCC vs. herbicide-treated strip were not that positive in another trial on Cabernet Sauvignon (Hickey et al., 2016), where under-the-row red fescue curtailed pruning weight per vine by 26% and reduced average yield/ha by 6.1 t. Notably, a similar trial carried out on Riesling in the Finger Lakes Region (NY) showed that none of UTCC treatments made of resident vegetation, buckwheat (*Fagopyrum esculentum*) and annual ryegrass (*Lolium multiflorum*) was effective at modifying vine vigour, yield, and juice characteristics vs. a weed-free under-the-row strip maintained with glyphosate application (Jordan et al., 2016).

Adoption of grassing and cover crops in temperate Mediterranean climates is still regarded with suspicion due to the soil water and nutritive competition they exert on the grapevines within a vineyard ecosystem. The challenge here is especially daring, as even in such areas, tillage and resident vegetation are, the most adopted practices, with the former contributing to soil loss structure, erosion and fast degradation of organic matter and, the latter to excessive resource competition, even when limited to the alleyway area (Celette et al., 2009; Diti et al., 2020; Novara et al., 2018; Santos et al., 2020; Steenwerth and Guerra, 2012). In most of the vineyards planted in North-Central Italy, experiencing frequent drought in summer, mowed resident vegetation is by far preferred to any sown cover crop, for obvious practical and economic reasons (Diti et al., 2020). As already shown in the case studies about humid climates, the introduction of cover crops in non-irrigated Mediterranean vineyards has generated quite contrasting results. Once specified that UTCC is still a mostly unexplored option in such environments, the most studied comparison is when inter-row tillage is replaced with grassing, native or sown (Abad et al., 2021). The ideal outcome is that vine vigour is moderately decreased, yield maintained, or slightly curtailed and grape composition enhanced, especially as higher total soluble solids (TSS), anthocyanins, and other phenolics. Similar patterns were found in several studies (Baumgartner et al., 2008; Delpuech and Metay, 2018; Lee and Steenwerth, 2013; Linares Torres et al., 2018; Mercenaro et al., 2014; Monteiro and Lopes, 2007; Volaire and Lelièvre, 2010).

Conversely, introducing mid-alley resident or sown cover crops instead of tillage has brought disappointing results such as significant yield reductions with slight or no quality improvement (Cruz et al., 2012; Pou et al., 2011) or severe limitation of leaf gas exchanges (Cataldo et al., 2020; Mattii et al., 2005). Hence, the above scenario is even more puzzling if it is considered that eventual irrigation use might fully offset differences between cover crops vs. till practice (Steenwerth et al., 2013).

Italy ranks second in the world in acreage under organic vineyards (107,000 ha in 2019, equivalent to 16.5% of the total grape surface), testifying to sensitiveness towards a sustainable practice to increase biodiversity and maintain/promote ecosystem services (Borsato et al., 2020; Sandhu et al., 2010). However, floor management of organic vineyards sited in temperate and Mediterranean areas subjected to summer drought and high-temperature peaks clashes with the forbidden use of herbicides and the quite common trait of unfeasible or highly restricted use of irrigation water (Döring et al., 2019). Under such circumstances, the adoption of full tillage with any inherent disadvantage is still widespread, whereas the well-accepted alleyway resident vegetation is often conducive to excessive competition towards the vines.

The purpose of the present 4-year study is to test and recommend effective inter-row tillage/grassing combinations to regulate vine balance, maintain yield and improve grape composition in a non-irrigated organic vineyard.

2.3 Materials And Methods

2.3.1 Plant material and experimental layout.

The trial was conducted over four seasons (2017–2020) in a non-irrigated Barbera (*Vitis vinifera* L.) vineyard grafted onto 420A rootstock established in 2001 in Castelnovo Val Tidone, Colli Piacentini wine district, La Pernice Estate, (44°97’ N, 9°42’ E, 213 m a.s.l.), Italy. The vineyard is located on a south-facing versant at a moderate longitudinal slope (5%), with North–South (NS) oriented rows and vines trained to a single-cane vertically shoot positioned (VSP) Guyot trellis at a spacing of 2.4 m x 1 m (inter- and intra-row) for a density of 4166 vines/ha. Each vine had a bud-load of about eight nodes; the cane raised 90 cm from the ground with three pairs of top catch wires for a canopy wall extending about 1.5 m above the main wire. Standard regional protocol for organic viticulture was applied in all the trial years. The canopy was mechanically trimmed once shoots outgrew the top foliage wire.

While no shoot trimming was needed in 2017, trimming in the following years occurred on 12 June 2018, 8 July 2019, 10 June, and 20 July 2020. During each season, daily minimum, mean and maximum temperature (°C) and rainfall (mm) were recorded by a weather station located within the vineyard.

A randomized complete-block design with four blocks each containing one replicate per treatment was used. Every treatment x block combination was applied on a 200-260 m^2 area (28-36 m length and 7.2 m width) with at least 40-56 experimental vines identified along the two inner rows of a group of four adjacent rows excluding the initial and final 4 m sectors assumed as buffer zone. Four experimental vines per treatment x block combination were then randomly identified and assumed as sub-replicates for the entire trial duration. The five inter-row treatments were: i) permanent native grass (PG); ii) tillage (T); iii) alternate permanent native grass and tillage (AGT); iv) alternate permanent native grass and winter cover crop (AGC) and v) temporary native grass (TG).

2.3.2 Inter-row management and floristic assessment

In all treatments, under trellis, weed suppression was achieved every season by repeated tillage applied on a 60 cm wide soil strip after budburst, at berry lag-phase, and post-harvest. Permanent native grass (PG) had already been established at the end of the training period since 2003. In the tillage (T) treatment, the inter-row was spaded regularly in accordance with grass growth and weather conditions to limit native grass development; therefore, three runs per year were scheduled concurrently to the under-trellis tillage. The third treatment (AGT) corresponded to a mixed-management system, as native grass was alternated to soil tillage every second mid-row, thus reducing the fraction of soil surface covered by the resident vegetation. In AGC, a cover crop mixture was sown in the fall, with the following composition: Italian ryegrass (*Lolium multiflorum,* 20%), Triticale (x *Triticosecale*, 15%), Oat (*Avena sativa,* 15%), rye (*Secale cereale* L. 13%), vetch (*Vicia sativa* L., 10%), field pea (*Pisum sativum arvense* L., 10%), sainfoin (*Onobrychis viciifolia,* 5%), crimson clover (*Trifolium incarnatum,* 4%), horseradish (*Armoracia rusticana,* 4%), white mustard (*Brassica alba,* 2%), and Indian mustard (*Brassica juncea,* 2%). The alleys were spaded and harrowed to break up clods and create an adequate seedbed. Sowing was performed with a pneumatic seeder on 14 October 2016, 3 November 2017, 3 November 2018, and 13 February 2020 (the latter was postponed due to unfavourable soil conditions in autumn 2019). The cover crop was then mowed in the season following the sowing, when different species were around the flowering stage and swards were left in place with a combined mulching and green manuring function. TG consisted in suppressing post-harvest the inter-row resident vegetation through spading and harrowing, while waiting for natural regrowth in spring. Mowing of the native grass growing in the inter-rows of PG, AGT, AGC, and TG was performed when average grass height exceeded about 35 cm and cut swards were likewise left in place.

Year-round active soil cover crop soil coverage (S_{cc}) was calculated on the basis of between row distance (2.4 m, 0.6 m of which pertaining to the under trellis strip), as well as spatial fraction of tilled vs grassed soil and within season changes of tilled to grassed soil ratios in treatments AGC and TG. In greater details, in AGC annual temporary winter grassing provided significant additional soil cover in February, March and April when termination was performed as described above. Conversely, in TG, inter-row permanent grass was tilled mid-October and significant native grass regrowth occurred, on average, mid-February the following season. Due to such relative spatial and temporal changes of the grassed to tilled soil ratios, year-round calculated S_{cc} values were: 75.0% (PG), 0% (T), 37.5% (AGT), 46.9% (AGC) and 41.7% (TG). It must be noted that all percentages are calculated as static grassed soil fractions over total and neither dynamics in grass establishment in AGC and TG nor mid-row cover heterogeneity were considered.

Assessment of floristic composition in the inter-row space was made in June 2019 in PG, T, and AGC treatments by recording species inventory and abundance along three transects of about 60 meters for each block. T was included in the assessment, as the repeated tillage was not effective in preventing/removing resident vegetation, whereas, in AGC, an assessment was performed on the new native grass cover emerging after slashing of the winter grass. Plants were identified directly in the field or on samples and taken to the laboratory according to Italian Vascular Flora (Abbate et al., 2001). Plants were usually classified at the species level, although some of them were classified only at the genus level, as at the time of assessment, they were damaged, or their development was incomplete. Identified species were also grouped according to Raunkiaer classification (Raunkiaer, 1934) for the assessment of the biological spectra of each treatment. Abundance was defined in three classes scored as 1: < 50%; 2: 50–75% and 3: > 75% according to soil coverage with respect to other species.

2.3.3 Soil profile and analysis

The vineyard soil is characterised by silty alluvial sediments dating back to the middle to upper Quaternary period and deposited on a paleo-surface highly representative of the first terrace of the Emilia Romagna hills, where several wine districts are located. Based on the Regional Soil Map at 1:50,000 scale (Regione Emilia-Romagna, 2018), the vineyard falls within the CTD1/RIV1 Map Unit, as described by the delineation number 8622, corresponding to the consociation of the “Cittadella” and “Rivergaro” silty-loamy soils, the former with 1–5% slope grade.

Soil organic matter (SOM) was assessed before treatment application on the standard practice of PG as well as at the end of the four-year experiment. On 23 August 2016, twelve sampling points located in the alley between two adjacent rows were identified and drilled down to 0.30 m depth with a Dutch auger. Six composed samples were reunited to represent 0–0.15 m and 0.15–0.30 m depths. A post-trial survey was run on 18–19 January 2021, when a batch of 20 composed samples corresponding to every treatment x block combination was collected at both 0–0.15 m and 0.15–0.30 m depth intervals. All samples were sent to an external laboratory for determination of SOM by elemental analysis. In January 2021, soil bulk density (g cm^{-3}) in the 0.10–0.20 m layer was assessed according to the regional protocol for soil description (Regione Emilia-Romagna, 2002). For each treatment x block combination, undisturbed soil cores were sampled in triplicate using sample rings of 98.125 cm^3 in volume. The rings were carefully excavated, cleaned of soil adhering to the ring, and taken to the laboratory where dry weight was registered. No pre-trial samples were taken for estimating soil bulk density.

To link the experimental site to the Regional Soil Map at 1:50,000 scale, a soil profile down to 1.40 m depth was opened on 16 July 2019. For each horizon layer, a soil sample was collected for further determination of chemical and physical properties performed by the above-mentioned laboratory (Table 2.1). According to USDA – Natural Respource Conservation Siervice Service, gran sized based soil classification was of the Silty Clay Loam type (García-Gaines and Frankenstein, 2015).

2.3.4 Single-leaf gas exchange and water status assessment

In each season, leaf sampling was performed on different dates for assessing leaf function at fruit set, lag-phase of berry growth, and veraison. On two test vines, two leaves inserted at median shoot level (7–10th node) were measured for a total of 80 leaves. Assimilation (A, µmol m^{-2} s^{-1}), transpiration (E, mmol m^{-2} s^{-1}), and stomatal conductance (g_s, mol m^{-2} s^{-1}) were assessed with the portable gas-exchange analyser LCi-SD (ADC BioScientific Ltd, Hoddesdon, UK) featuring a broad-leaf chamber having a 6.25 cm^2 window. All readings were taken in the morning (10:00–13:00 solar time) under clear sky and saturating light conditions at ambient air temperature and CO_2 concentration. The same leaves were removed, and their midday stem water potential (ψ_{MD}) measured using a Scholander pressure chamber (3500 Model, Soilmoisture Equip. Corp., Santa Barbara, CA). On the same days, pre-dawn leaf water potential (ψ_{PD}) was measured at sunrise (~ 04.00) with the same method, although this time the leaves were sampled from basal shoot positions.

2.3.5 Vegetative growth, yield components, and grape composition at harvest

In each season, four representative shoots per treatment (one shoot per block) were removed at veraison when vegetative growth was almost complete. Thereafter, all main and lateral leaves were counted, and their size measured through a leaf-area meter (LI-COR 3000 Bioscience, Lincoln, NE). After leaf fall, the total number of nodes per vine on main and lateral canes was counted. Leaf area (LA) per vine was subsequently calculated by combining the mean leaf size and the corresponding node number, by keeping separate main and lateral wood contributions. One-year-old pruning mass was recorded at winter pruning keeping separate contributions for main and lateral canes. Source-to-sink vine balance was calculated as either the Ravaz index (yield-to-pruning weight ratio given as kg/kg) and the LA-to-yield ratio (m^2/kg).

Each year, usually at mid-May, total shoot and inflorescence numbers per vine were recorded and node fruitfulness was given as inflorescences/shoot ratio. Harvest was performed on 6 September 2017, 19 September 2018, 23 September 2019 and 6 September 2020, when, in the standard soil management treatment (PG) grape maturity showed TA ≤ 8.5 g/L and TSS was between 23 and 24 °Brix. At harvest, yield per vine was measured with a portable field scale, cluster number per vine recorded, and mean cluster mass calculated accordingly.

A three-basal-cluster sample was collected from each tagged vine, brought to the laboratory, and processed for subsequent determinations. Clusters were individually weighed, and their compactness was expressed as the ratio of total berry fresh mass to rachis plus the total main shoulders length ratio (g/cm). From the three clusters, 10 berries were collected and immediately frozen at -20°C. From each berry, the skin was carefully removed with a razor blade and a small metal spatula, stored and lyophilised for the determination of anthocyanins and phenolics by HPLC. Extraction was performed according to Downey and Rochfort (2008): 0.100 g of freeze-dried skin was extracted in 1.0 mL of 50% (v/v) methanol in water for 15 min with sonication. Anthocyanins and flavonols were analysed as prescribed by Poni et al. (2017), where the mobile phase consisted of a gradient mixture of solvent A (0.85% phosphoric acid solution) and solvent B (acetonitrile).

A second 50-berry subsample was used to determine the concentration of total anthocyanins and phenols after Iland (1988), expressed as mg/g of fresh berry mass. The remainder of each three-cluster sample was crushed, and the resulting musts were immediately analysed for total soluble solids concentration (TSS, as Brix), pH, titratable acidity (TA as g/L), and yeast available nitrogen (YAN). TSS concentration was determined using a temperature-compensating refractometer (RX-5000 ATAGO U.S.A., Bellevue, WA), pH was assessed with a pH-meter CRISON GLP 22 (Crison, Barcelona, Spain) and TA was measured by titration with 0.1 N NaOH to a pH 8.2 endpoint and expressed as g/L of tartaric acid equivalents. The YAN concentration was determined through the formol titration method as reported by Gump et al. (2002). The must potassium (K^+) concentration was measured by an ion-selective electrode (Model 96-61, Crison). The quantification of organic acid concentration in musts was assessed with a 0.22 µm polypropylene filter for HPLC and directly injected. Separation was performed in isocratic conditions using an Allure Organic Acid Column, 300 × 4.6 mm, 5 µm (Restek, Bellefonte, PA, USA). Organic acids were identified using authentic standards; quantification was based on peak areas and performed by external calibration with standards.

2.3.6 Statistical analysis

Data were subjected to a two-way analysis of variance (ANOVA) assuming vineyard floor management as the main factor, using IBM SPSS Statistics 27 (SPSS Inc., Chicago, USA). The year was considered as a random factor and the error term for the soil treatments was the year x treatment interaction mean square (Gomez and Gomez, 1984). The year x treatment interaction was tested over the pooled error and discussed only in case of significance. Normal distribution for each parameter was checked with the Kolmogorov–Smirnov test ($p < 0.001$). Homogeneity of error variances for data taken over different years was assessed with Bartlett's test. In the case of the significance of the Fisher test, means were compared by the Student-Newman Keuls (SNK) test, at $p < 0.05$.

Repeated measures of the same parameters (A and g_s rates) taken at different dates along the season were analyzed with the Repeated Measure analysis of variance routine embedded in the XLSTAT software package (Addinsoft, New York, NY, USA). The least squared (LS) mean method at $p<0.05$ was used for multiple comparisons within dates. The same XLSTAT package was used to perform regression analyses and R^2 calculations. Distributions of soil organic matter and soil bulk density data were displayed using box-and-whisker plots (Mead et al., 2017).

2.4 Results

2.4.1 Weather trends, soil status, and floristic composition

Active heat summation given as growing degree days (GDD) and calculated for the period 1 April–30 September was between the highest at 1912 °C (2018) and the lowest at 1744 °C (2019) – data not shown. 2019 was the wettest season, with 584 mm of rain *vis-a-vis* the minimum of 257 mm recorded in 2017. However, 2019 had an unseasonal hot month in June, when the daily T max peaked at 36.7 °C, whereas in the other seasons, peak temperatures were always recorded in July and/or August. To resemble seasonal trends, annual rainfall varied between the lowest value of 2017 (493mm) to the highest in 2019 (939mm) whilst in 2018 and 2020 intermediate precipitation values were registered (725 and 632mm, respectively).

Different soil horizons at the trial site showed a quite uniform textural composition which, averaged over the 0–1.40 m total depth, resulted in 5% sand, 59% silt, and 36% clay. Soil organic matter (SOM) showed a fast decrease with soil depth and was ≤ 0.5% at soil depths ≤ 0.6 m. Soil pH and lime contents were within ranges not conducive to iron chlorosis, sometimes observed in the area (Table 2.1).

Table 2.1. Variation of chemical and physical soil properties of the Barbera vineyard depending on soil horizons described along the profile excavated in July 2019.

Horizon	Depth (m)	Sand (%)	Silt (%)	Clay (%)	pH	Total lime (%)	Active lime (%)	SOM (%)	K_2O (mg/kg)	P_2O_5 (ppm)	Total N (g/kg)
Ap1	0-0.15	5	58	37	6.44	1.1	0.90	1.86	183	22.3	0.80
Ap2	0.15-0.30	6	63	31	6.89	1	< 0.50	0.88	131	24.2	0.73
Ap3	0.30-0.60	5	55	40	6.99	1.1	0.99	0.76	92	19.6	0.61
Bt1	0.60-0.90	4	54	42	7.04	1.1	0.51	0.46	64	19.7	0.69
Bt2	0.90-1.10	4	57	39	7.85	1	0.97	0.26	27	20.9	0.64
Bt3	1.10-1.40	4	70	26	8.09	1	0.71	0.19	26	26.1	0.54

Floristic composition and species abundance evaluated in treatments PG, T and AGC are shown in Table 2.2. The total number of grass species detected in PG and AGC plots were 34 and 35 respectively, against 13 species described in the tilled alleys. The highest frequency of Raunkiaer life-form categories were found across treatments for hemicryptophites (from 34.3% in AGC to 41.2% in PG) and for therophytes (from 20.0% in T to 37.1 in AGC).

Pre-trial SOM at 0–0.15 and 0.15–0.30 m soil depth intervals was 2.17 and 1.7% respectively (data not shown). Vineyard floor management influenced SOM in the 0–0.15 m layer varying between 2.2% (PG) and 1.6% (T) (Figure 2.1A). Topsoil layer SOM was not increased in AGT and AGC as compared to T, whereas a slight increase was recorded for TG, which aligned with PG. Conversely, SOM was frequently < 1.5% at 0.15–0.30 m depths, and general overlapping among boxes shows that treatments effect was quite mild. Although soil bulk density was not assessed pre-trial in PG, end of trial readings showed its value significantly reduced in T, AGC and TG vs. PG and AGT (Figure 2.1B). Notably, box-plots for PG and T showed a high level of skewness; PG had a positively skewed distribution, whereas T's data group was negatively skewed.

2.4.2 Vegetative growth, yield components, and grape composition at harvest

With all the treatments sharing the same under trellis management (light tillage for periodic weed removal), inter-row management affected leaf area per vine which, for data pooled over the 4 years, was the highest for T (2.6 m^2), lowest for PG (1.7 m^2, i.e., 35% less) and intermediate for the remaining soil treatments (Table 2.3). The same order of differences was found for total pruning weight per vine and its components (main and lateral pruning weight) although the latter was curtailed in PG by 70%, compared to T. However, each pruning weight component showed a significant ($P < 0.01$) treatment x year (T x Y) interaction which is partitioned in Figure 2.2A–D. The nature of the interaction is mostly explained by the different behaviour of PG over seasons, as compared to the other practices. Taking lateral pruning weight as a reference (Figure 2.2B), it is rather evident that in PG, a major suppression of lateral growth is found regardless of differences in precipitations recorded in May and June, when vegetative growth is the most active: in 2019, this reached 249 mm, as against the lowest quantity of 106 mm scored in 2017. Conversely, lateral formation in all the remaining treatments was more responsive to rainfall, and in the quite wet 2019, all managements leveled at around 150 g/vine. Possibly, due to the homogenizing effect due to shoot trimming, the same effect was quite confounded when the main pruning weight was considered (Figure 2.2A).

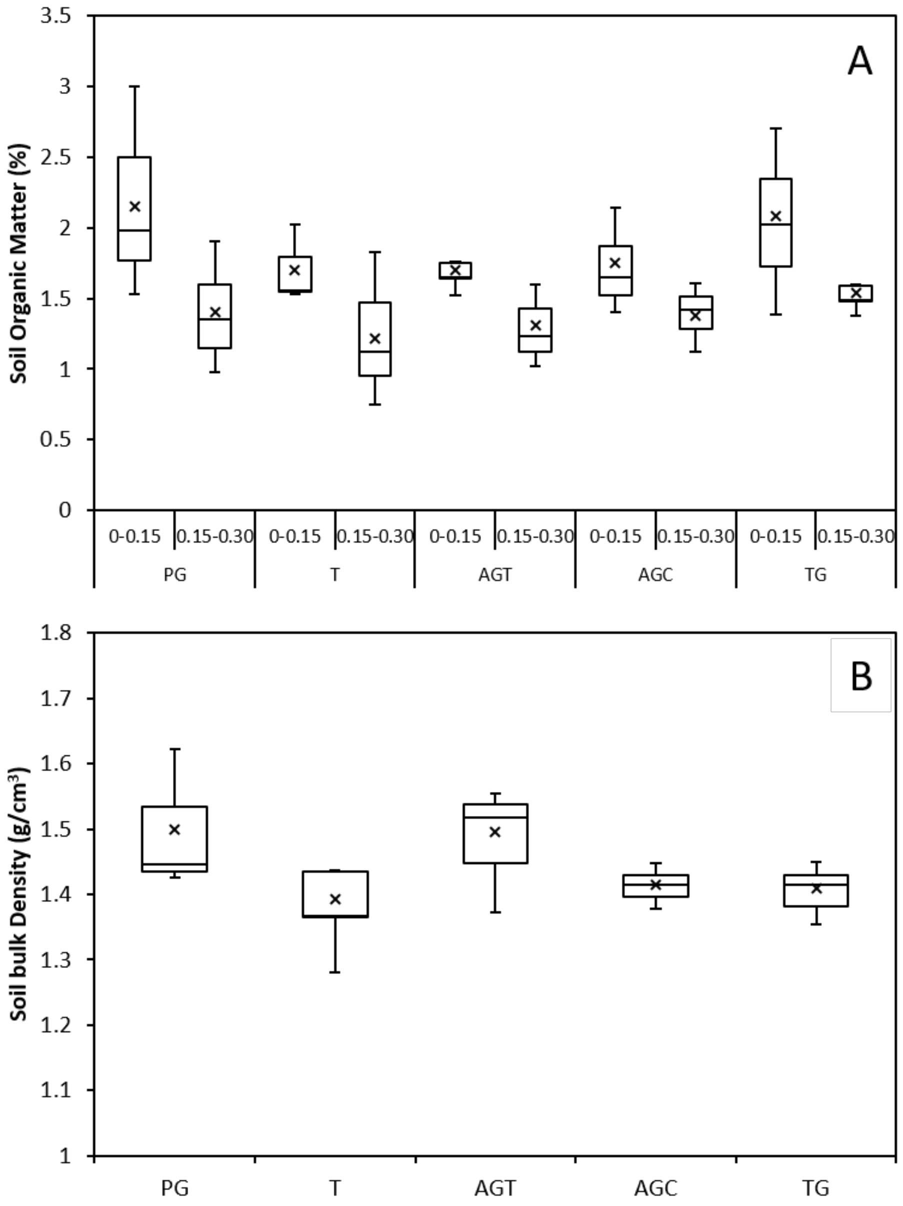


Figure 2.1. Box plots of soil organic matter (A) and soil bulk density (B) values determined on soil samples taken at 0–0.15 m and 0.15–0.30 depth for each treatment combination (n = 20). Samples are representative of end-of-trial situation (January 2021). Each box contains the middle 50% of data values and the median is marked with a horizontal line across the box. The lines either side of the box show the range of the lower (Q1) and upper (Q3) quartile of data values. Vertical bars protruding from the box indicate minimum and maximum values, whereas x is the data mean.

Table 2.2. Floristic composition and species abundance described for treatments PG, T and AGC in May 2019. Single species abundance is expressed as based on three classes scored as 1: <50%; 2: 50-75% and 3: >75%. The bottom row shows total number of species identified within treatment.

FAMILY	SPECIES	PG	T	AGC
AMARYLLIDACEAE J.St.-Hil.	*Allium spp*	2	2	3
APIACEAE Lindl.	*Daucus carota* L.	2	1	3
ASTERACEAE Bercht. & J.Presl	*Bellis perennis* L.	2	-	-
	Chicorium spp.	-	-	1
	Cirsium arvense (L.) Scop.	-	1	1
	Crepis spp.	3	2	3
	Erigeron canadensis L.	-	-	2
	Helminthotheca echioides (L.) Holub	1	-	2
	Lactuca saligna L.	3	1	3
	Lactuca virosa L.	1	-	-
	Matricaria chamomilla L.	1	1	1
	Taraxacum officinale (group) sin. *Taraxacum* sect. *Taraxacum*	3	1	2
BORAGINACEAE Juss.	*Heliotropium europaeum* L.	1	-	1
BRASSICACEAE Burnett	*Capsella bursa-pastoris* (L.) Medik.	1	-	-
	Raphanus sativus L.	-	-	1
CARYOPHYLLACEAE Juss	*Stellaria media* L.	3	2	2
CONVOLVULACEAE Juss.	*Convolvulus arvensis* L.	3	-	3
EUPHORBIACEAE Juss	*Euphorbia characias* L.	2	2	1
FABACEAE Lindl.	*Medicago spp.*	1	-	2
	Trifolium campestre Schreb.	1	-	2
	Vicia sativa L. s.l.	2	-	3
GERANIACEAE Juss.	*Geranium robertianum* L.	3	1	3
PLANTAGINACEAE Juss	*Plantago lanceolata* L.	2	-	3
	Plantago major L. s.l.	2	-	1
	Veronica persica Poir.	2	-	2
POACEAE Barnhart	*Alopecurus myosuroides* Huds.	-	-	1
	Avena fatua L.	1	-	-
	Avena sativa L. s.l.	3	2	3
	Bromus hordeaceus L. s.l.	2	-	-
	Cynodon dactylon (L.) Pers.	1	-	-
	Dactylis glomerata L.	3	-	-
	Digitaria ischaemum (Schreb. ex Schweigg.) Schreb. ex Muhl. subsp. *ischaemum*	-	-	1
	Festuca spp	2	2	1
	Hordeum vulgare L.	2	-	3
	Lolium multiflorum Lam.	1	-	2
	Lolium perenne L.	3	-	3
	Poa spp	-	1	1
	Poa trivialis L.	-	-	1
	Setaria viridis (L.) P. Beauv. s.l.	-	-	1
	x Triticosecale	-	-	1
POLYGONACEAE Juss.	*Rumex crispus* L.	3	3	3
	Rumex obtusifolius L. s.l.	1	2	-
VERBENACEAE J.St.-Hil.	*Verbena officinalis* L.	2	-	2
TOTAL		34	15	35

Table 2.3. Leaf area components per vine (main, lateral, total) and winter pruning weight components per vine (main, lateral, total) recorded over four years (2017-2020) on the field-grown cv. Barbera grapevines subjected to different interrow soil practices.

	Main leaf area/vine (m^2)	Lateral leaf area/vine (m^2)	Total leaf area/vine (m^2)	Main pruning weight/vine (g)	Lateral pruning weight/vine (g)	Total pruning weight/vine (g)
Treatment (T)						
PG	1.58c	0.22c	1.70c	307b	32c	339c
T	2.10a	0.50a	2.60a	440a	105a	545a
AGT	2.06ab	0.27b	2.33b	364bc	79ab	443b
AGC	2.03ab	0.32b	2.35b	377b	80ab	457b
TG	1.90b	0.37b	2.17b	359bc	65b	424b
F-prob	**	**	**	**	**	**
Year (Y)						
2017	2.18a	0.41a	2.59a	241d	18c	259c
2018	2.23a	0.38	2.61a	524a	59b	583a
2019	1.56b	0.40a	1.96b	298c	148a	446b
2020	1.75b	0.21c	1.96b	407b	68b	474b
F-prob	**	**	**	**	**	**
TxY	ns	ns	ns	**	**	**

Within column, in case of significant F test, mean separation was performed by Student-Newman-Keuls (SNK) test. * = p <0.05, ** p < 0.01, ns = not significant.

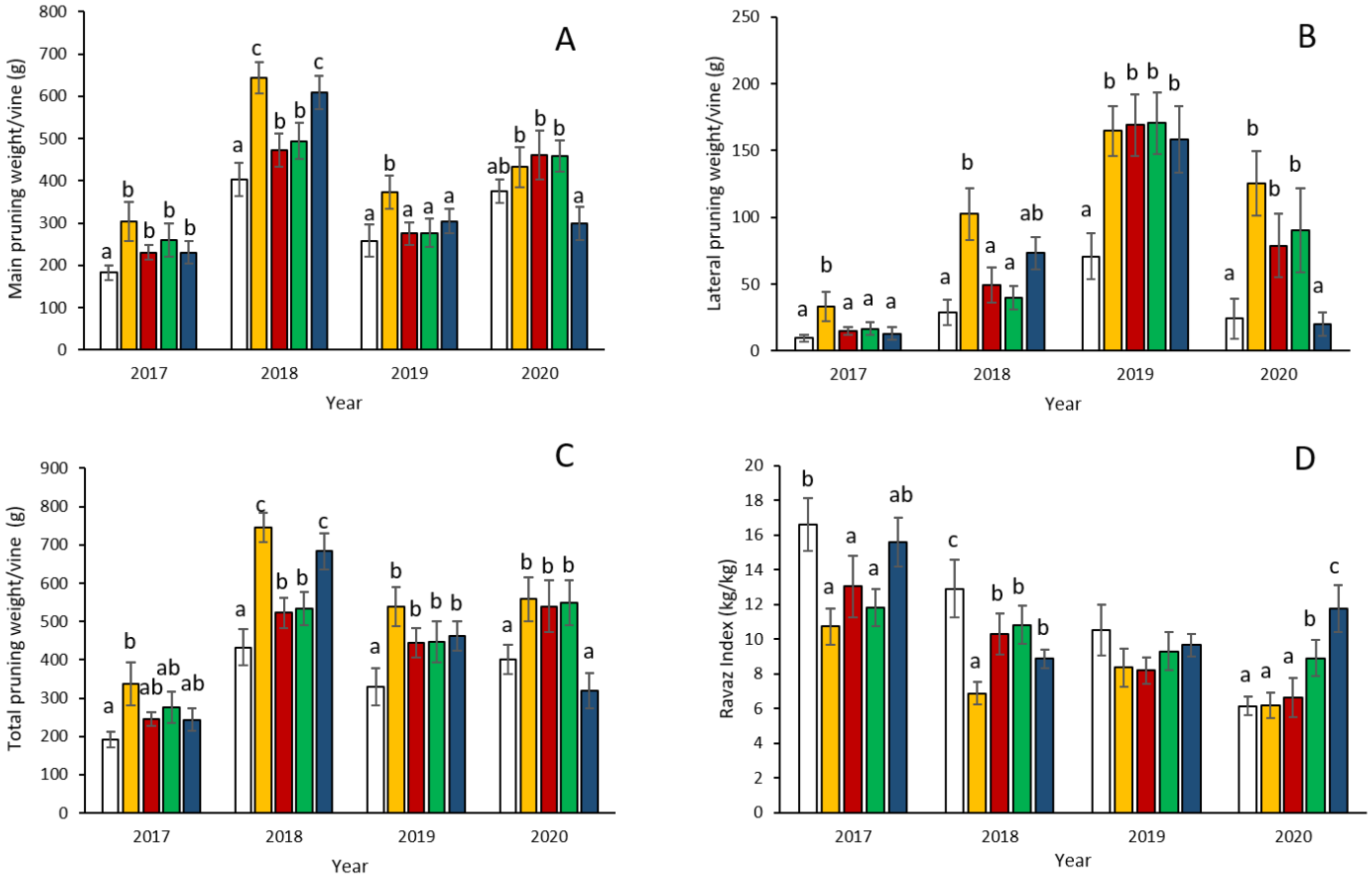


Figure 2.2. Partitioning of the significant year x treatment interactions recorded for main pruning weight/vine (A), lateral pruning weight/vine (B), total pruning weight/vine (C) and the Ravaz index (D). Colour codes for soil treatments are: ☐ = PG; 🟧 = T; 🟥 = AGT; 🟩 = AGC; 🟦 = TG. Data are means for each year x treatment combination (n = 4) and vertical bars are standard errors (SE).

The yield per vine and its main components were not that responsive to inter-annual variation, as only the main effects were found (Table 2.4). Starting with very high uniformity in cluster number per plant (19–20), T, AGC, and TG treatments had the highest yield per vine, setting around 4 kg to correspond to 16.7 t/ha. Conversely, in PG and AGT, yield reduction vis-a-vis the T treatment was 14% and 9% respectively. Interestingly, the relative variation among soil treatments of the two yield components of berry and cluster weight was essentially the same. Regardless of how vine balance was expressed, the highest amount of source per unit of crop was reached in the T treatment (leaf area-to-yield ratio = 0.68 m^2/kg and Ravaz Index = 7.0), whereas the most source-limited treatments were PG and TG. Processing of the Ravaz Index data described a significant T x Y interaction (Figure 2.2D) showing the highest sensitivity to seasonal conditions of the vine balance in PG, with a variation coefficient (CV) of 39.5 %, whereas the most stable practices were T and AGC (CV around 24%), although recently introduced.

Table 2.4. Yield components, cluster characteristics and vine balance given as leaf area-to-fruit ratio and Ravaz index (yield-to-pruning weight ratio) recorded over four years (2017 - 2020) on the field grown cv. Barbera grapevines subjected to different interrow soil treatments.

	Clusters/ shoot	Clusters/ vine	Cluster weight (g)	Berry weight (g)	Yield/ vine (kg)	Cluster compactness (g/cm)	Leaf area-to-yield ratio (m^2/kg)	Ravaz Index (kg/kg)
Treatment (T)								
PG	1.4	20	165b	2.01b	3.31b	15.1	0.51b	9.8a
T	1.5	19	202a	2.23a	3.84a	17.5	0.68a	7.0c
AGT	1.5	20	178b	2.02b	3.56ab	14.7	0.65a	8.0b
AGC	1.4	19	211a	2.22a	4.01a	16.8	0.59b	8.8ab
TG	1.5	19	213a	2.24a	4.05a	16.8	0.54b	9.5a
F-prob	ns	ns	**	**	**	ns	**	**
Year (Y)								
2017	1.4b	18b	175b	2.20a	3.15b	16.7a	0.82a	12.1a
2018	1.7a	25a	215a	2.21a	5.38a	17.1a	0.48c	9.2b
2019	1.3b	18b	206a	2.14a	3.71b	16.5a	0.53c	8.3bc
2020	1.3b	18b	178b	2.03b	3.21b	14.4b	0.61b	6.8c
F-prob	**	**	**	**	**	*	**	**
T x Y	ns	ns	ns	ns	ns	ns	ns	**

Within column, in case of significant F test, mean separation was performed by Student-Newman-Keuls (SNK) test. * = $p < 0.05$, ** $p < 0.01$, ns = not significant.

When year-round soil cover crop coverage (%) resulting from the different tilled/grassed combinations in the five treatments was correlated with pruning weight/vine, leaf area/vine, and yield/vine (Figure 2.3A) it was apparent that the two vegetative variables yielded a very close correlation ($R^2 = 0.93$ and 0.85, respectively), whereas the correlation was looser for yield/vine ($R^2 = 0.21$) and the fitted linear model non-significant. PW was reduced by 38% at 75% S_{cc} vs. 0% S_{cc} of the T treatment), whereas yield/vine was curtailed by 15% only in PG vs. T.

Different floor management affected the final grape composition (Table 2.5) and for most of the variables, a significant T x Y interaction was found. In terms of the main effects for data pooled over years, PG had the highest TSS (24.2 Brix) and AGT the lowest (22.1 Brix). However, in the dry and moderately cropped 2017 season, all treatments had an unrestricted sugar accumulation approaching the physiological threshold of 25 °Brix, whilst in the remaining years, PG maintained higher TSS than any other practice, with the maximum gains, compared to T and AGT (Figure 2.4A).

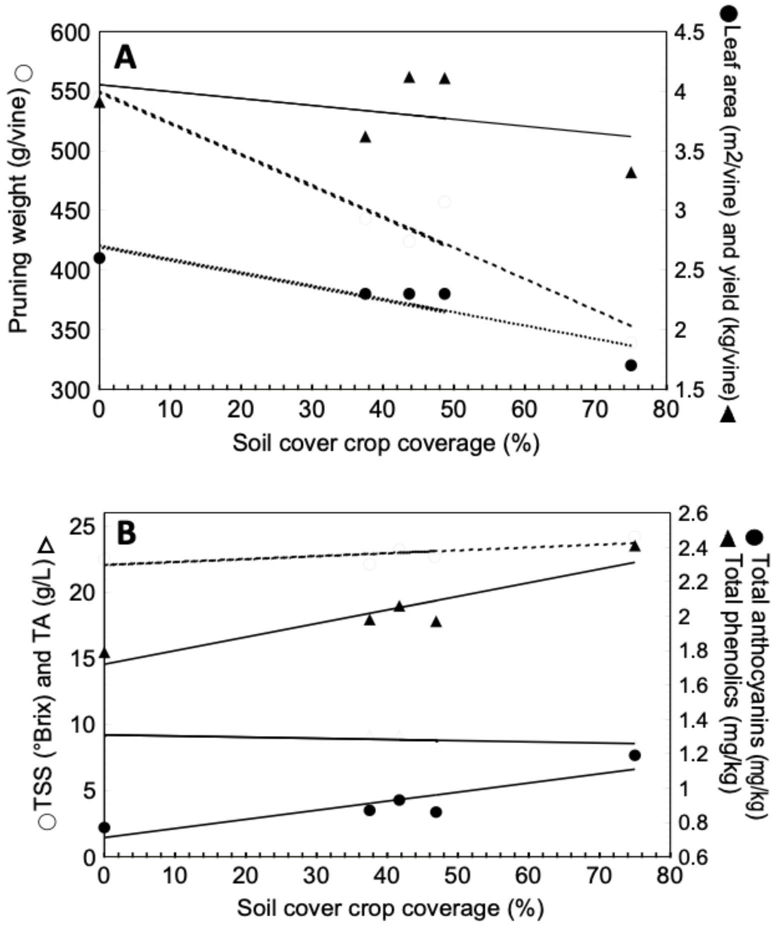


Figure 2.3. Panel A: linear regressions of soil cover crop coverage (%) vs. total pruning weight/vine, total leaf area/vine, and total yield/vine. Each data point is the mean pooled over years (n = 4). Linear model equations were: $Y = -2.6276x + 549.28$, $R^2 = 0.93$ (○), F prob = 0.007; $y = -0.0112x + 2.699$, $R^2 = 0.85$, F prob = 0.020 (●) and $y = -0.0058x + 4.054$, $R^2 = 0.21$, F prob = 0.400 (▲). Panel B: linear regressions of soil cover crop coverage vs. TSS, TA, total anthocyanins and total phenolics. Each data point is the mean pooled over years (n = 4). Linear model equations were: $y =0.00218x +22.07$, $R^2 = 0.52$, F prob = 0.168 (○); $y = -0.088x +9.22$, $R^2 = 0.52$, F prob = 0.170 (△); $y = 0.00531x +0.712$, $R^2 = 0.79$, F prob = 0.044 (●), $y = 0.0079x +1.72$, $R^2 = 0.90$, F prob = 0.020 (▲).

This pattern was essentially mirrored by the TA data (Figure 2.4B) that was quite uniform in 2017 (between 7.5 and 8 g/L across soil treatments), whereas in the following seasons, PG maintained a lower TA pool mostly contributed by a differential response in terms of malic acid (Figure 2.4C). Again, except for 2017, malic acid concentration in the final must of PG vines was quite drastically curtailed, compared to most of the other year x treatment combinations. Must K^+ concentration determined at harvest was rather uniform across treatments, but for AGC showing a reduced K accumulation in the berries (Table 2.5). Yeast Available Nitrogen (YAN) also resulted in a significant T x Y interaction, again emphasising low and similar values among treatments in 2017 and, especially in 2018 and 2019, a quite severe YAN starvation in the PG vines *vis-à-vis* the other treatments (Figure 2.4D).

Table 2.5. Must composition and total yeast available nitrogen (YAN) recorded over four years (2017-2020) at harvest on the field grown cv. Barbera grapevines subjected to different interrow soil treatments. TSS = total soluble solids, TA = titratable acidity, YAN = Yeast Available Nitrogen.

	TSS (°Brix)	pH	TA (g/L)	Tartrate (g/L)	Malate (g/L)	YAN (mg/L)	K^+ (mg/L)	Anthocyanins (mg/g)	Phenolics (mg/g)
Treatment (T)									
PG	24.2a	2.97	8.42b	10.30a	1.38d	100c	1475a	1.19a	2.41a
T	22.5b	3.05	9.06a	8.78b	2.08a	180a	1352a	0.77c	1.79c
AGT	22.1b	2.98	9.14a	10.21a	1.74bc	161a	1351a	0.87b	1.98b
AGC	22.7ab	3.04	8.63ab	9.03b	1.62c	135b	1165b	0.86b	1.97b
TG	23.2ab	3.09	9.11a	10.46a	1.90ab	162a	1405a	0.93b	2.06b
F-prob	**	ns	**	**	**	**	**	**	**
Year (Y)									
2017	25.0a	3.05	7.36c	5.74c	1.38c	75d	1127c	1.03a	2.30a
2018	20.8d	2.99	9.08b	10.38b	1.16d	217a	880d	1.00a	2.06b
2019	21.6c	3.04	9.32ab	10.52b	2.47a	90c	1332b	0.90b	1.98b
2020	24.5b	3.04	9.68a	12.24a	1.98b	201b	2036a	0.78c	1.84c
F-prob	**	ns	**	**	**	**	**	**	**
T x Y	**	ns	**	ns	**	**	ns	**	**

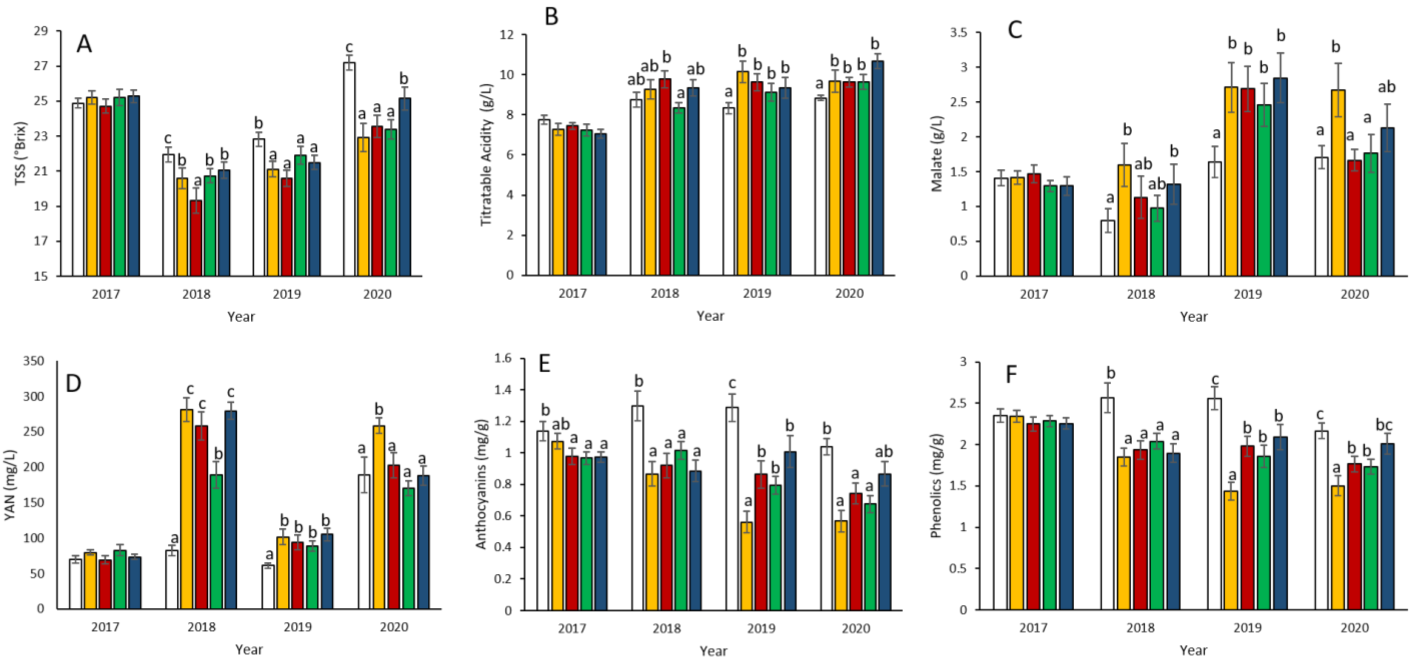
Within column, in case of significant F test, mean separation was performed by Student-Newman-Keuls (SNK) test. * = p <0.05, ** p < 0.01, ns = not significant.

Total anthocyanins and phenolics concentrations determined at harvest did confirm significant year effect across treatments (Figures 2.4E, F). In general, PG stood out as the most efficient in colour accumulation regardless of season, with amplified differences in 2018, 2019 and 2020, especially when compared to T (Figure 2.4E). The closest treatment to PG was TG scoring 0.93 mg/kg of anthocyanins, still significantly lower than the 1.19 mg/kg reached by PG for data pooled over years. The total phenolic concentration closely mirrored the pattern just described for total anthocyanins concentration (Figure 2.4F).

Correlating year-round soil cover crop coverage (%) with TSS, TA, total anthocyanins and phenolics (Figure 2.3B) showed that the two phenolic maturity variables, i.e., total anthocyanins and total phenolics had a quite close linear correlation vs S_{cc} (R^2 = 0.79 and 0.90, respectively), whereas the correlation was less tight for TSS and TA (R^2 = 0.52 for both) and the linear models fitted to the data did not reach the 5% probability level.

Assessment of different flavonols and anthocyanidins carried out each year on berry skins highlighted that PG consistently affected the main flavonols, as myricetin 3-O-glucoside and quercetin 3-O-glucoside were higher than any other treatment for data pooled over the four harvest seasons (Table 2.6). PG also retained the highest anthocyanidin concentration, although the largest difference from the other treatments was found for delphinidin-3-O-glucoside and petunidin-3-O-glucoside. The treatments did not affect the anthocyanin profile by keeping similar proportions among single anthocyanidins.

Figure 2.4. Partitioning of the significant year x treatment interactions recorded for total soluble solids (A), titratable acidity (B), malate (C), yeast available nitrogen (D), total anthocyanins (E) and total phenols (F). Colour codes for soil treatments are: □ = PG; 🟧 = T; 🟥 = AGT; 🟩 = AGC; 🟦 = TG.



Data are means of each year x treatment combination (n = 4) and vertical bars are standard errors (SE).

2.4.3 Leaf gas exchange and water status

In each season, single-leaf gas exchange rates were taken at dates corresponding to fruit-set, lag-phase of berry growth, and the onset of veraison. In 2017, predawn leaf water potential (ψ_{pd}) showed scant variation over the progressing season with values between -0.38 and -0.55 MPa, whereas in the remaining seasons, values that are more negative were recorded along subsequent stages (Table 2.7). The most stressful values (down to -0.87 MPa in AGT and PG) were recorded at veraison in 2018. Looking at year x dates data sets, it was apparent that very limited treatment effects were found for $\psi_{pd} \geq -0.40$ MPa, whereas when this threshold was overcome, floor management tended to nicely separate with T being the least stressed, PG the highest, and the remaining treatments often setting at intermediate values.

A somewhat similar scenario was shown by the midday leaf water potential (ψ_{md}) values showing limited among-treatments variation for $\psi_{md} \geq -1.0$ MPa and, beyond this threshold, lower values in PG vs. T for 2017 and 2019. Such difference did not reach significance in 2018 and 2020.

Across treatments, the leaf assimilation (A) rate showed a progressive decline over each season, and the largest reductions were seen in 2018 and 2020 (Table 2.7). Overall, for each year, within-date treatment differences were rather occasional or inconsistent. As a confirmation, when regressing A rates vs. ψ_{pd} (Figure 2.5A–D) and ψ_{md} (Figure 2.5E–H) although a quadratic model closely fit the data in each season (R^2 varying from 0.52 to 0.90 for A vs. ψ_{pd} and from 0.48 to 0.94 for A vs. ψ_{md}) no specific grouping could be assigned to a given treatment, whereas data points referring to different measuring dates were separated. Among years, the main difference was that while the fruit-set readings taken in 2018, 2019, 2020 started from optimal ψ_{pd} values (around -0.2 MPa), in 2017, fruit-set assessment already had ψ_{pd} down to -0.4 MPa.

Leaf stomatal conductance (g_s) showed somewhat higher responsiveness to soil treatments within each season, again without providing evidence of a consistent differential behaviour among treatments. Significant linear or quadratic models were fit to g_s vs. ψ_{pd} (R^2 varying from 0.70 to 0.94) and to g_s vs. ψ_{md} (R^2 varying from 0.83 to 0.93) (Figure 2.6A–D and 2.6E–H).

Intrinsic water use efficiency, derived as the A/g_s ratio also resulted in occasional differences among soil treatments, confirming that no practice stood out for either improved or diminished WUE_i (Table 2.7). Correlating leaf gas exchange and water stress variables with fraction of S_{cc} (yearly soil treatment data pooled over the three timings of sampling within each season) did not yield any significant fit and R^2 calculated for linear models ranged between 0.002 (S_{cc} vs g_s), and 0.28 (S_{cc} vs Ψ_{MD}).

Table 2.6. Skin flavonols and anthocyanins concentration over four years (2017-2020) on field grown cv. Barbera grapevines subjected to different interrow soil practices.

	Myricetin 3-O-glucoside (mg/g)	Quercetin 3-O-glucoside (mg/g)	Myricetin (mg/g)	Kaempferol 3-O-glucoside (mg/g)	Delphinidin 3-O-glucoside (mg/g)	Cyanidin 3-O-glucoside (mg/g)	Petunidin 3-O-glucoside (mg/g)	Peonidin 3-O-glucoside (mg/g)	Malvidin 3-O-glucoside (mg/g)	Acylated (mg/g)	Coumarated (mg/g)
Treatment (T)											
PG	0.626a	2.207a	0.013	0.068a	3.043a	0.879a	3.606a	0.915	13.931a	4.797a	4.637a
T	0.423b	1.641b	0.012	0.052b	1.640b	0.626ab	2.100b	0.696	9.076b	3.437b	3.554b
AGT	0.441b	1.831b	0.011	0.048b	2.225b	0.778ab	2.762b	0.837	11.863b	4.294ab	4.676a
AGC	0.440b	1.920b	0.015	0.060ab	1.844b	0.569b	2.422b	0.664	10.506ab	3.751b	3.875ab
TG	0.465b	1.885b	0.015	0.058ab	2.193b	0.677ab	2.740b	0.754	11.342ab	4.128ab	4.175ab
F-prob	**	**	ns	**	**	*	**	ns	**	**	*
Year (Y)											
2017	0.698a	2.120b	0.011b	0.075a	2.469a	0.571b	2.895ab	0.585b	10.494b	4.177b	3.913b
2018	0.503b	1.521c	0.004c	0.039b	2.447a	0.600b	3.315a	0.769ab	15.607a	5.255a	5.945a
2019	0.381c	1.572c	0.007bc	0.039b	2.288a	0.872a	2.752b	0.972a	11.542b	3.965b	3.805b
2020	0.328c	2.403a	0.022a	0.078a	1.517b	0.780ab	1.898c	0.763ab	7.515c	2.855c	2.986c
F-prob	**	**	**	**	*	**	**	**	**	**	**
TxY	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

Within column, in case of significant F test, mean separation was performed by Student-Newman-Keuls (SNK) test. * = $p < 0.05$, ** $p < 0.01$, ns = not significant.

2.5 Discussion

Long-term comparison of five different floor management techniques in a mature Barbera vineyard showed that while single-leaf gas exchange and water status in each treatment were overall mildly affected, vine performance as vegetative growth, crop load, and grape quality were significantly impacted. A common feature of all the treatments was an under-the-row mechanical tillage (60 cm wide row strip over a 2.4 m between row spacing) to eliminate weeds in an organic viticulture scenario. Therefore, observed differences are due to the different inter-row soil management that featured varying combinations in space and time of tillage, native grass, and sown cover crop.

The response of PW to varying S_{cc} fits nicely with the three-year data reported on Shiraz grapevines growing in a French Mediterranean climate under no irrigation where 0, 30%, 60% and 100% S_{cc} treatments were imposed (Delpuech and Metay, 2018), indicating that both vine vigour given as PW and yield/vine linearly decreased as the S_{cc} increased. However, in our study, this trend was not confirmed for yield/vine (Table 2.4), as AGC and TG, despite an S_{cc} of about 43–48%, had the same yield of T (0 S_{cc}). A related consequence was that the two vine balance indices calculated as LA/Y ratio and Ravaz Index were primarily driven by the variation in vegetative growth, rather than yield. Then, the strange scenario deserving explanation is why both AGC and TG had LA/Y and Ravaz index values almost identical to PG (Table 2.4), yet achieved 20% higher yield mostly due to heavier clusters and berries and higher canopy efficiency expressed as total sugar per vine (i.e., the product of yield/vine x TSS) that was 15% higher in AGC and TG, compared to PG.

The first hypothesis that can be proposed is that especially when referring to the LA/Y ratio as a well-recognized index for vine balance (Kliewer and Dokoozlian, 2005; Poni et al., 2018), the “total” leaf area does not necessarily reflect “functional leaf area” and therefore, at the same LA/Y ratios, different outcomes are possible simply because of differences in light exposure, age, health status, etc., foliage “quality“ might differ (Mabrouk and Sinoquet, 1998). In our study we have evaluated seasonal assimilation rates of primary leaves accounting for about 85% of total leaf area (Table 2.3) and found occasional differences across treatments to indicate that overall leaf “quality” was also similar, and that the above hypothesis should be rejected.

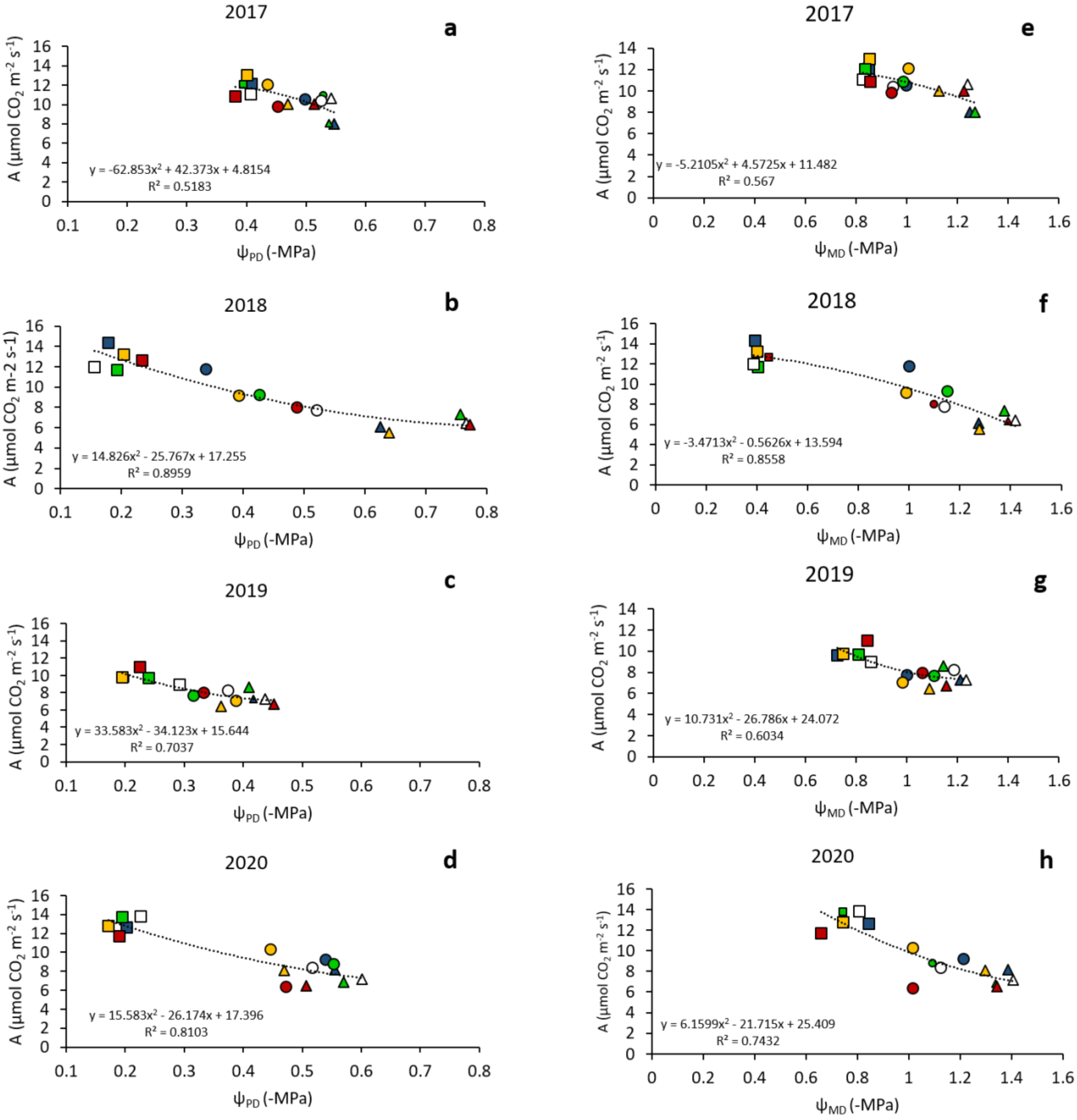


Figure 2.5. Left panels: curvilinear regressions between leaf assimilation rates (A) and pre-dawn leaf water potential (ψ_{pd}) calculated for 2017 (a), 2018 (b), 2019 (c), and 2020 (d). Right panels: curvilinear regressions between leaf assimilation rates (A) and midday leaf water potential (ψ_{MD}) calculated for 2017 (e), 2018 (f), 2019 (g), and 2020 (h). Within each year, soil management and seasonal sampling timing data were pooled over. Equations and R^2 values are shown within each panel. Colour codes for soil treatments are: ☐ = PG; 🟧 = T; 🟥 = AGT; 🟩 = AGC; 🟦 = TG. Within each treatment colour code, squares represent data taken at fruit-set, circles represent data taken at lag-phase and triangles represent data taken at the onset of veraison. Each data point is the mean of eight leaves.

Further support to such conclusion is given by the overall low vine vigour across treatments proved by either PW/vine, which is slightly less than 0.5 kg/m of a canopy, a threshold considered to be an almost perfect balance for an undivided canopy (Kliewer and Dokoozlian, 2005) and by the fraction of lateral leaf area accounting for only 15% of total leaf area (Poni and Giachino, 2000). Consequently, the proposition has to be ruled out that any treatment tested in this study caused excessive vigour, creating in turn issues of internal canopy shading as previously described in a Barbera vineyard from the same region characterised by a relevant within-field variability (Gatti et al., 2017). Therefore, total LA/Y resumes full validity under our trial condition, and it should be simply recognised that the same LA/Y ratios can be achieved with different combinations of the two terms; in other words, AGC and TG can assist a higher crop level with a correspondingly higher leaf area, without significantly impacting gas exchange and leaf water status. Moreover, in our study, WUE_i, albeit showing a typical large variability with higher values associate to lower g_s (Tortosa et al., 2019) was not a discriminant among treatments.This latter conclusion shifts attention to the degree of competition for water and nutrients that the grass root systems can exert towards the consociated grapevines. As shown in previous works, such competition does not simply relate to the fraction of cover crop soil coverage to total vineyard surface but includes other factors such as total precipitation available in the summer (Cruz et al., 2012; Mattii et al., 2005; Steenwerth et al., 2013), soil depth (Delpuech and Metay, 2018), floristic composition of the resident vegetation or the sown cover crop (Muscas et al., 2017; Pou et al., 2011; Sweet and Schreiner, 2010; Trigo-Córdoba et al., 2015; Volaire and Lelièvre, 2010) and physical/spatial interaction to be established in the soil volume where grapevine roots start to get in conflict with the grassroots (Fleishman et al., 2021). This latter interaction is especially interesting and should distinguish two different situations: a) an under-trellis cover crop is established, and immediate competition is triggered with the grapevine roots and b) grass occupies only mid rows and, with an under-the-trellis bare soil strip, competition will increase as long as the grapevine roots grow laterally. Case (a) has been studied quite extensively (Centinari et al., 2016; Fleishman et al., 2019; Klodd et al., 2016; Wheaton et al., 2008) and results converge toward a deeper grapevine root system having less fine root production and lifespan, as well as a marked decrease in overall absorptive root length. Case (b) applies to our study which, however, is served by fewer pieces of literature. However, Pool and Lakso (1994) reported in a 5-year study (where grapevine root development was assessed along a grid of 1.0 m soil depth and 1.2 m from the row axis to mid-row in two treatments) that the total herbicides and under-the-row herbicides (80 cm strip width) combined with alley managed with orchard grass (*Dactilis glomerata* spp.).

Interestingly, when in this second treatment the grapevine roots reached the competition zone, the tendency was to grow decidedly into deeper soil layers, whereas in the bare soil control, over 95% of the grapevine roots were contained in the upper 0–40 cm soil layer.

A comprehensive four-year study (Celette et al, 2008) comparing the water dynamics in a vineyard featuring a perennial cover crop, annual cover crop or the use of chemical weed control nicely showed that the rooting of a permanent cover crop was deeper than that of an annual crop, with a higher root density. Consequently, the soil volume exploited by the cover crop was larger and the grapevine was forced to explore deeper soil layers. Finally, Linares Torres et al. (2018) ascertained, in long term study (8 years) carried out in a irrigated vineyard planted in the semi-arid conditions of Madrid, that an inter-row annual cereal treatment was associated to a high grapevine root density between 0.4 and 0.8 m, whereas an herbicide treatment showed the lowest mean root density at the same depths.

An ideal term of comparison for our work is the study by Bordoni et al. (2019), comparing in a similar environment in Italy soil and grapevine root characteristics of the PG, T, and AGT treatments. They concluded that AGT achieved the highest root density and the strongest root reinforcement (Schwarz et al., 2013), up to 45%, compared to PG and up to 67–73% in comparison to T. However, considering the PG, T, and AGT performances in our study, AGT essentially played as an intermediate between PG and T for vine vigour and yield and, TSS at harvest was slightly less than that recorded on T (Tables 2.3 and 2.4 and Figure 2.4). Thus, no additional advantages seem to derive from a technique that aims at concurrently controlling excessive competition due to PG adoption while limiting the negative impacts on soil structure and health due to full tillage.

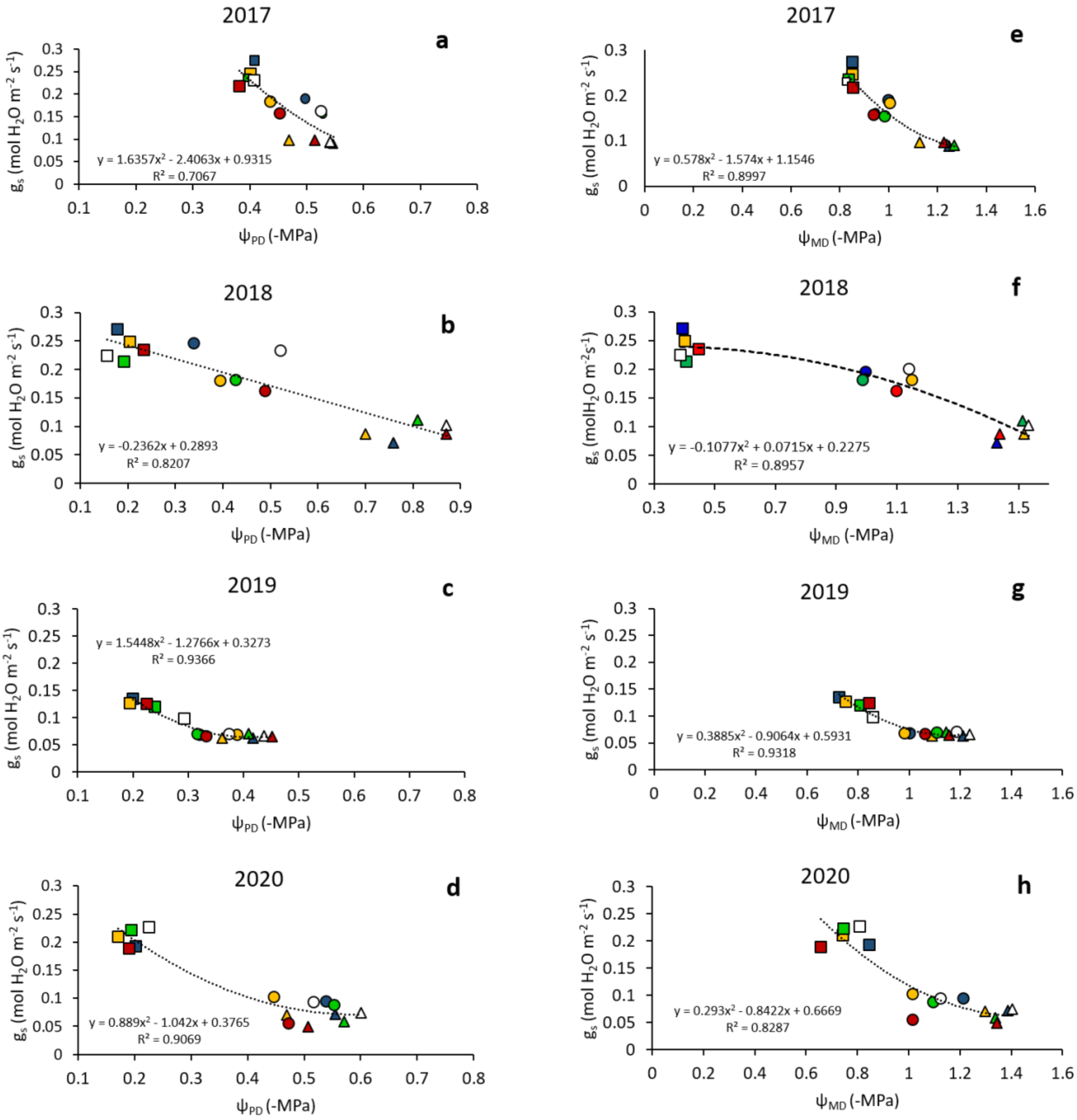


Figure 2.6. Left panels: curvilinear regressions between leaf stomatal conductance rates (g_s) and pre-dawn leaf water potential (ψ_{PD}) calculated for 2017 (a), 2018 (b), 2019 (c) and 2020 (d). Right panels: curvilinear regressions between leaf stomatal conductance rates (g_s) and midday leaf water potential (ψ_{MD}) calculated for 2017 (e), 2018 (f), 2019 (g), and 2020 (h). Within each year, soil management and seasonal sampling timings data were pooled over. Equations and R^2 values are shown within each panel. Colour codes for soil treatments are: ⬜ = PG; 🟧 = T; 🟥 = AGT; 🟩 = AGC; 🟦 = TG. Within each treatment colour code, squares represent data taken at fruit-set, circles represent data taken at lag-phase and triangles represent data taken at the onset of veraison. Each data point is the mean of eight leaves.

Attention must be devoted to the twin treatments of PG and AGT, namely TG and AGC, respectively. In the first comparison (i.e. PG vs. TG), it is noteworthy that breaking the native grass after harvest and leaving its new establishment to spontaneous regrowth in the spring significantly increased vigour (as total LA or PW) and yield per vine (Figure 2.2), whereas a moderate, albeit significant, constraint was observed in TSS, total anthocyanins and phenolics at harvest (Figures 2.4A, E and F). Although the timing of grass termination (post-harvest) does not directly interfere with vine development and ripening, such a simple technique is viable when vine capacity has to be pushed while maintaining vineyard access from early spring until post-harvest. It is quite likely that fall termination would favour faster/more efficient replenishment of water field capacity, which might positively affect the dynamics of water stress development during the season. In two out of the four trial seasons (2019 and 2020), TG registered less negative ψ_{pd} at the fruit set assessment, thus supporting this hypothesis (Table 2.7).

The second twin comparison (i.e., AGT vs. AGC), up to 4 years of observations, showed no significant variation in vine vigour and yield (Tables 2.3 and 2.4), with grape maturity too quite similar (Table 2.5).

This is not surprising if we consider that effects due to temporary winter grassing terminated in the spring with mulching might require several years before becoming significant, being associated with long-term responses, compared to green manuring (Dobrei et al., 2016; Rotaru et al., 2011). Further confirmation is provided by end-of-trail SOM content, which too did not differ. Interestingly, though, soil bulk density seemed to be more responsive, and decreased in AGC, indicating that the beneficial effects on the soil physical properties due to the drilling effect of the temporary cover crop mixture might be prompter than any nutritional effects. According to previous research, this evidence might be primarily associated with an increased soil porosity induced by soil harrowing that was repeated annually before cover crop seeding. De la Fuente et al. (2015) described similar bulk density and infiltration rates for winter cover crops and tilled soil, whilst Belmonte et al. (2018), discussing results collected over a 22-year experiment, reported that the alley combining sown cover crop and tillage was associated with the highest aggregate loss. Concerning nutritional aspects, the same study affirms that significant variations of SOM are bound to long-term assessment. Then, in theory, the presence of a tall cover crop in spring might use more water early in the season; however, water status assessed each season at fruit set confirms that no major limitations occurred in AGC vs. AGT (Table 2.7).

Table 2.7. Leaf assimilation rate (A), stomatal conductance (g_s) , water use efficiency (A/g_s), pre-dawn and midday leaf water potential recorded over four years at three dates during each season (2017-2020) on field-grown cv. Barbera grapevines subjected to different interrow soil practices.

		Ψ Pre-dawn (MPa)			Ψ Midday (MPa)			Assimilation rate (μmol CO_2 $m^{-2}s^{-1}$)			Stomatal conductance (mol H_2O $m^{-2}s^{-1}$)			Water Use Efficiency (μmol CO_2 mol^{-1} H_2O)		
2017	DOY	167	187	201	167	187	201	167	187	201	167	187	201	167	187	201
	PG	-0.41	-0.53c	-0.54b	-0.83	-0.95	-1.24b	11.059	10.406	10.587	0.230b	0.163ab	0.094	48.1ab	63.8a	112.6
	T	-0.40	-0.44a	-0.47a	-0.85	-1.00	-1.13a	13.006	12.113	9.969	0.246b	0.184ab	0.096	52.9a	65.8a	103.8
	AGT	-0.38	-0.45ab	-0.52b	-0.86	-0.94	-1.23b	10.871	9.846	9.977	0.217b	0.158b	0.096	50.1ab	62.3a	103.9
	AGC	-0.40	-0.53c	-0.54b	-0.84	-0.98	-1.27b	12.031	10.918	8.017	0.236b	0.155b	0.089	51.0ab	70.4a	90.1
	TG	-0.41	-0.50bc	-0.55b	-0.85	-1.00	-1.25b	12.096	10.578	7.976	0.274a	0.190a	0.088	44.1b	55.7b	90.6
2018	DOY	172	194	215	172	194	215	172	194	215	172	194	215	172	194	215
	PG	-0.16	-0.52b	-0.87a	-0.39	-1.14b	-1.53	11.965	7.779b	6.403	0.224	0.195a	0.102a	53.4	40.0b	62.8b
	T	-0.20	-0.39ab	-0.70b	-0.40	-0.99a	-1.44	13.186	9.170b	5.465	0.249	0.181ab	0.086ab	53.0	50.1ab	63.5b
	AGT	-0.18	-0.49b	-0.87a	-0.38	-1.10b	-1.52	12.928	8.012b	6.373	0.245	0.163b	0.086ab	52.8	49.1ab	74.1b
	AGC	-0.19	-0.43ab	-0.81ab	-0.41	-1.15b	-1.51	11.663	9.283b	7.280	0.214	0.182ab	0.111a	54.5	51.0ab	65.6b
	TG	-0.18	-0.34a	-0.76ab	-0.39	-1.00a	-1.43	14.318	11.819a	6.078	0.271	0.200a	0.071b	52.9	59.1a	85.6a
2019	DOY	172	205	220	172	205	220	172	205	220	172	205	220	172	205	220
	PG	-0.29b	-0.37ab	-0.44bc	-0.86c	-1.18c	-1.24b	8.949	8.264	7.256	0.098	0.071	0.065	91.3a	116.4	111.66
	T	-0.20a	-0.39b	-0.36a	-0.75ab	-0.98a	-1.09a	9.761	7.073	6.371	0.126	0.069	0.062	77.5bc	102.5	102.7
	AGT	-0.23a	-0.33a	-0.45c	-0.84c	-1.06ab	-1.16ab	10.994	7.999	6.658	0.124	0.067	0.064	88.7ab	119.4	104.3
	AGC	-0.24a	-0.32a	-0.41b	-0.81bc	-1.11bc	-1.15ab	9.690	7.722	8.575	0.119	0.070	0.069	81.4ab	110.3	124.3
	TG	-0.20a	-0.32a	-0.42b	-0.73a	-1.00a	-1.21ab	9.572	7.788	7.242	0.135	0.069	0.062	70.9c	112.9	116.8
2020	DOY	176	202	212	176	202	212	176	202	212	176	202	212	176	202	212
	PG	-0.23c	-0.52b	-0.60c	-0.81b	-1.12b	-1.41	13.792	8.363a	7.115	0.227a	0.094a	0.074	60.8	89.0	96.2
	T	-0.17a	-0.45a	-0.47a	-0.74ab	-1.02a	-1.30	12.777	10.328a	8.063	0.210ab	0.103a	0.070	60.8	100.3	115.2
	AGT	-0.19ab	-0.47a	-0.51ab	-0.66a	-1.02a	-1.35	11.713	6.383b	6.458	0.189b	0.056b	0.048	62.0	113.9	134.5
	AGC	-0.20b	-0.55b	-0.57c	-0.75ab	-1.09ab	-1.34	13.739	8.819a	6.776	0.222ab	0.088a	0.058	61.9	100.2	116.9
	TG	-0.20b	-0.54b	-0.56bc	-0.85b	-1.21c	-1.39	12.623	9.223a	8.101	0.193b	0.095a	0.071	65.4	97.1	114.1
	Y x T F prob	**	*	**	**	**	*	**	**	**	**	**	**	**	**	**

Within column, in case of significant F test, mean separation was performed by Student-Newman-Keuls (SNK) test. * = p <0.05, ** p < 0.01, ns = not significant.

Considering the soil type profile characteristics of our study, the absence of main root hindrance factors along the soil would indicate, on the one hand, potentially deep soil for root colonization and on the other, the exponential decrease in SOM and K_2O with increasing depth, suggesting that any floor management technique that might shift the grapevine roots to grow into deeper layers will find poorer soil, even with simultaneous possible higher water availability. If these two factors counteract each other, it is likely that vine vigour may not change considerably, depending on such a root shift. However, such interaction can be ideally verified with under-the-row cover crop directly competing with the grapevine root system (Fleishman et al., 2021), which was not the case in our study.

Floristic composition assessed in PG, T, and AGC showed that the number of grass species was highly reduced in the grass regrowth detected in the tilled plots. Besides, this latter treatment totally lacked some families, compared to PG and AGC (e.g., Boraginaceae Juss, Brassicaceae Burnett, etc) while Polygonaceae Juss was the most abundant with the Rumex L. to prevail, a species well known for flourishing in disturbed environments (Meadly, 1958).

The second pillar of discussion is centered around soil management effects on technological and phenolic maturity (Tables 2.5 and 2.6). The desired final composition of the Barbera grapes in the area, where still or sparkling Barbera wine types are sought by consumers, should have a sugar concentration of at least 22.5 Brix, TA between 8 and 10 g/L and, in case of still wines, the highest reachable total anthocyanins content.

Except for must pH, all variables showed significant variation across soil treatments. At first sight, higher leaf area-to-yield ratio at harvest in T vs PG (Table 2.4) clashes against higher efficiency of PG in sugar accumulation. However, significant Y x T interaction found for TSS strongly suggests that, in our trial, the sugaring process responded to other mechanisms and that year-to-year variability played a role. When putting in direct comparison significant T x Y interaction for TSS and lateral pruning weight per vine (Figure 2.2B and Figure 2.4A) it is sharp that in 2017, the levelling of all treatments around the 25 Brix threshold links to a very limited lateral growth, as no treatments exceeded 50 g of fresh mass per vine; conversely, in the more vigorous 2019 and 2020 seasons, large differences in total lateral pruning weight/vine of PG vs. others corresponded to wide differences in sugar accumulation. In agreement with previous studies (Keller et al., 1999; Poni and Giachino, 2000) very limited lateral development registered in PG might have promoted preferential post-veraison accumulation of assimilates into berries. Moreover, in the case of T, the higher cluster and berry weight might have contributed to the higher dilution of the berry solutes and the lower TSS (Coombe et al., 1987; Roby et al., 2004).

Extending this analysis to other key parameters for technological maturity, due to the well-known relationship linking malic acid degradation to cluster exposure to high light intensity and temperature (Ford, 2012), the likely open canopies with very few laterals developing in 2017 enhanced malic acid degradation (Figure 2.4C), irrespective of soil treatment (concentrations at harvest never exceeded 1.5 g/L). As could be expected, this gap in malic acid concentration between PG and any other treatment was maintained also in 2019, when treatments other than PG slightly exceeded 150 g of lateral cane weight per vine, and PG reached only 61 g. This should be regarded as an inherent weakness of PG; while it is true that due to the intrinsic attitude of Barbera at maintaining high TA even under warm and dry conditions (Bernizzoni et al, 2009), lower malic acid in PG was a minor shortcoming in this study, which could become a matter of serious concern in white cultivars where acid preservation is a must if sparkling winemaking is envisaged.

A further weakness of PG is that YAN at harvest is close to the threshold (100 mg/L) (Figure 2.4D), below which increased risk of sluggish/stuck/slow fermentations, increased production of undesirable thiol and higher alcohols, and low production of esters and long-chain volatile fatty acids are likely (Bell and Henschke, 2005). Similar results were obtained on Pinot noir grown in the Willamette Valley (Oregon), where on a three-year basis, a grass treatment consisting of *F. rubra* L. sown in the alleys vs. full tillage achieved YAN at harvest between 58 and 84 mg/L vs. the 172–196 mg/L measured in the tilled plots (Reeve et al., 2016). Though, significant Y x T interaction found in our study (Figure 2.4D) indicates that PG sensitivity to lower YAN was especially pronounced in 2018 and 2019 which scored the highest precipitation in spring or over the whole season, respectively, whereas no differences were found in dry and hot 2017. Hypothesis is that, as previously reported in Abad et al. (2021) and Sweet and Schreiner (2010) cover crops effect of YAN at harvest is a complex function of seasonal weather course that through regulation of the relative growth of legume and/or grass species and potential of soil N uptake in spring might determine quite different outcomes at harvest.

AGC also stood out as the only effective treatment for reducing berry potassium concentration at harvest (Table 2.5). The proposed hypothesis is that the temporary winter cover crop drained some potassium from the soil, rendering it unavailable for uptake by grapevine roots (Table 2.2). An interesting work by Witter and Johansson (2001) investigating potassium uptake from the subsoil by green manure species showed that a ryegrass/clover mix (20% of the former used in the mix of the present study) removed up to 88kg of K/ha from the topsoil and subsoil. Dunlop et al. (1979) showed that ryegrass retains much greater maximum rate of K influx than white clover (*Trifolium repens* L.);

it is also known that rye (13% in our mix), is a species with high demand in potassium (White, 1993). Finally, the floristic composition of the permanent grassing (Table 2.2) shows more abundant ryegrass presence in AGC vs. PG and T.

Mitigating K uptake in the vine and thus lowering the risk of excessive must and wine pH is among the main challenges posed by climate change. Must pH values above 4 are readily reached in hot climates and have been recorded in traditional cool climates too (Mira de Orduña, 2010). *Inter alia*, several authors have suggested that higher temperatures lead to increased potassium levels (Coombe et al., 1987) due to increased evaporative demand and thus to soil-root-plant transport (Mpelasoka et al., 2003). However, in the vineyard, Boulton (1980) has clarified that the main process leading to higher grape pH and lower TA is the uptake of K and Na. Then, in an environment conducive to excessive K uptake due to high natural soil availability or increased evaporative demand, using temporary winter grass to reduce the K pool available for uptake might be successful.

The phenolic maturity was also impacted by the different soil management treatments and, most notably, total phenolics showed the tightest correlation towards S_{cc} (Figure 2.3B). Our results confirm a common trait of several studies, where cover crop-based treatments achieved increased berry skin total phenols and anthocyanins (Coniberti et al., 2018; Lee and Steenwerth, 2013; Monteiro and Lopes, 2007; Pérez-Álvarez, 2017; Steenwerth and Guerra, 2012), compared to tillage or herbicides treatments. In our study, there could be several reasons why PG had the best phenolic ripeness and T the worst (Figure 2.4F), like colour formation dragged by higher TSS, more advanced ripening due to less vegetative competition, smaller berries in PG leading to a more favourable skin-to-berry ratio, and improved cluster light exposure. This latter is confirmed by the notable increase in flavonols, namely quercetin 3-O glucoside (Table 2.6), whose synthesis is known to be greatly enhanced by light intensity increase at the cluster level (Matus et al., 2009; Price et al., 1995). Flavonols are yellow pigments playing an important role in the stabilisation of young red wines, through the co-pigmentation interaction with anthocyanidins. However, when excessive (e.g. in red wine > 60 mg/L), they contribute to an unpleasant perception of astringency and bitterness (Gutiérrez-Escobar et al., 2021) and can also originate precipitates during bottle storage.

Anthocyanin composition was altered in the shaded fruit, which had a greater proportion of the dioxygenated anthocyanins, the glucosides of cyanidin and peonidin. Anthocyanin composition was altered in the shaded fruit, which had a greater proportion of the dioxygenated anthocyanins, the glucosides of cyanidin and peonidin. In terms of anthocyanidins composition, the deoxygenated anthocyanin forms – the glucosides of cyanidin and peonidin – were overall less affected by the soil treatments, compared to the tri-oxygenated forms (Table 2.6). If we assume that the lower vine vigour shown by the PG vine also allows more light penetration in the fruiting area, then our results agree with those on Shiraz (Downey et al., 2004) where boxing applied on clusters to exclude light since flowering favoured the above change vs. the well-exposed clusters.

2.6 Conclusions

The main objective of this study carried out in an organically managed vineyard was to test and validate floor management treatments capable of minimising well-known disadvantages of either tillage or native vegetation, while focusing on a balance between the two techniques with variations in space and time. This happened under the same under-the-trellis management (i.e. tillage), thus emphasising the role of inter-row soil management. High expectations from the AGT strategy were partially disregarded, as this treatment is set almost in an intermediate position between the two extremes, without assuring any significant marginal gain. Conversely, modulating PG into TG via a temporary removal of the resident vegetation in the fall and AGT into AGC by growing a winter cover crop terminated in the spring as green manuring, gave the highest yield at adequate technological and phenol ripeness.

In these two pairs of comparisons, TG also assured higher YAN levels for more regular must fermentation in two out of the four trial seasons, whereas AGC proved to be effective at mitigating K^+ accumulation in berries. Data taken over 4 years in a non-irrigated vineyard did not show any major limitations in leaf gas exchange and water status across treatments, as most of the observed changes were primarily season-related.

2.7 Acknowledgments

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Chapter 3. Published Manuscript 2: A low-cost portable chamber based on Arduino micro-controller for measuring cover crops water use

3.1 Abstract

Cover crop adoption is growing in sustainable vineyard management to replace tillage and limit its ecosystem disservices. However, water use of such crops must be known and low-cost (~ 210$), yet fast reading, portable and accurate equipment is therefore needed for multipoint measurements in the field. Using an Internet of Things (IoT) approach, in this study we provide details for setup, calibration and operational data of a very low cost, small, closed type chamber. Chamber calibration was performed either as instantaneous evaporation (E) rates under laboratory condition (25 runs, 2 minutes each) and daytime cumulative evapotranspiration (ET) rates performed outside in small pots sown with different cover crops or managed with light tillage. In both cases chamber's derived water loss rates were validated against a gravimetric method. A very close linear relationship between gravimetric vs chamber values was found for lab and outdoor calibration runs (R^2 = 0.96 and 0.99, respectively) within ranges of 0-0.8 mm h^{-1} (lab) and 0-23 mm d^{-1} (outdoor). Ideal measurement time window was estimated at 60 seconds after "time zero" set at 15 second upon chamber positioning. Under any condition, chamber heating never exceeded 2 °C above air temperature. In the warmest hours of the day (i.e. from 11:00 to 16:00) *Festuca arundinacea* hourly ET was 0.56 mm while *Lotus corniculatus* and wet soil tillage registered 0.71 and 0.69 mm h^{-1}, respectively. Dry soil tillage showed ET values between 0.2 and 0.3 mm h^{-1}. The proposed device represents an effective IoT application as total cost of the needed components does not reach a total amount of 200 euros and its size as well as flexibility of use makes it an ideal tool for fast multipoint readings of soil and grass water losses in the field.

3.2 Introduction

Vineyard cover cropping is a sustainable soil management practice extensively used in many of the world's viticultural areas (Celette et al., 2008). It is in fact one of the recommended practices to promote environmental sustainability and to face climate change impacts in vineyards (Celette and Gary, 2013; Diti et al., 2020; Schultz and Stoll, 2010). The adoption of cover crops allows for achieving many ecosystem services (ES), including: i) improvement of soil fertility and physical features; ii) better soil water retention capacity and water infiltration rates; iii) improved pest and native weed control together with iv) environmental and social benefits (e.g. carbon sequestration, biodiversity conservation and landscape aesthetics) (Garcia et al., 2018). Thus, cover cropping usage has widely been assessed in a variety of soils and climate conditions across the world and to name a few: Italy (Ferrero et al., 2005; Pardini et al., 2002), Spain (Marques et al., 2010; Ruiz-Colmenero et al., 2013) France (Celette and Gary, 2013; Ripoche et al., 2010), South Africa (Fourie et al., 2017), Australia (Danne et al., 2010; Nordblom et al., 2020) and United States (Steenwerth and Guerra, 2012).

However, together with ecosystem services, that may lead to a food production promotion, some ecosystem disservices (EDS) may be generated which, contrariwise, tend to hinder it (Von Döhren and Haase, 2015). Competition for soil resources (e.g. water and nutrients) is a good example of cover crop disservice (Celette and Gary, 2013; Klodd et al., 2016). In fact, only a small percentage of farmers are planting cover crops in semi-arid areas due to the disadvantages often outweighing the advantages (Medrano et al., 2015). This happens to be even more important in a viticultural context of climate change where, along with a quite certain global warming, higher frequency of hot spells and slightly reduced total precipitations are expected over most land areas on daily and seasonal timescales (Pachauri et al., 2014). In viticultural areas, these changes would lead to a reduction of the water available to plants and, as a likely consequence, occurrence of significant summer drought will increase especially in traditionally non irrigated districts with negative influences on both grape and wine quality (Mirás-Avalos and Intrigliolo, 2017; Pagay et al., 2016). Within such a scenario, the demand for irrigation will rise. To more wisely schedule irrigation events, a more comprehensive knowledge of the dynamic and magnitude of water used by all the vineyard ecosystem components (i.e. vines, grass and soil) is needed (Centinari et al., 2013). The same need applies to other orchard systems including apple (Mobe et al., 2020), olive (Novara et al., 2021).

Previous studies have mainly focused on quantifying whole vine’s transpiration rate using approaches such as sap flow gauges (Braun and Schmid, 1999; Dragoni et al., 2006) or canopy enclosure systems (Poni et al., 2014). However, to determine the total evapotranspiration (ET) of the vineyard ecosystem, the amount of water used by both the bare soil and/or the cover crop needs to be incorporated. The contribution of these two components (i.e., soil and cover crop) to the vineyard water use can be very significant also depending on other interfering factors (e.g. training system, between row distance, etc.). However, data available regarding direct measurements of the amount of water used by a grass cover in a vineyard are still quite limited (Centinari et al., 2012; Lopes et al., 2004; Uliarte et al., 2013). Lopes et al. (2004) used a portable gas exchange system to measure cover crop transpiration rates and showed that contribution to vineyard evapotranspiration can vary from less than 1 mm d^{-1} m^{-2} for *Festuca rubra* subsp. *rubra* to more than 4 mm d^{-1} m^{-2} for *Malva neglecta*.

Several methods can be used to measure ET fluxes, each with advantages and limitations. Cover crop ET can be gravimetrically determined by using a mini-lysimeter (ML), which represents an alternative solution to field lysimeters as it can be used in a limited space situation (such as that available between the vine rows) (Bremer, 2003; Lakso et al., 2019). A ML consists of some kind of container filled with a soil core covered with the same vegetation of the surrounding area and inserted into the ground to the point of being even with the adjacent soil surface. The containers used may be plastic pots (Centinari et al., 2013) provided there are holes at the bottom for the drainage of the water. In this method, MLs are irrigated, allowing time for the extra water to drain and then weighed several times in the following days. Loss in mass during the interval between weighing is attributed to ET.

Micrometeorological techniques, such as the eddy covariance, are other methods that can be used for cover crop ET measurements and have a clear advantage in continuous measurements without disturbing (i.e. indirect method) the micro-environment of the measured field (Müller et al., 2009). However, this method does not apply to small-scale experiments (Baldocchi, 2014).

Conversely, chamber enclosure still holds as a non-invasive method for small scale readings (Steduto et al., 2002). Here, a transparent chamber is placed over vegetation or soil, and gas fluxes are estimated from the concentration changes of the gases through the chamber. Typically, chamber methods are classified into two categories: closed vs open chambers (Garcia et al., 1990; Wagner and Reicosky, 1992).

In an open chamber, the gas is continuously pumped into and out of the chamber through openings. The difference of water vapour concentration between the chamber inlet and outlet is measured and used to determine the ET flux. Measurements can be obtained continuously over a time period from a few days to the whole growing season. However, complex systems are required in order to maintain the micro-climate inside the chamber reasonably close to ambient (Corelli-Grappadelli and Magnanini, 2019; Poni et al., 2014). Moreover, open chambers portability is usually limited and the air flow fed to the chambers needs to be carefully measured.

Even though Centinari et al. (2009) successfully used an open chamber system to determine *Festuca arundinacea* water use in a vineyard, a closed chamber has been designed to better suit the need of portability (Luo et al., 2018) and, as such, it can be quickly moved among several sampling locations in the field. A closed chamber system estimates gas fluxes by measuring the rate of change in gas concentrations in the chamber air in a short period of time, while the chamber is closed. To minimize chamber-induced canopy micro-climate changes, rapid measurements for brief periods (i.e. 1 minute or so) should be used (Nomura et al., 2019).

There is some controversy concerning the accuracy of the closed chamber method when comparisons have been made with other ET measurement techniques. A few studies have shown a good agreement between the daily ET obtained with a closed chamber system and the Energy Balance Bowen Ratio method (Luo et al., 2018; McLeod et al., 2004; Steduto et al., 2002). Contrariwise, other studies have reported that chamber derived water use rates overestimated by about 25% amounts derived from a gravimetric (Grau, 1995) or eddy-covariance (Stannard and Weltz, 2006) approach. However, comparing the results obtained in these studies is complicated because of the different characteristics (e.g., shape, size, etc.) of the closed chambers used.

The rates of change in gas concentrations are frequently assumed to be constant, and the linear regression function (LR) has usually been fit to the measured changes of gas concentrations to estimate gas fluxes in closed chamber systems (Wagner and Reicosky, 1992). However, the non-linear nature of changes in gas concentration, due to the diminishing concentration differences of the gas between the measured subject (e.g. soil, cover crop) and the chamber air during the chamber closure has been recognized (Nomura et al., 2019). Several studies have argued that the use of LR can lead to underestimation of gas fluxes (Kutzbach et al., 2007; Langensiepen et al., 2012). To minimize this flux underestimation (Wagner and Reicosky, 1992) proposed the quadratic regression function (QR). According to that, the flux can be estimated by the first derivative of the function at time zero (i.e., immediately after the chamber closure).

Although LR and QR might fit well to observed changes of concentration, these conventional regression functions might still be conducive to underestimations of fluxes due to the dynamic characteristics of a concentration sensor (i.e., response lag and dead time) as argued by Nomura et al. (2019). This problem is often connected to usage of low cost sensors since rapid-response concentration sensors tend to be expensive and this has limited the applicability of the closed chamber method.

Considering the need to develop low-cost yet reliable and fast-enough concentration sensors, the objectives of this study are to: i) describe a new, custom-built and low-cost closed chamber system for vineyard cover crop ET flux measurements; ii) perform proper calibration; and iii) provide examples of the kind of datasets and degree of accuracy that the system can achieve.

3.3 Materials and methods

3.3.1 Chamber description and setup

The chamber design consists of: i) a cylindrical structure made of waterproof 2mm thick polyvinyl chloride (PVC) sheet; ii) a lower plastic frame and iii) an upper conical lid with a 0.5cm thick rubber gasket for sealing (Fig. 3.1).

The chamber has a ground surface of 491 cm^2 and a height of 57cm, with a total volume of 28L and it is operated as a closed system. It was designed to work on top of a steel collar previously inserted 3 cm onto the soil.

The chamber is made of PVC because of its uniform transmissivity at 400-800 nm wavelength light and because it is lightweight, low-cost and easy to handle and to seal with solvent glue.

Chamber light transmittance properties as well as variation of the diffuse-to-direct light ratio were checked during a summer clear day using a BF2 Sunshine Sensor (Delta-T Devices Ltd, Cambridge, UK) placed horizontally inside and outside the chamber. Recorded values (mean ± SE, n = 10) for direct and diffuse radiation outside the chamber were 1368 ± 2.80 and 580 ± 1.35 $\mu mol\ m^{-2}\ s^{-1}$, respectively, and 1128 ± 6.98 and 486 ± 1.87 $\mu mol\ m^{-2}\ s^{-1}$ inside it. Therefore, light transmission through the chamber was reduced by 17% whereas the diffuse-to-direct light ratio remained unchanged at around 43%.

The lid was designed flat instead of the spherical shape used in other commercial chambers, for easier manufacturing and handling in the field, and to reduce the measurement time due to a lower chamber volume (Ladrón De Guevara et al., 2015).

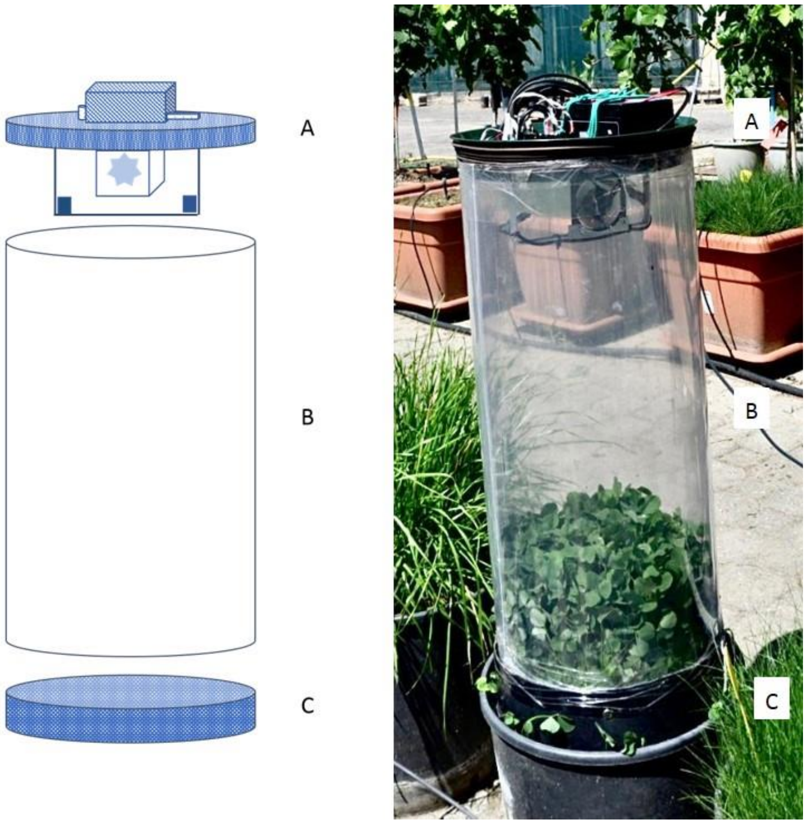


Figure 3.1. Portable closed chamber whose main components are: A) upper conical lid on which electronics and sensors are mounted; B) cylindrical polyvinyl chloride structure; C) lower plastic frame.

All the chamber components were fixed on the lid and connected to the Arduino1 Rev3 micro-controller (Smart Projects, Ivrea, TO, Italy). Actual components (A) and the wiring (B) of the chamber electronic system are shown in Fig. 3.2.

For a more flexible field operation, the electrical and electronics systems were designed to be of low current drain, compatible with being powered from a rechargeable battery or directly from a tablet or a computer. The software was developed using Arduino platform (1.6.11 version). After turning on the micro-controller and the brushless fan, with the chamber temporarily kept at about 50 cm above the ground, chamber humidity stabilisation has to be reached, usually taking no more than 45 seconds. Then, the chamber is lowered on the pot (perfect fit is needed) or on the steel frame, previously inserted onto the soil. Data recording was programmed at 15 seconds interval and the duration of the calculation window was set at a maximum of 120 seconds. The initial lag and mixing time was estimated as 15 seconds (i.e. 15 seconds from the chamber closure) from which the calculation time window (TW) started.

Current cost of each item needed to build and configure the chamber is shown in Table 3.1.

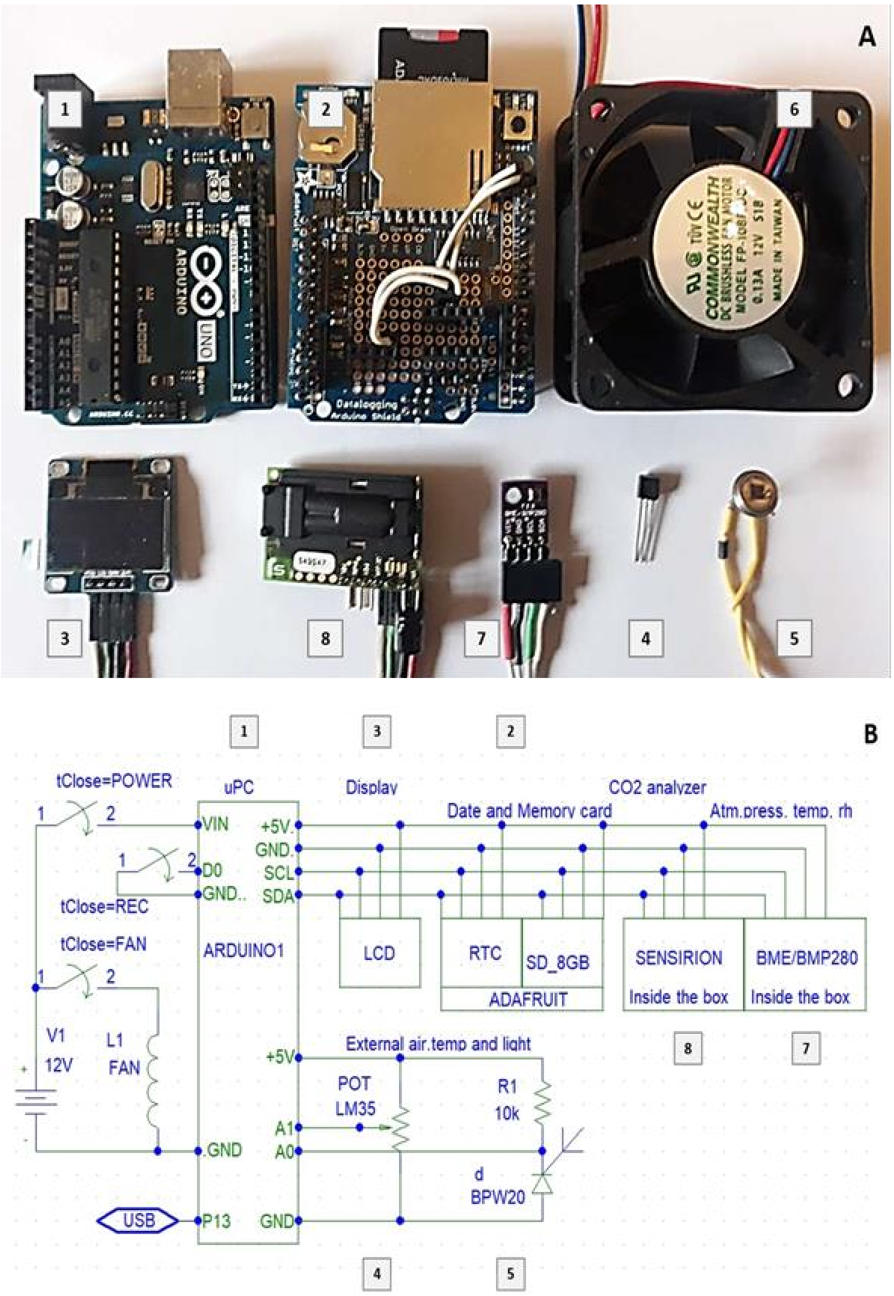


Figure 3.2. Actual components (A) and wiring (B) of the chamber electronic system. Components are: 1) Arduino1 Rev3 micro-controller; 2) Adafruit Assembled Data Logging shield for Arduino (Adafruit industries, NY, USA) made of an SD mass memory, a clock and a dater; 3) OLED 96C 0.96′′ Display (Futura Group s.r.L. divisione elettronica, Gallarate, VA, Italy); 4) an outside-the-chamber temperature sensor LM335 (STMicroelectronics, GE, Switzerland); 5) a Silicon Photodiode BPW20RF (Vishay Intertechnology Inc., PA, USA) and a 12 V 7Ah rechargeable battery (not shown); 6) brushless fan (Commonwealth Industrial Corporation, Taiwan); 7) pressure, temperature and humidity sensor GY BME280 (Bosch Sensortec GmbH, RT, Germany) and 8) CO_2, temperature and humidity sensor Sensirion SCD30 (Sensirion AG, ZH, Switzerland). The holes for inserting the sensor and the fan cable in the chamber were sealed with solvent glue.

Table 3.1. List of components and relative current costs (USD and Euro) for assessing ET fluxes. Material and equipment quantities are calculated for one single portable closed chamber system.

Item	Item (No.)	Cost (Euro)	Cost (USD)
PVC sheet	1	2.33	2.82
Plastic conical frame	1	1.2	1.45
Plastic conical lid	1	0.99	1.20
Rubber gasket	1	2.5	3.03
Steel frame	1	6.9	8.35
Battery	1	16	19.36
Brushless fan	1	6.77	8.19
Arduino1 Rev3 micro-controller	1	22.5	27.23
Sensirion SCD30	1	58.56	70.86
GYBMEP BME/BMP280	1	8.5	10.29
Display OLED96C 0.96’’	1	11	13.31
Adafruit Assembled Data Logging shield for Arduino	1	17.02	20.59
Temperature sensor LM335	1	0.51	0.62
Silicon Photodiode BPW20RF	1	6.5	7.87
Other electronic material	14	12.8	15.49
Total cost		174.08	210.64
Workload cost[1]		24	29.04

[1] Workload cost for 1 ha sampling where 40 readings are assumed to be needed.

3.3.2 Evapotranspiration calculation

The chamber sensor GY BME280 (Bosch Sensortech, Milan, Italy) measures pressure (p), temperature (T) and relative humidity (RH).

Relative humidity is defined as the ratio, expressed as a percentage, of the actual water vapour pressure (p_w) to the saturation water vapour pressure (p_{ws}) at a given temperature:

$$RH = \frac{p_w}{p_{ws}} \quad (1)$$

While RH (%) is given by the sensor every 15 seconds interval, p_{ws} (Pa) can be calculated from the Antoine equation:

$$p_{ws} = exp[\, A - \frac{B}{T+273.15} - C\, ln(T + 273.15)] \quad (2)$$

$$A = 65.81 \qquad B = 7066.27 \qquad C = 5.976$$

Where T is the chamber temperature (°C) given by the sensor and A, B and C are constant values. The p_{ws} value at the chamber micro-climate condition is calculated every 15 seconds.

Both RH and p_{ws} are known and from Eq. (1) and the actual water vapour pressure (p_w) can be calculated as:

$$p_w = p_{ws} * \frac{RH}{100} \quad (3)$$

Then, according to Dalton's law of partial pressures, the partial pressure water moles (n_w) is obtained from the relation:

$$n_w = \frac{n * p_w}{p} \quad (4)$$

Where p_w (Pa) is the actual water vapour pressure, p (Pa) is the total pressure of the gas mixtures present in the chamber measured at 15 seconds intervals by the sensor and n (mol) is the number of moles that can be held inside the chamber volume V (l).

From the ideal gas equation where:

$$V_m = \frac{R * T}{p} = \frac{V}{n} \quad (5)$$

n (mol) can be obtained as:

$$n = \frac{V}{V_m} \quad (6)$$

Then, V_m can be calculated from Eq. (5) using sensor measured T (°C) and p (Pa) and the gas constant R (8.324 L Pa K^{-1} mol^{-1}). V_m is then expressed as L mol^{-1}.

Once n_w (mol) is calculated from Eq. (4), the amount of water "lost" (g), i.e. evaporated, every 15 seconds from the ground surface covered by the chamber can be estimated as

$$H_2O = n_w * 18.015 \quad (6)$$

Where 18.015 g mol^{-1} is the water molecular weight. The amount of water evaporated every 15 seconds is then expressed as mm m^{-2}.

Then, a quadratic regression (QR) model was applied (Wagner and Reicosky, 1992)

$$y = at^2 + bt + c \quad (7)$$

Where y is the water evapotranspiration flux; a, b and c are fitted parameters and t is the sampling time. The use of QR is appropriate since the water vapour concentration inside the chamber increases with time, leading to a decreasing water vapour deficit which reduces the measured ET.

In order to determine the instantaneous ET, the calculation time window (TW) disregards the very first record and "time zero" is considered to be the reading taken at 15 s after chamber positioning. Therefore, the time window comprised between time zero and 60 seconds afterwards is the one used for inferring ET calculation.

Of these 60 seconds curve, the slope is calculated through the first derivate of the QR according to the following equation:

$$\frac{dy(0)}{dt} = b \quad (8)$$

The instantaneous ET (i.e., *b*) is then expressed as mm h^{-1} m^{-2} (usually referred to as mm h^{-1}) and when needed, converted into mm d^{-1} m^{-2}.

3.3.3 Chamber calibration

A mass balance method was used in a laboratory test to determine the accuracy and stability of the chamber measurements. Calibration was initially performed comparing evaporation (E) from a water-soaked cloth measured using the canopy chamber against its gravimetric water loss measured with a precision balance. The weighing system consisted of an electronic scale (model PS 2100.R2.M, Radwag, Radom, MZ, Poland) with a resolution of 0.01 g. The calibration consisted of 25 chamber runs each lasting 120 seconds. For each run, the water lost from the cloth was calculated using the equations previously described, whereas the gravimetric loss was determined by weighing the cloth before and right after the chamber measurements. The data were expressed as hourly rate of evaporation (mm h^{-1}).

A second round of calibration testing was made outdoors on 22 July 2020 at the Università Cattolica del Sacro Cuore (Piacenza, Italy, 45°2'N; 09°42'E) on eight pots (0.27 m deep with an internal diameter of 0.26 m). Pots were filled with clay-loam soil having 35% sand, 36% silt and 29% clay. After (Saxton et al., 1986)the total available water was calculated at 0.14 cm cm^{-1}, whilst field capacity and wilting point were 32.8 and 18.7 % vol, respectively.

Four different pot management practices were tested with two replicates each. The four treatments were: i) wet soil tillage (WST); ii) surface-dry soil tillage (ST); iii) *Lotus corniculatus* (LC) and iv) *Festuca arundinacea* (FA) grassed soils (Fig. 3.3). Both *L. corniculatus* and *F. arundinacea* were pot seeded in April 2020 and by the time calibration readings were taken both cover crops had 100% soil coverage and no weed species growth was recorded (Fig. 3.3).

To facilitate grass establishment and avoid any water deficit, a single dripper was fitted in each pot delivering 350 mL of water 3 times a day. Automated irrigation was stopped one day before the calibration test and, the day before, 1L of water was given to each pot. In order to include in the comparison a tilled treatment having a dry surface, in ST irrigation was interrupted 8 days before measurements.

Pot soil management consisted of one mowing event and one soil tillage. On the 6 July 2020, the cover crop in the four pots assigned to LC and FA treatments was hand-trimmed to 4 cm. On the same day, soil tillage was implemented in the WST and ST treatments. Surface soil was lightly cultivated using a three-tooth rake.

Calibration consisted of one full day experiment. Each pot was weighed in the morning at the beginning of the calibration run and then the chamber measurements were conducted every 2 hours (from 9:00 to 19:00). Daily water loss was estimated as the area underneath the regression curve that represented best fit to each pot diurnal evapotranspiration (ET) pattern. Whereas the gravimetric loss was determined by weighing the pot at the beginning and end of the day. Data were then expressed as daily rate of evapotranspiration (mm d^{-1}).

The steel frame was omitted in this pot calibration and the chamber was directly laid on the pot surface having a diameter allowing a perfect fit without gas leak risks.

During each measurement, the air temperature and relative humidity outside and inside the chamber was measured at 15 seconds intervals with the air VPD calculated accordingly. For each pot ET assessment, PAR was recorded through the silicon photodiode positioned on the upper lid facing the outside of the chamber.

Data were recorded on the SD card and simultaneously visualized in the small display set on the lid or directly on the tablet connected through a USB-cable. All data were collected only under clear sky conditions.

3.3.4 Pot experiment

To demonstrate ability of the new sensor to detect even small differences in water use by different cover crops as compared to two control treatments in a different condition (i.e. wet and dry bare soil) a further experiment was conducted on 23 July 2020 on twelve pots as previously described in the outdoor calibration test. The same four different soil management treatments were tested, this time with three replicates each.

Before mowing (6 July 2020), the above-ground biomass of 15 plants from each cover crop treatment (i.e. five plants per pot per treatment) was collected. Grass height was measured and once scanned, green leaf area was estimated using the image-analysis Image J software (National Institutes of Health, Bethesda, MD, USA). Leaf area index (LAI) was then calculated as m^2 of green leaf area per m^2 of ground area. Cover crop LAI on the experiment day was estimated using the linear relationship between cover crop height and LAI obtained for both *F. arundinacea* ($y= 0.1358x + 0.6749$, $R^2=0.94$) and *L. corniculatus* ($y= 0.1389x + 0.8867$, $R^2=0.91$). The portable chamber operating as a closed system was used, as already illustrated, to assess the difference in terms of water loss. No further gravimetric confirmation was conducted in this pot experiment based on the quite encouraging outcome from the pot calibration itself.

Data were expressed as hourly rate of evaporation (mm h^{-1}) and converted in daily rate (mm d^{-1}) for further evaluations.

Figure 3.3. View of the treatments tested ad: A) *F. arundinacea*; B) *L. corniculatus*; C) bare soil.

3.3.5 Statistical analysis

The degree of variation around means was given as a standard error (SE). Both linear (LR) and quadratic (QR) regression analysis were used when appropriate. Statistical analysis was performed, and ANOVA was used to evaluate potential significance of mean differences in diurnal trends of evapotranspiration. Means separation was obtained through Student Newman Keuls (SNK) test at 5% probability.

3.4 Results and discussion

3.4.1 Laboratory and pot calibration of the chamber system

The plot of the gravimetric evaporation data versus the corresponding values obtained using the chamber system yielded a highly significant linear relationship ($R^2 > 0.96$) for both the laboratory and the pot system calibration test (Fig. 3.4 A, B). However, plotting chamber derived E (mm h^{-1}) vs gravimetric evaporation from the water filled cloth, showed that with increasing cloth drying the chamber measure tended to increasingly underestimate E data, especially above the 0.4 mm h^{-1} threshold.

This is not surprising as due to the quite small chamber volume (28L) progressive RH build up within the chamber is expected to decrease evaporative demand around the wet cloth. Sticking to closed chamber systems, the nearest comparison in the literature is the one reported by Luo et al. (2018) who worked on different field crops using chambers of different sizes. Although, their smallest chamber was 1395 L in volume, therefore much bigger than the one we used. Using the same calibration methodology and plotting data over the same range of E values (i.e., 0-0.85 mm h^{-1}) they found no chamber underestimation at relatively high values. Yet, at the same time, they also confirmed that the closest relationship was held from 0 to 0.4 mm h^{-1} E rates. Despite the huge difference in chamber size, this is in close agreement with our data, and it suggests that, regardless of chamber volume within the above specified limits, reliable instantaneous E measures up to about 0.4 mm h^{-1} can be granted by a closed chamber system. Taken on a daily basis and assuming an average of 10 hours of not limited transpiration, the calculated amount of about 4 mm d^{-1} would be able to accommodate even the transpiration rates of the most luxurious grass (Lopes et al., 2004). When the same water cloth calibration was carried out, again for a very similar range of water loss rates (0-0.6 mm h^{-1}) and at two different air flow rates (9.2 and 21.6 L s^{-1}), using an open chamber system with a volume of 196 L (Centinari et al., 2009) the underestimation found in our study vanished. This confirms that, when using a closed chamber system with a limited chamber volume, identifying optimal timing for data recording before E estimation becomes increasingly biased towards RH build up inside the chamber is crucial.

Figure 3.4B presents the daytime cumulative chamber and gravimetric evapotranspiration (ET) values performed outside in small pots sown with two different cover crops and two bare soil controls (one wet and the other one with dry surface) whereas Figure 3.5 shows an example, for the 4 treatments of ET (A), relative humidity (B), temperature (C) and VPD (D) variation over the 120 seconds readings taken at 16:00, having time zero set at 15 s after chamber positioning.

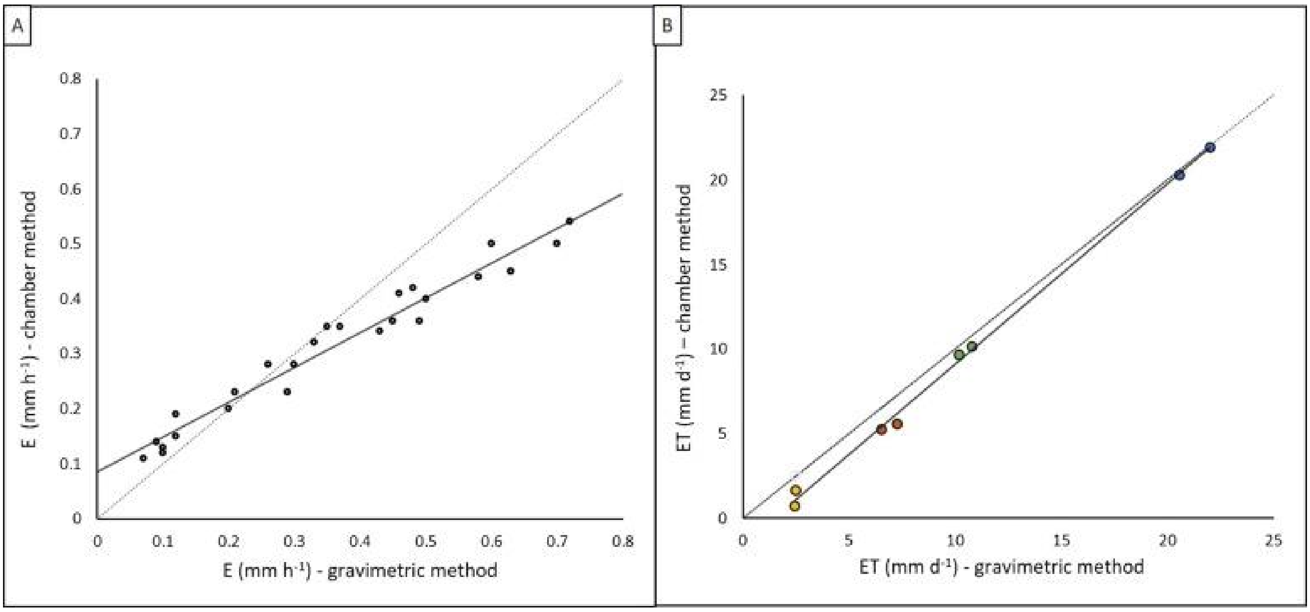


Figure 3.4. Linear regression analyses between (A) the cloth water loss (hourly averages of evaporation) measured gravimetrically and estimated by the chamber and (B) cumulated pot daily water loss measured gravimetrically and determined as the area under the daily ET curves estimated by the chamber. Four different treatments are represented in (B): *L. corniculatus* (blue circles), *F. arundinacea* (green circles); wet soil (orange circles) and surface-dry soil (yellow circles). Linear regression equations are (A) y = 0.6325x + 0.0856, R^2 = 0.9611 and (B) y = 1.0649x – 1.5513, R^2 = 0.9979. Dotting indicates the 1:1 line.

Despite slight underestimations of the actual gravimetric daily water loss when below 5 mm d^{-1}, the chamber measurements showed a very close linear fit (R^2=0.99), and the regression line was not significantly different from the 1:1 line (Fig. 3.4B). These results are quite encouraging since, in agreement with Lou et al. (2018), it is confirmed that increasing the time scale of measurements (in our case from instantaneous to daily ET values) is quite helpful to smooth out the error inherent to instantaneous readings. When examining the dynamic of ET (mm m^{-2}) of the 4 treatments within the 120 s time window (Fig. 3.5A), it is apparent that maximum ET gain occurred over the first 60 s (+88%, +88%, +84% and +83%) as compared to time zero for LC, FA, WST and ST, respectively) with a tendency to become linear during the second 60 s half. Clearly, low evaporation rates recorded in the surface dry soil treatment were not able to quickly saturate the chamber volume and maintained a more linear trend over the recording time window. Not surprisingly, similar patterns were also found for RH increase (Fig. 3.5B), however it is worth noticing that LC, FA and WST tended to overlap over similar RHs as a likely result of fast chamber humidification. Correctness of our time window length is confirmed by the air heating pattern inside the chamber (Fig. 3.5C) showing that it never exceeded 2 °C as compared to time zero and saturated in all treatments after the 60 seconds time window. This shows that the wet bulb temperature is reached after 60 seconds of chamber closure as it coincides with the adiabatic saturation temperature (Stull, 2011).

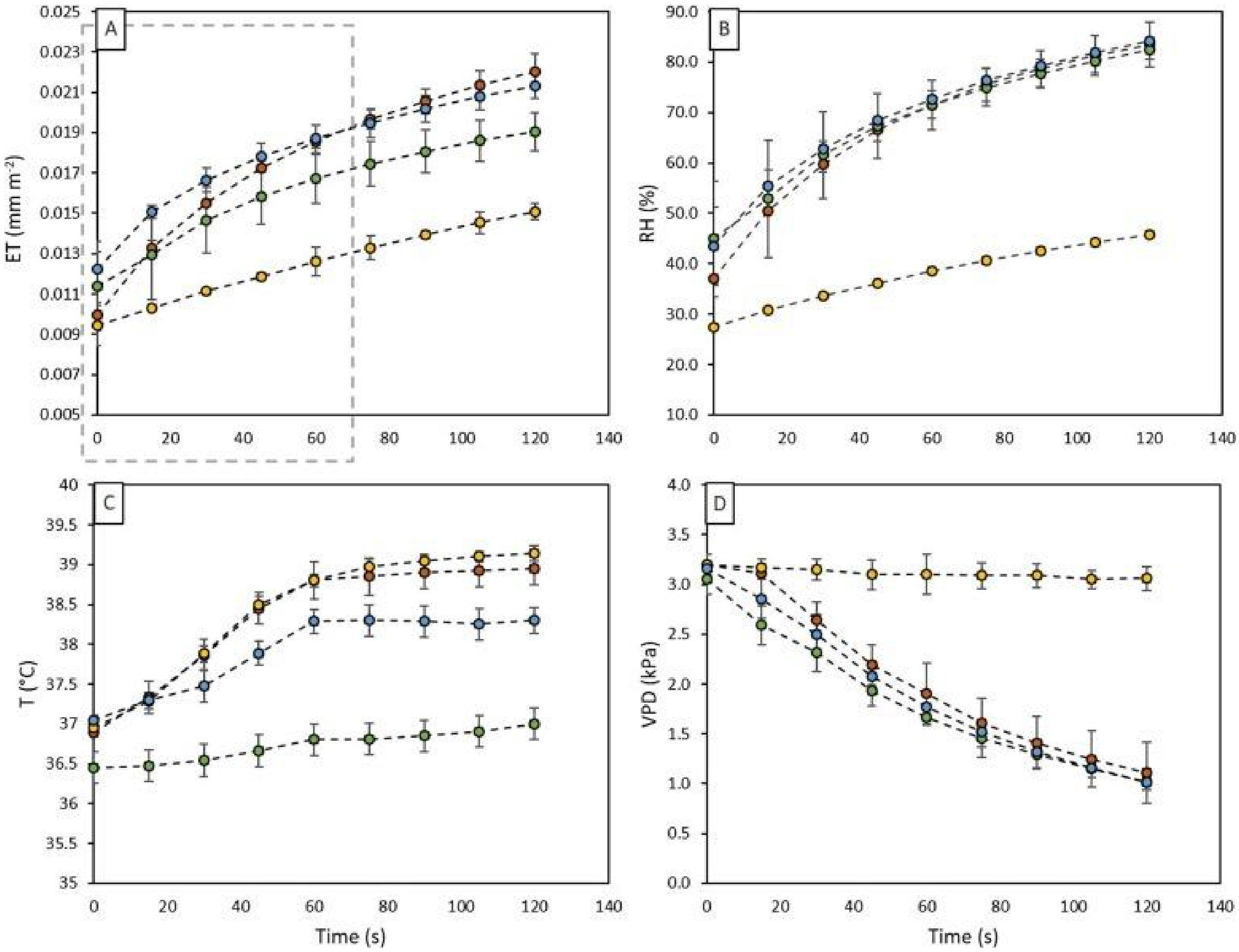


Figure 3.5. (A) Representative ET trends (mm m^{-2}) for *L. corniculatus* (LC, blue circles) and *F. arundinacea* (FA, green circles) cultivated soil, wet soil tillage (WST, orange circles) and surface-dry soil tillage (ST, yellow circles). The calculation time window (TW) is referred to the first 60 s of the water loss curve as described by the following quadratic equation: (LC) $y=-1E-06x^2 + 0.0002x + 0.0123$, $R^2 = 0.99$; (FA) $y = -6E-07x^2 + 0.0001x + 0.0113$, $R^2 = 0.99$; (WST) $y = -1E-06x^2 + 0.0002x + 0.01$, $R^2 = 0.99$; (ST) $y = -1E-07x^2 + 6E-05x + 0.0094$, $R^2 = 0.99$. ET values are mean ± SE (n = 2). Inside the chamber trends for relative humidity (B), air temperature (C) and VPD (D) measured for the four treatments during the 120 s of chamber closure are also shown. Relative humidity, Temperature and VPD values are mean values ± SE (n = 2). Data refer to reading taken at 16:00 on 22 July 2020.

3.4.2 Cover crop and soil water loss in the pot experiment

Data collection performed on 23 July at five times during the day from 9:00 to 19:00 occurred at an ambient relative humidity (RH) varying between 32 and 53% (Fig. 3.6A) while air temperature changed from 31.1 °C recorded at 9:00 to the peak of 38.6 °C registered at 13:00 (Fig. 3.6B). As a result, daily VPD varied from a minimum of 1.8 kPa to a maximum of 3.3 KPa recorded at 16:00 (Fig 3.6C).

As expected, upon chamber placement, the RH increase was faster and higher in grassed pots and lower in the surface-dry soil tillage (Fig. 3.6A). After 60 seconds of chamber closure, FA and LC RH ranged between 80 to 90%, whilst ST relative humidity never exceeded 70%. RH increase in ST was higher in the first part of the day (i.e., 9:00 and 11:00) while in the afternoon ST relative humidity recordings were very close to the outside values. That is probably due to some dew accumulation during the night on the soil surface that is progressively lost during the day. Conversely, inside the chamber RH increase in WST closely paralleled that of grassed pots albeit recording slightly lower rates.

The temperature increase (ΔT) inside the chamber never exceeded 2°C more than the one outside (Fig. 3.6B) therefore falling within a range of acceptable alteration as compared to surrounding environment (Garcia et al., 1990). The recorded ΔT was similar to the one reported in other studies using closed chambers (Grau, 1995; Guidolotti et al., 2017). However, it is quite relevant that, in our study, reasonable chamber heating was obtained under conditions of high radiation load and VPD; conversely Grau (1995) worked in an environment where ambient temperature never exceeded 22 °C therefore making the issue of chamber heating less significant. Indeed, our results point out that, even in a small, closed chamber, a good control of chamber heating can be achieved also in very warm days provided that rapid measurements for brief periods (1-2 minutes) are used.

Within each single measurement session, significant differences between the wet soil tillage (WST) and the dry soil tillage (ST) treatments were found (Fig. 3.7). For data pooled over the five periods of measurements ET in ST was curtailed by around 30% as compared to WST. The maximum gap between these two treatments was reached during the hottest time periods (i.e., 13:00 and 16:00). Although in our study we did not measure the daily evaporation rates of a wet soil that is left to dry out, our maximum WST water loss rate is similar to what was reported by Wythers et al. (1999) where recently irrigated bare soil daily evaporation rates were assessed to be as high as 7.5 to 9 mm d^{-1} and to remain around 4.5 to 7.5 mm d^{-1} for the first 6 days of the experiment. This was assessed for a clay-loam type soil that is also the same soil type used in our study. In our experiment, daily averaged ET values of WST were 5.43 mm while ST, which was monitored 9 days after the last irrigation event, registered 1.2 mm d^{-1} of water loss. Evaporation rates measured in the field on bare soil with an open chamber system after two rain events (a condition that approximates to our WST treatment) were around 3.3 mm d^{-1} (Centinari et al., 2013) a value that is a good match with the daily average amount of 0.54 mm h^{-1} measured in our study.

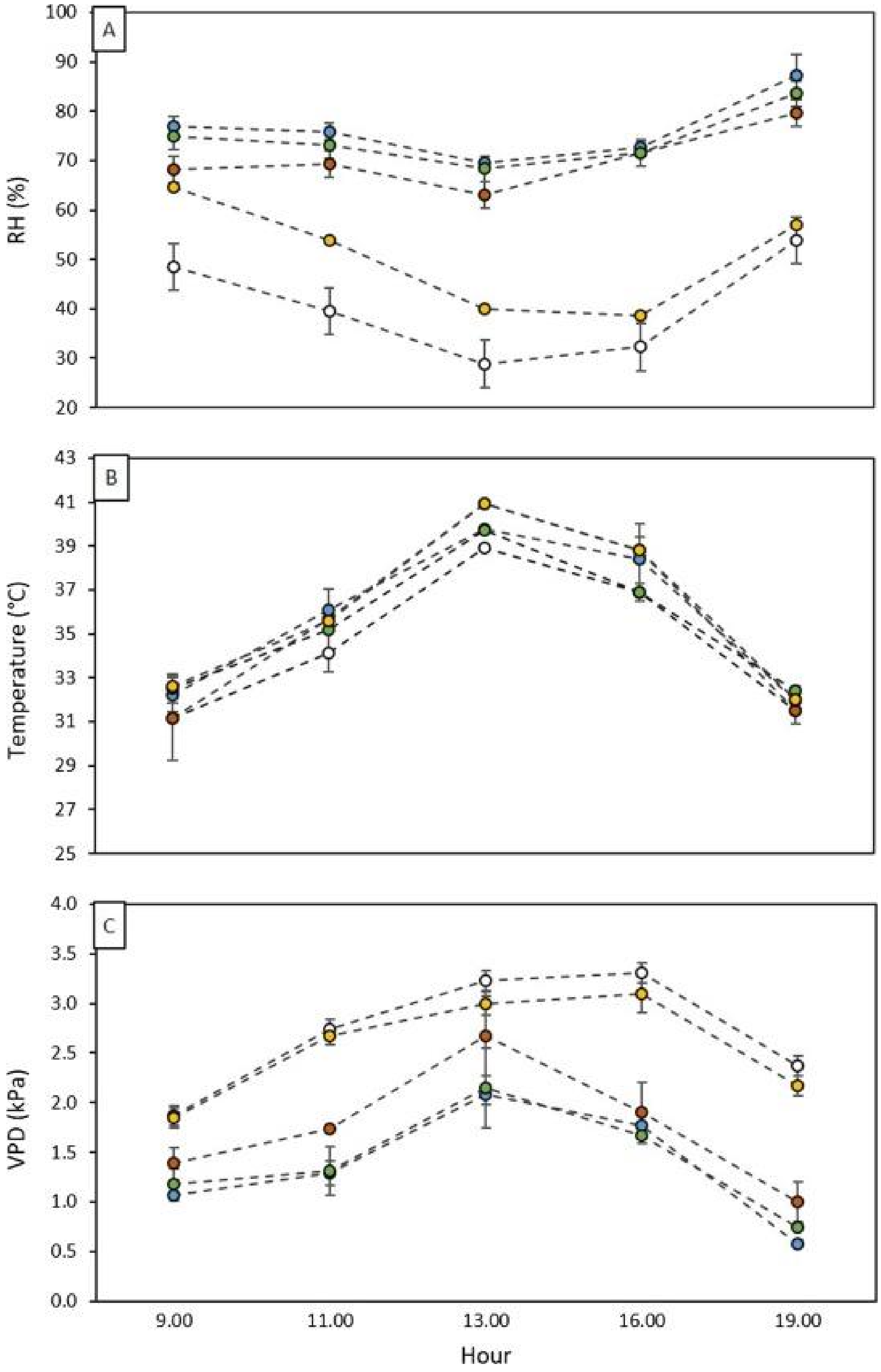


Figure 3.6. Diurnal trends for relative humidity (A), air temperature (B) and VPD (C) measured on 23 July outside the chamber (open circles) and inside the chamber for wet bare soil (WST, orange circles), surface-dry soil tillage (ST, yellow circles) and grassed soil with *L. corniculatus* (LC, blue circles) and *F. arundinacea* (FA, green circles) after 60 s of chamber closure. Relative humidity, Temperature and VPD values are mean values ± SE (n = 3).

Indeed, variability in such comparisons should take into account: i) the changes in resistance to evaporation due to differences in soil texture; ii) the amount of energy available able to drive the evaporative process (e.g., soil light interception depending upon time of the day and interaction with the grapevine canopy) and iii) the amount of water available to evaporate (Wythers et al., 1999). It is agronomically relevant from our WST recorded hourly values that a wet soil can have evaporation rates as high as those of *L. corniculatus* cover crop (LC) and even more than *F. arundinacea* (FA). For example, in the warmest hours of the day, WST and LC registered values as high as 0.58 and 0.78 mm h^{-1}, respectively at 11:00 and 13:00, while FA mean hourly ET never exceeded 0.45 and 0.70 mm h^{-1} in the same time period (Fig. 3.7). It has been shown that water loss trend from a wet soil has a typical exponential dynamic showing that 8-10 days after a wetting event the water loss becomes negligible (Wythers et al., 1999).

Daily evaporation rates measured in ST ranged from 0.18 to 0.30 mm h^{-1} showing a statistical difference with any other treatments at any timing of measurement. The daily evaporation under ST observed in the current study and estimated as the area underneath the regression curve that represented best fit to the diurnal ET pattern was of 1.2 mm d^{-1}. This value is lower than the one found in a vineyard trial as reported in Centinari et al. (2013) where tilled soil registered a water loss of 1.97 mm d^{-1}. Moreover, water loss from ST was fairly constant during the day and did not follow, for instance, air VPD which was maximum at 16:00 (Fig. 3.6D) when ET was lower than the rate measured at 11:00 (Fig. 3.7). This behaviour is helpful when trying to estimate how evaporation from dry soil can contribute to whole-vineyard water balance where tillage is still the most frequent practice (Wythers et al., 1999).

FA hourly rates of ET ranged from 0.3 to 0.7 mm h^{-1} showing higher values than ST (ranging from 0.2 to 0.3 mm h^{-1}) yet lower than those of LC and WST as daily VPD increased. For instance, in the warmest hours of the day (i.e., from 11:00 to 16:00) FA mean hourly ET was of 0.56 mm while LC and WST registered 0.71 and 0.69 mm h^{-1}, respectively. Indeed, when comparing cover crop water use across different experiments, differences in LAI should be taken into account. Similar differences between FA and LC are also presented in Grau (1995) where FA during the day registered values that were 20 to 30% less than those recorded on the legume grass. It is quite encouraging that FA water use estimated by Grau (1995) refer to a grass height (16-18 cm) and a LAI (2.8-3.6) quite similar to the conditions of our study where the same parameters set at 13.5 cm and 2.5, respectively. Our measured daily FA water consumption is also in close agreement with data reported by Litvak and Pataki (Litvak and Pataki, 2016) while being higher than the ones recorded in Centinari et al. (2009).

That is probably due to different water availability: while in Litvak and Pataki (2016) ET of irrigated turf grass reached a maximum of 10.4 mm d^{-1}, in Centinari et al. (2009), daily water consumption of *F. arundinacea* sown in mid rows of a non-irrigated mature vineyard was estimated at 3 to 4 mm d^{-1}. Our FA recorded ET values of 9 mm d^{-1} are therefore higher than the ones recorded in Centinari et al. (2009) but very close to what assessed by Litvak and Pataki (2016). Moreover, Centinari et al. (2009) worked under field conditions which were conducive to a slower grass regrowth after cutting: they had 10 cm regrowth upon 21 days after slashing, while we reached 13.5 cm after 17 days. This would confirm likely sub-optimal water supply condition in Centinari's experiment. Our FA daily water loss rates also match quite closely data reported by Uliarte et al. (2013) for the same species. ET rates they reported for a hot summer day (T = 38.1 °C and PAR = 1582 μmol m-2 s-1) within the time window between noon and 15:00 are around 320 $gH_2O\ m^{-2}\ h^{-1}$ against about 250 $gH_2O\ m^{-2}\ h^{-1}$ estimated in the current experiment from 13:00 to 16:00. Others have estimated up to 6.8 mm d^{-1} of water used for tall fescue (*F. arundinacea*) under continuously well-watered conditions using a simulation model (Qian et al., 1996).

L. *corniculatus* cover (LC) registered, together with the WST, the highest values of water loss. LC ET values ranged between 0.29 (i.e., at 9:00) and 0.8 mm h^{-1} (i.e., at 16:00). This is in agreement with what found by Grau (1995) where L. *corniculatus* registered over the day values as low as around 3 mmol $H_2O\ m^{-2}\ s^{-1}$ in the early morning (i.e., 9:00) reaching a peak value of about 11 mmol $H_2O\ m^{-2}\ s^{-1}$ at 14:00. Notably, Grau's work refer to readings taken at the beginning of flowering on a 16-18 cm tall LC cover with a LAI of 4.6-5.0, whereas we were at the same same phenological stage yet with grass height of 29.6 cm and a LAI of 5. Shorter grass height in Grau's experiment might explain why their ET increase during the morning was slower than the rate recorded in our experiment.

In terms of a more general discussion on the type of data that the chamber can deliver, as well as modalities and accuracy of sampling, it is inherent the usefulness of this kind of equipment that bursts from the following items: i) vineyard management is rapidly evolving towards sustainable soil management where tillage or native grass are increasingly replaced by sown cover crops (Diti et al., 2020b); ii) proper selection of these implies that their water use, before and after slashing, is known in order to limit water competition towards the associated grapevines and iii) in several instances, presence of grass cover (either native or sown) in a vineyard is a largely neglected factor in terms of contribution to the whole vineyard seasonal water budget and need to be precisely determined and incorporated into this budget. It has been shown that a fraction of water use accounted for/by grass covers in a vineyard ecosystem can represent up to 30-40% of the total water use (Uliarte et al., 2013).

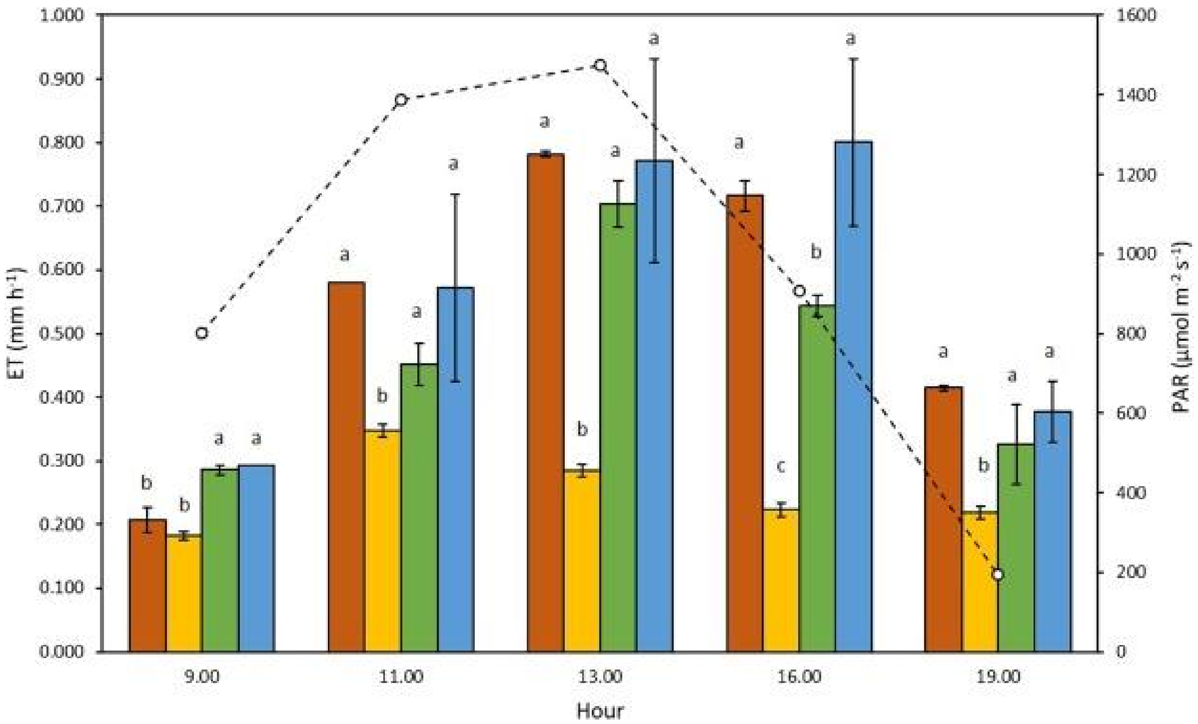


Figure 3.7. Diurnal trends of evapotranspiration (ET, mm h^{-1}) for the wet bare soil (brown), surface-dry soil tillage (yellow), *F. arundinacea* (green) and *L. corniculatus* (blue). Open circles indicate hourly PAR (µmol $m^{-2}s^{-1}$). Data were collected on 23 July. ET are means ± SE (n = 3). For a given hour different letters indicate significant differences among treatments (SNK test, $p < 0.05$).

3.5 Conclusions

In this study a low-cost, custom-made closed portable chamber was tested under controlled and semi-controlled conditions (i.e., laboratory and an outdoor pot-lot). The plot of the gravimetric evaporation data versus the corresponding values obtained using the chamber system yielded a highly significant linear relationship for both the laboratory and the pot system calibration test (R^2 equal to 0.96 and 0.99, respectively). We infer that running calibration under ambient conditions (as opposed to controlled) greatly reduce chamber biases and provide best accuracy. The chamber proved to be a reliable, efficient and accurate way to measure ET for a range of time scales (i.e., instantaneous and cumulated daily) under bare soil conditions and sown crops of *L. corniculatus and F. arundinacea*. Moreover, the chamber is low cost, easy to set up and can be transported rapidly across experimental plots. Therefore, the newly proposed system enables, for example, fast multipoint evaluations of ET fluxes at a very reasonable budget. Future studies will include validation of the chamber method over a range of cover crop varieties and orchard field conditions.

Chapter 4. Published Manuscript 3: A comparative study of fifteen cover crop species for orchard soil management of water uptake, root density and soil aggregate stability

4.1 Abstract

Increasing the use of cover crops (CCs) is a necessity in sustainable viticulture, although it might clash with possible excessive competition towards vines. Especially in a climate-change scenario, the latter feature should be minimized while maintaining ecosystem services. Aimed at identifying CCs for vineyard floor management, the trial characterized several species according to their evapotranspiration (ET) rates, root growth patterns, and soil aggregate stability potential.

The study was performed in 2020 in Piacenza (Northern Italy) on 15 CC species grown in pots kept outdoor and classified as grasses (GR), legumes (LE) and creeping (CR). Together with bare soil (control), they were arranged in a complete randomized block design. CCs ET was assessed through a gravimetric method and using a closed portable chamber, starting before mowing and then repeated 2, 8, 17 and 25 days thereafter. Above-ground dry biomass (ADW), root length density (RLD), root dry weight (RDW) and root diameter class length (DCL) were measured, and mean weight diameter (MWD) was calculated within 0-20 cm depth.

Before mowing, ET was the highest in LE (18.6 mm day^{-1}) and the lowest in CR (8.1 mm day^{-1}) the latter being even lower than the control (8.5 mm day^{-1}). The high ET rates shown by LE were mainly related to very fast development after sowing, rather than to a higher transpiration per unit of leaf area. After mowing, the 15 species' ET reduction (%) plotted vs leaf area index (LAI, m^2 m^{-2}) yielded a very close fit ($R^2 = 0.94$), suggesting that (i) a linear decrease in water use is expected anytime starting with an initial LAI of 5-6, (ii) a saturation effect seems to be reached beyond this limit.

Selection of cover crop species to be used in the vineyard was mainly based on diurnal and seasonal water use rates as well as dynamic and extent of root growth patterns. Among GR, *Festuca ovina* stood out as the one with the lowest ET due to its "dwarfing" characteristics, making it suitable for a permanent inter-row covering. CR species confirmed their potential for under-vine grassing, assuring rapid soil coverage, lowest ET rates, and shallow root colonization.

4.2 Introduction

Vineyards are frequently established on inherently poor soils (Coll et al., 2011) and subjected to intensive management practices, threatening soil functions and associated ecosystem services (Diti et al., 2020c; Garcia et al., 2018; Salomé et al., 2016). Moreover, the Mediterranean climate is often characterized by severe summer droughts associated with short, yet heavy rainstorms in autumn-spring, favouring the run-off of surface waters (Rodrigo-Comino et al., 2018; Salomé et al., 2016), soil degradation and erosion (González-Hidalgo et al., 2007; Ruiz-Colmenero et al., 2011). High surface water runoff due to short and heavy rainstorms in autumn-spring removes the more fertile topsoil layer, reducing soil organic matter (SOM) content and carbon (C) sequestration, nutrients availability and water-holding capacity leading to an overall decrease in soil fertility and crop productivity (Dennis C. Flanagan et al., 2013). In addition, following SOM loss, soil aggregates tend to break down more easily and soil erodibility worsens (le Bissonnais and Arrouays, 1997; Wu and Tiessen, 2002). Lastly, surface runoff and resulting soil erosion are the main routes through which fertilizer and pesticide residues reach surface waters (Dennis C. Flanagan et al., 2013).

Conventional vineyard soil management affects soil properties (Gatti et al., 2022; Salomé et al., 2016). Mechanical weeding may induce physical degradation of vineyard soils (Coulouma et al., 2006; Ruiz-Colmenero et al., 2011), and modify soil biological communities at different trophic levels (Schreck et al., 2012). Conversely, vineyard cover cropping is considered a sustainable soil management strategy, as it boosts essential ecosystem services of soil (Garcia et al., 2018), including surface water infiltration (Basche and DeLonge, 2019), C sequestration (Freibauer et al., 2004), and reduced soil erosion (López-Vicente et al., 2020; Novara et al., 2011; Ruiz-Colmenero et al., 2011). Further, cover crops (CCs) can help to protect soil from water and/or wind erosion, as they improve soil aggregate stability (Goulet et al., 2006) and protect them from the raindrops impact (Dabney et al., 2001).

CCs can also help enhancing/maintaining a favourable soil structure and stable porosity in vineyards (Ferrero et al., 2005) as root development and turnover directly influence subsoil structure, increasing macro-porosity. During growth, roots exert pressure which generates a reorganization of the soil pore network (Kolb et al., 2012). After root decomposition, root-dug channels remain empty, forming bio-pores (Jones et al., 2004; Leonard and Andrieux, 1998). Consequent to increased soil macro-porosity, soil surface hydraulic conductivity, water infiltration, and sub-soil refilling usually improve during the rainy season (Gaudin et al., 2010; Wassenaar et al., 2005). During a rainfall event, if the soil becomes saturated, the hydraulic conductivity of the soil surface decreases, leading to surface water runoff (Garcia et al., 2018). Such a decrease is partly counteracted by the presence of a CC (B. A. Joyce et al., 2002). Further, CC leaf area reduces the kinetic energy of raindrops and promotes water infiltration as the staying time of water at the soil surface increases (Wassenaar et al., 2005).

The improved rainfall infiltration rate and enhanced soil water storage promoted by CCs might warrant additional soil water storage (Gaudin et al., 2010). This is especially significant in areas where precipitation occurs over a relatively short time in a series of heavy rainfall events (Garcia et al., 2018), as in the Mediterranean area. However, vine growers in the Mediterranean regions are still quite reluctant to use CCs due to concerns about water and nutrient competition with the main crop (Celette et al., 2009; Celette and Gary, 2013) as the above-mentioned additional water budget could be rapidly used (i.e., transpired), partly or totally by the CC itself (Celette et al., 2008).

Typically, the most common technique of cover cropping involves the management of native species as readily available and inexpensive (Diti et al., 2020; Pardini et al., 2002) yet, usually being the most competitive for both water and nutrients (Celette and Gary, 2013; Porqueddu' et al., 2000).

To mitigate or remove competition, CC is often terminated in spring with tillage (Diti et al., 2020). Nonetheless, as a negative side effect of this decision, several benefits bound to the permanent cover of the vineyard soil (e.g., facilitated machine transit with wet soil, reduced soil erosion, etc.) are lost (Biddoccu et al., 2020; Diti et al., 2020). Therefore, identifying appropriate strategies (i.e., CC species and adoption of the best cultural practices) to maintain the permanent soil cover benefits, while reducing CC competition in vineyards, is still necessary.

According to the literature, mowing can be used as a useful short-term water preservation strategy (Celette and Gary, 2013; Centinari et al., 2013). After mowing, sward residual mass left *in situ* further protects the soil from erosion and runoff (Baumhardt and Blanco-Canqui, 2014; Prosdocimi et al., 2016), and improves soil health in the short term (Warren Raffa et al., 2021), while reducing water competition and soil evaporation (Centinari et al., 2013; Lopes, 2018).

To exploit as many positive externalities as possible and to reduce the potential problems associated with the presence of CC in a vineyard, it is advisable to switch from the use of native species to sown (i.e., selected) ones (Pardini et al., 2002). Moreover, when a high risk of water competition towards the consociated vine is assessed, the selection of the appropriate type of CCs becomes crucial, favouring those featuring reduced above-ground biomass and root development (Pardini et al., 2002), assuming such characteristics to be conducive to a lower water consumption (Delpuech, 2013; Porqueddu' et al., 2000).

Unfortunately, to date, the winegrowers' demand for low-competitive species is still largely unmet (Delpuech, 2013). Agroscope (Changins - Wädenswil, Switzerland) has initiated the selection and propagation of low-competition genotypes for use in vineyards (Delabays et al., 2006; Delabays and Spring, 2000). Moreover, the desirable ideotype should possess some other important characteristics: i) good establishment capacity and resistance to repeated trampling; ii) homogeneity and long-lasting soil cover; iii) effective weed control; iv) perennial habitus (to reduce seeding cost); v) reduced aerial development (to reduce maintenance and vineyard interventions) and vi) summer growth lag followed by autumn recovery.

Among almost fifty species, tested best results were obtained with *Hordeum murinum* and, to a less extent, *Trifolium subterraneum* and *Trifolium repens* (Delabays and Spring, 2000). Other studies have shown that perennial species (e.g., *Trifolium repens*) tend to be more competitive with vines, compared to annuals with spontaneous reseeding (Delabays et al., 2006). Not surprisingly, the less competitive ones are also those with greater difficulty in ensuring the establishment of the sward and maintaining a good soil coverage over time, often being invaded by native grass within two or three seasons (Delpuech, 2014).

This pot trial is, to the best of our knowledge, the first case of a comparative screening to evaluate water use, root characterization, and anti-erosion potential of a large number of herbaceous species potentially targeted for vineyard use. Together with some already used CCs, such as grasses (GR) and legumes (LE), new creeping (CR) ones were included in the study for their potential interest as living mulches under the trellis.

The present study aimed to compare different CC species for (i) assessing water loss (use) before and after mowing, (ii) characterize root traits and clarify their effects on soil aggregation, and (iii) identify the most recommended species for vineyard cover cropping.

4.3 Materials and methods

4.3.1 Plant material and experimental layout

The study was conducted in 2020 at the Department of Sustainable Crop Production, Università Cattolica del Sacro Cuore (Piacenza, Northern Italy, 45° 2' N; 09° 42' E) on 64 pots of 15 L volume (0.27 m deep, with an internal diameter of 0.27 m) kept outdoor in a pot-lot. Pots were filled with clay-loam soil having 35% sand, 36% silt and 29% clay. Field capacity, permanent wilting point, and soil bulk density were estimated at 32.8%, 18.7% and 1.42 g cm^{-3}, respectively(Saxton et al., 1986).

The experiment was set up as a randomized complete block design (RCBD) with four replicates and sixteen treatments: a control (i.e., bare soil) and fifteen CCs, which were tested as divided into three groups: i) grasses, ii) legumes, and iii) creeping plants (Table 4.1).

CCs' sowing rate was computed according to a previous germination test (Table 4.1) and all CCs were manually seeded on 20 April 2020. By the time the first measurements were made, they all had 100% soil coverage and no weed growth was recorded. To aid plant establishment and avoid any water deficit, throughout the trial period, each pot was supplied with 350 mL of water three times per day (i.e., 55% of available water) delivered by an automated single dripper. Automated watering was stopped a day before ET measurements and the exact amount of 1 L pot^{-1} was given manually.

During the trial season, pot management consisted of two mowing events and two tillage operations. On 6 July and 20 September 2020, grasses and legumes were hand-mowed to ~4 cm above soil surface while creeping plants were only trimmed, as it concerns the aerial biomass exceeding the pot's edges, as they are not supposed to be trimmed in height under open field conditions. On the same days, light soil tillage (around 3 cm depth) was performed in the bare soil pots using a three-tooth rake.

Table 4.1. Cover crop tested are here shown as divided into the three groups defined. The seeding rate (g m^{-2}) was defined according to the label guidelines of each species and computed according to the germination test. Total above-ground dry clipped biomass (ADW_TOTAL; g m^{-2}) -i.e. the equivalent of the two mowings made.

Cover crop group	Cover crop	Seeding rate (g m^{-2})	ADW_TOTAL (g m^{-2})
Legumes	*Trifolium michelianum* Savi cv. Bolta	0.96	937.65
	Medicago polymorpha L. cv. Scimitar	1.20	428.17
	Medicago lupulina L. cv. Virgo	4.20	1087.24
	Medicago truncatula Gaertn. cv. Paraggio	1.73	780.86
	Lotus corniculatus L. cv. Leo	1.56	2121.11
Grasses	*Festuca arundinacea* Schreb. cv. Thor	38.50	630.41
	Festuca ovina L. cv. Ridu	24.00	277.20
	Festuca rubra L. var. *commutata* Gaud. cv. Casanova	24.00	447.85
	Poa pratensis L. cv. Tetris	8.40	598.82
	Lolium perenne L. cv. Playfast	7.00	352.86
Creeping	*Glecoma hederacea* L.	2.92	1035.25
	Hieracium pilosella L.	0.73	102.80
	Dichondra repens J.R.Forst. & G.Forst	4.00	218.11
	Sagina subulata (Swartz) C. Presl	0.07	0.00
	Trifolium subterraneum L. cv. Denmark	3.24	23.21

4.3.2 Evapotranspiration measurements, above-ground biomass and roots sampling

CCs' ET measurement was performed through a gravimetric method as, at each measuring date, all pots were weighed at 8 a.m. and 7 p.m. with an electronic scale with a resolution of 0.01 g. The daily CC ET (mm d^{-1}) was calculated as $\Delta W/S$, where ΔW is the change in the pot mass between the 2 daily weights, and S is the surface area of the pot (Centinari et al., 2013). ET rates measured were then referred to per square meter of removed leaf area (ET_{LEAF}) when needed. ET rates were also assessed through the closed chamber method after Capri et al. (2021). Daily maximum, mean and minimum air temperature (°C), together with daily precipitation (mm), were monitored throughout the experiment, and data were collected from an automated meteorological station positioned next to the experiment pot-lot.

On 6 July and 20 September 2020, the hand-cut biomass was collected and placed in a ventilated oven at 105 °C until constant weight, and then the above-ground dry weight was measured as first (ADW_MW1) and second (ADW_MW2) mowing. Total above-ground dry weight (ADW_TOTAL) was calculated as the sum of the two cuts.

Before mowing, the above-ground fresh biomass of 20 plants from each tested CC (i.e., five plants per pot) was sampled and the equivalent leaf area was measured using the image-analysis Image J software (National Institutes of Health, Bethesda, MD, USA) (Capri et al., 2021). The sampled biomass was then dried in a ventilated oven at 105 °C until constant weight. Cover crop LAI on the first day of ET measurement was estimated fitting the ADW_MW1 in the linear regression leaf area vs dry weight linear regression obtained for all CC tested (Table 4.2).

For CR alone, as the above-ground biomass was clipped exclusively when exceeding the pot borders, LAI was estimated through pot photo-analysis. The total leaf number per pot was counted on the photo prints (despite the surface pot being completely covered, leaves were clearly visible as just a few overlaps occurred) and multiplied by CC mean leaf area (known from the leaf sampling mentioned above).

Root sampling was conducted on September 29 with a self-constructed "Shelby" tube sampler of known volume (6.88 cm diameter and 23.2 cm length) that was inserted into the soil to reach 0.2 m depth. Soil samples for each pot were taken at an intermediate position between the edges and the centre of the pot. Each soil core was divided into two layers: 0-10 cm and 10-20 cm soil depths. Two more samples per pot were then taken at the end of the trial, on 4 February 2021, with a tubular soil sampler (2.5 cm diameter) for aggregate stability analysis. The litter (if present) was removed, and each soil core was divided into 0-10 and 10-20 cm depths. Soil samples were passed through an 8 mm sieve through gentle breaking (Denef et al., 2007), air-dried and stored at room temperature for subsequent determinations.

4.3.3 Root characterization

Soil cores were stored at -20 °C until root separation and analysis were carried out. After defrosting, samples were kept in a solution of oxalic acid (2%) for 2 hours to facilitate the separation of roots from soil (Fiorini et al., 2018). Soil samples were then washed and cleaned. The roots were recovered from the water using a 2 mm sieve (Fiorini et al., 2018). Finally, the roots were hand-cleaned from organic particles, immersed in 10% (v/v) ethanol solution(Monti and Zatta, 2009) and stored at +4 °C. For scanning, roots were placed on a transparent plastic tray. Distilled water was added to the tray to facilitate the layout of the root and minimise overlapping.

The roots' images were acquired by a scanner (Epson Expression 10000xl, 600 dpi) equipped with a double light source to avoid root overlapping (Chimento and Amaducci, 2015). The software WinRHIZO Reg 2012 (Regent Instrument Inc., Quebec, Canada) was used to determine RLD (cm cm^{-3}) and the root diameter (RD, mm). RLD within each diameter class – namely the DCL (mm cm^{-3}) – was calculated for very fine (DCL_VF, <0.075 mm), fine (DCL_F, 0.075-0.2 mm), medium (DCL_M, 0.2-1.0 mm) and coarse (DCL_C, > 1.0 mm) roots, as adapted from Reinhardt and Miller (1990). Moreover, RDW (mg cm^{-3}) was gravimetrically determined after drying the roots in a ventilated oven at 60 °C until constant weight.

Table 4.2. Leaf area (cm^2) vs dry weight (g) linear regression and R^2 for each cover crop tested.

Cover crop group	Cover crop	Linear regression	R^2
Legumes	*Trifolium michelianum* Savi cv. Bolta	y = 353.43x + 0.3896	0.89
	Medicago polymorpha L. cv. Scimitar	y = 191.1x + 0.9518	0.46
	Medicago lupulina L. cv. Virgo	y = 215.49x + 0.539	0.67
	Medicago truncatula Gaertn. cv. Paraggio	y = 177.78x + 0.6999	0.84
	Lotus corniculatus L. cv. Leo	y = 171.41x + 0.2284	0.80
Grasses	*Festuca arundinacea* Schreb. cv. Thor	y = 94.141x + 0.4729	0.73
	Festuca ovina L. cv. Ridu	y = 199.78x + 0.0821	0.53
	Festuca rubra L. var. *commutata* Gaud. cv. Casanova	y = 99.508x + 0.3949	0.69
	Poa pratensis L. cv. Tetris	y = 116.24x + 0.2004	0.83
	Lolium perenne L. cv. Playfast	y = 114.93x + 0.3919	0.72
Creeping	*Glecoma hederacea* L.	y = 199.06x + 1.2761	0.74
	Hieracium pilosella L.	y = 202.01x + 1.1024	0.85
	Dichondra repens J.R.Forst. & G.Forst	y = 178.36x + 0.23	0.92
	Trifolium subterraneum L. cv. Denmark	y = 130.35x + 2.4772	0.37

4.3.4 Soil aggregate distribution and mean weight diameter

Subsamples of 80 g were dipped into deionized water for 5 minutes and wet sieved. Three sieves of 2000 µm, 250 µm, and 53 µm meshes were used to separate the four aggregate fractions: LM (>2000 µm), sM (250-2000 µm), m (53-250 µm) and s+c (<53 µm). Each fraction was isolated by manually moving the sieve up and down 50 times. After each phase, soil aggregates remaining on the top of the sieve were transferred onto an aluminium pan, oven dried at 105 °C and weighed. Water and soil passing through the sieve were poured onto the smaller sieve mesh, thus starting the next phase (wet-sieving).

All fractions were corrected for sand content, and the MWD was calculated according to van Bavel (1950) as follows:

$$MWD = \sum_{i=1}^{n} x_i \, w_i \qquad (1)$$

where x_i is the mean diameter of each aggregate-size fraction separated by sieving, and w_i is the proportion of each sand-free aggregate-size fraction out of the entire sample weight.

4.3.5 Statistical analysis

All data were subjected to a one-way analysis of variance (ANOVA) using IBM SPSS Statistics 27 (SPSS Inc., Chicago, USA). In case of significance of the Fisher test, mean separation was performed through the Student-Newman Keuls (SNK) test ($p<0.05$).

Principal Component Analysis (PCA) was also carried out on 10 representative variables of both below and above-ground growth (RLD, DCL_VF, DCL_F, DCL_M, DCL_C, RDW, ADW_MW1, and ADW_TOTAL) and water use (UMW_ET and MW_ET_25) using the XLSTAT statistical package (Addinsoft, New York, NY, United States). The chosen PCA was a Pearson correlation matrix. The number of filter factors was set at 5 and the final data visualization was in the form of a distance bi-plot.

A correlation analysis was performed separately for the two soil depths considered (0-10 and 10-20 cm) to assess the relationship between root traits (RLD, DCL_VF, DCL_F, DCL_M, DCL_C, RDW) and aggregate size fractions (LM, sM, m, s+c, MWD), using the non-parametric Spearman rank coefficient (ρ). A p-value of 0.05 was considered significant for the test. We used R 4.0.3.(Pinheiro et al., 2007) with factoextra (Kassambara and Mundt, 2020) package for the Spearman's rank correlations, respectively.

4.4 Results

4.4.1 Evapotranspiration measurements and above-ground biomass

Figure 4.1 shows daily evapotranspiration (ET, mm day^{-1}) of each CC tested before mowing (DOY, day of the year, 184) and at 2, 8, 17 and 25 days after mowing (DOY 190, 196, 205 and 213); bare soil was also included as a reference. Before mowing, ET rates showed significant differences between and within the three groups. CR plants had a mean ET of 8.1 mm day^{-1}, which was lower, compared to the other two groups (10.6 and 18.6 mm day^{-1} for GR and LE, respectively) and the bare soil control (8.5 mm day^{-1}). On DOY 184, values as high as 9.4 (*Glechoma hederacea* L., GH) and 9.8 mm day^{-1} (*Trifolium subterraneum* L. cv. Denmark, TS) were found (Fig. 4.1), while ranging around 7 mm day^{-1}, *Dichondra repens* J.R.Forst. & G.Forst. (DR), *Hieracium pilosella* L. (HP), and *Sagina subulata* (Swartz) C. Presl (SS) ET were lower than soil evaporation itself.

On the same day, a large ET variation was recorded within the GR group as *Festuca arundinacea* Schreb. cv. Thor (FA) scored the highest daily ET values (13.4 mm day^{-1}), whereas in *Festuca ovina* L. cv. Ridu (FO), water loss was reduced by 45% (7.5 mm day^{-1}). Within the 15 CCs, LE registered the highest pre-mowing ET with *Trifolium michelianum* Savi cv. Bolta (TM) peaking at 22.6 mm day^{-1}. However, within LE, *Medicago polymorpha* L. cv. Scimitar (MP) showed ET values as low as 12.1 mm day^{-1} (Fig. 4.1). Two days after mowing, all tested CCs recorded ET values lower than 9 mm day^{-1} (Fig. 4.1). Moreover, water use reduction among LE ranged between 56% (*M. polymorpha,* MP) and 73% (*T. michelianum*, TM), such that *T. michelianum* (TM, 6.1 mm day^{-1}), *Medicago truncatula* Gaertn. cv. Paraggio (MT, 5.6 mm day^{-1}) and *M. polymorpha* (MP, 5.2 mm day^{-1}) registered ET values lower than the bare soil (7.0 mm day^{-1}). Even though registering a consistent ET reduction after mowing, GR retained ET rates slightly higher than bare soil, except for *F. ovina* (FO), which recorded the lowest at 6.3 mm day^{-1}. Subsequent samplings showed that most of the CCs had a progressive recovery in water use (Fig. 4.1) and data taken 17 days after mowing confirmed that *Lotus corniculatus* L. cv. Leo (LC) and all GR fetched pre-mowing ET rates. *Medicago lupulina* L. cv. Virgo (ML) registered a partial recovery with similar rates (about 13 mm day^{-1}) at 17 and 25 days after the mowing event. *F. ovina* and all remaining LE stayed below 10 mm day^{-1} with ET values close to the control until the end of the trial. At 17 days from grass cutting, under a quite high exceeding-the-pot biomass, both *G. hederacea* (GH) and *T. subterraneum* (TS) reached ET values as high as 12.0 and 11.4 mm day^{-1}, respectively. On the other hand, *D. repens* (DR), *H. pilosella* (HP), and *S. subulata* (SS) even though with slightly higher ET values than those registered at the beginning of the trial (DOY 184), remained close to the soil evaporation rates until DOY 213.

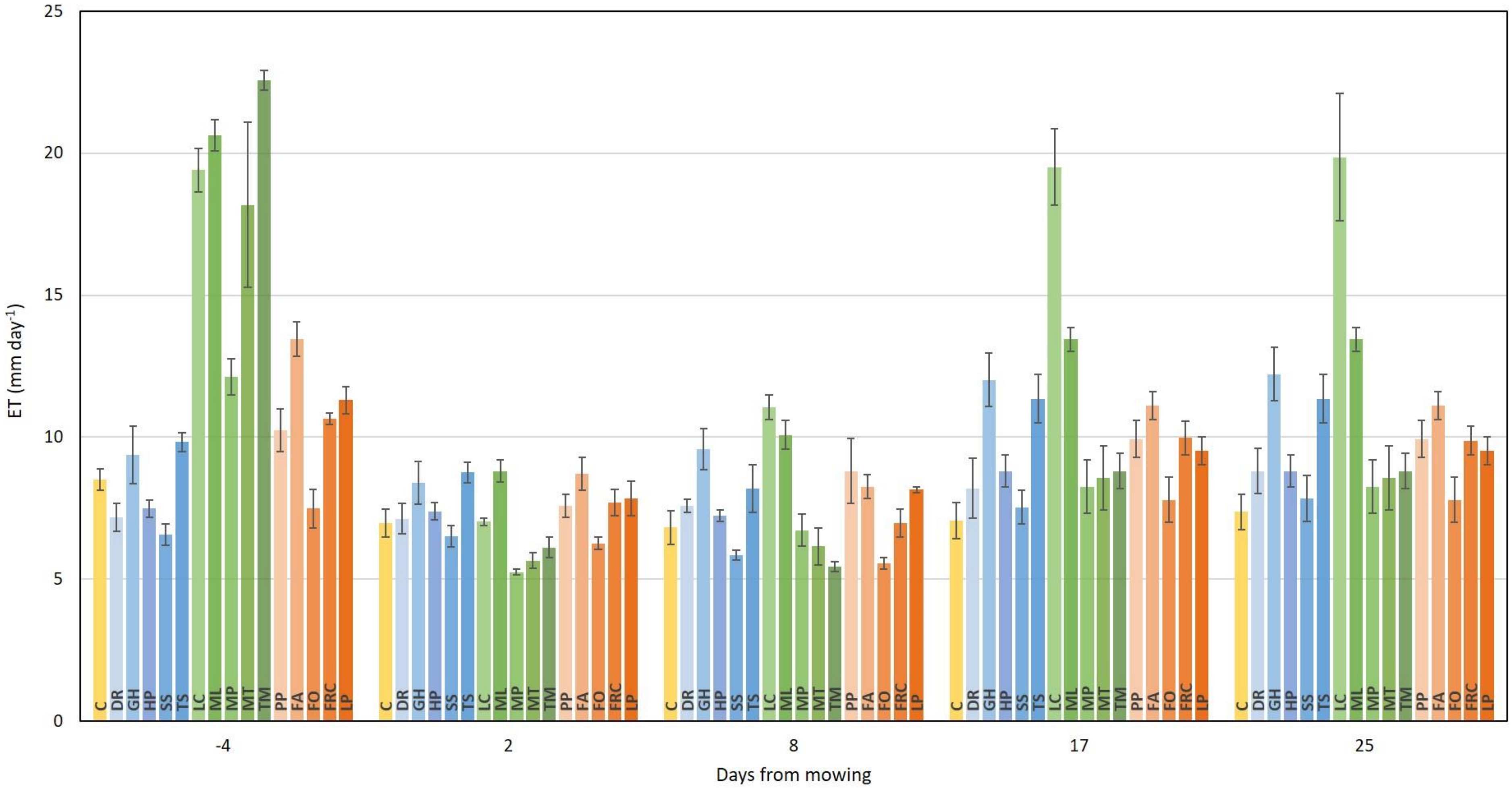


Figure 4.1. Vertical bars represent the daily water use as referred to unit of soil (ET, mm day^{-1}) for the bare soil (yellow) and all the cover crop species as divided into creeping plants (shades of blue), legumes (shades of green) and grasses (shades of orange). Evapotranspiration was measured though a gravimetric method before (i.e., -4) and at 2, 8, 17 and 25 days after mowing. ET data are mean values ± SE (n = 4).

Aboveground dry clipped biomass at the first mowing date (ADW_MW1, DOY 188) showed large differences among groups, as represented in Table 4.3. ADW_MW1 within LE was quite variable, as values ranged between 274.3 g m^{-2} (*M. polymorpha,* MP) and 750.0 g m^{-2} (*T. michelianum*, TM). With a mean value of 565.9 g m^{-2}, LE aboveground biomass was 80% higher than the mean GR ADW_MW1 (110.2 g m^{-2}). *F. ovina* (FO) scored the lowest value at 48.4 g m^{-2} among grasses, while within the creeping group, *G. hederacea* (GH) and *T. subterraneum* (TS) had biomass development outside the pot edges totalling 89.6 g m^{-2} and 23.2 g m^{-2}, respectively.

Leaf area index (LAI, m^{2} m^{-2}) at mowing showed the highest values in LE with LAI peaking at 12.4 (Table 4.3). Among GR, LAI did not show significant differences, being around 1.2. Concerning CR, LAI was assessed at 0.2 and 0.8 for *T. subterraneum* (TS) and *G. hederacea* (GH) respectively, while LAI estimated through photo analysis ranged between 1.3 (*D. repens,* DR) and 3.6 (*T. subterraneum* TS).

Table 4.3. Aboveground dry biomass clipped at the first mowing event (ADW _MW1), the corresponding leaf area surface index (LAI) and water use per leaf area unit (ET_{LEAF}) of all cover crops tested.

Cover crop group	Treatment (T)	ADW_MW1 (g m^{-2})	LAI (m^{2} m^{-2})	ET_{LEAF} (mm m^{-2} day^{-1})
Legumes	*Trifolium michelianum*	750.0 a	12.4 a	1.81 d
	Medicago polymorpha	274.3 c	2.5 c	4.92 bc
	Medicago lupulina	503.3 b	5.1 b	4.05 bcd
	Medicago truncatula	641.2 a	5.4 b	3.40 bcd
	Lotus corniculatus	660.7 a	5.3 b	3.65 cd
Grasses	*Festuca arundinacea*	161.8 cd	1.5 cd	8.83 a
	Festuca ovina	48.4 d	1.0 cd	7.75 a
	Festuca rubra commutata	125.3 cd	1.2 cd	8.54 a
	Poa pratensis	108.6 cd	1.3 cd	8.12 a
	Lolium perenne	106.8 cd	1.2 cd	9.22 a
Creeping	*Glecoma hederacea*	89.6 d	0.8 cd	3.68* bcd
	Hieracium pilosella	0.0 d	-	3.86* bcd
	Dichondra repens	0.0 d	-	5.46* b
	Sagina subulata	0.0 d	-	-
	Trifolium subterraneum	23.2 d	0.2 d	2.74* bcd

Lowercase letters indicate significant differences among treatments (SNK test, $p<0.05$) and * indicates ET_{LEAF} based on LAI estimated through photo analysis as creeping plants were not mowed.

Evapotranspiration per leaf area unit (ET_{LEAF}) was notably higher in GR, ranging between 7.75 (*F. ovina*, FO) and 9.22 (*Lolium perenne* L. cv. Playfast, LP) mm m^{-2} day^{-1} (Table 4.3). In descending order, ET_{LEAF} was the highest in *D. repens* (DR, 5.46 mm m^{-2} day^{-1}). Similar ET_{LEAF} was found when comparing some LE and CR species such as *M. truncatula* (MT, 3.40 mm m^{-2} day^{-1}), *M. lupulina* (ML, 4.05 mm m^{-2} day^{-1}), *G. hederacea* (GH, 3.68 mm m^{-2} day^{-1}), *H. pilosella* (HP, 3.86 mm m^{-2} day^{-1}) and *T. subterraneum* (TS, 2.74 mm m^{-2} day^{-1}). *T. michelianum* (TM), with 1.81 mm m^{-2} day^{-1} scored the lowest ET_{LEAF} of all species (Table 4.3).

Plotting LAI versus the before-mowing ET yielded a significant quadratic relationship (R^2>0.76) (Fig. 4.3A) which helped to distinguish two different data clouds. Till LAI values of about 6, the model was linear, having at its lower end all GR and CR species with the inclusion of *M. polymorpha* (MP) as a legume, while, at the other end, *M. truncatula* (MT), *L. corniculatus* (LC) and *M. lupulina* (ML) were grouped together. *T. michelianum* (TM) was isolated from all CCs at 22.56 mm day^{-1}.

When regressing the fraction of ET reduction, compared to pre-mowing values vs LAI (Fig. 4.2B), the same quadratic model achieved a very close fit ($R^2 = 0.94$, $p < 0.01$). CC grouping was similar to the patterns highlighted for ET, although more accurate predictions were reached at LAI, varying from 0 to 3. A linear ET reduction was shown when LAI removed through trimming ranged between 0 and 6, while thereafter, ET reduction was less than proportionate to the amount of LAI removed. This suggests an LAI of 5-6 as a benchmark, within which it is possible to maximise water use reduction after the trim.

4.4.2 Root growth and soil colonization

Root length density (RLD, cm cm^{-3}) determined for each CC at 0-10 cm and 10-20 cm depth is shown in Table 4.4. Within the topsoil layer, RLD of *Poa pratensis* L. cv. Tetris (PP), *Festuca rubra* L. var. *commutata* Gaud. cv. Casanova (FRC), and *F. arundinacea* (FA) peaked at 52.5; 53.7 and 59.0 cm cm^{-3}, respectively, whereas *M. polymorpha,* (MP), *M. truncatula* (MT), *T. subterraneum* (TS) and *T. michelianum* (TM) did not reach the 10 cm cm^{-3} threshold (Table 4.4). *L. corniculatus* (LC) recorded the highest RLD (29.7 cm cm^{-3}) at 0-10 cm among the LE species while being very close to *F. ovina* (FO, 30.3 cm cm^{-3}), which had the lowest RLD within the GR group. In the CR group, the highest and lowest RLD values within the top layer were found in *G. hederacea* (GH) and *T. subterraneum* (TS), at 26.9 and 7.4 cm cm^{-3} respectively (Table 4.4). Looking at the root colonization of the 10-20 cm soil horizon, *F. arundinacea* maintained the highest RLD (10.7 cm cm^{-3}), followed by *L. corniculatus* (7.9 cm cm^{-3}). Overall, very low RLD was recorded through this layer in all the remaining CCs.

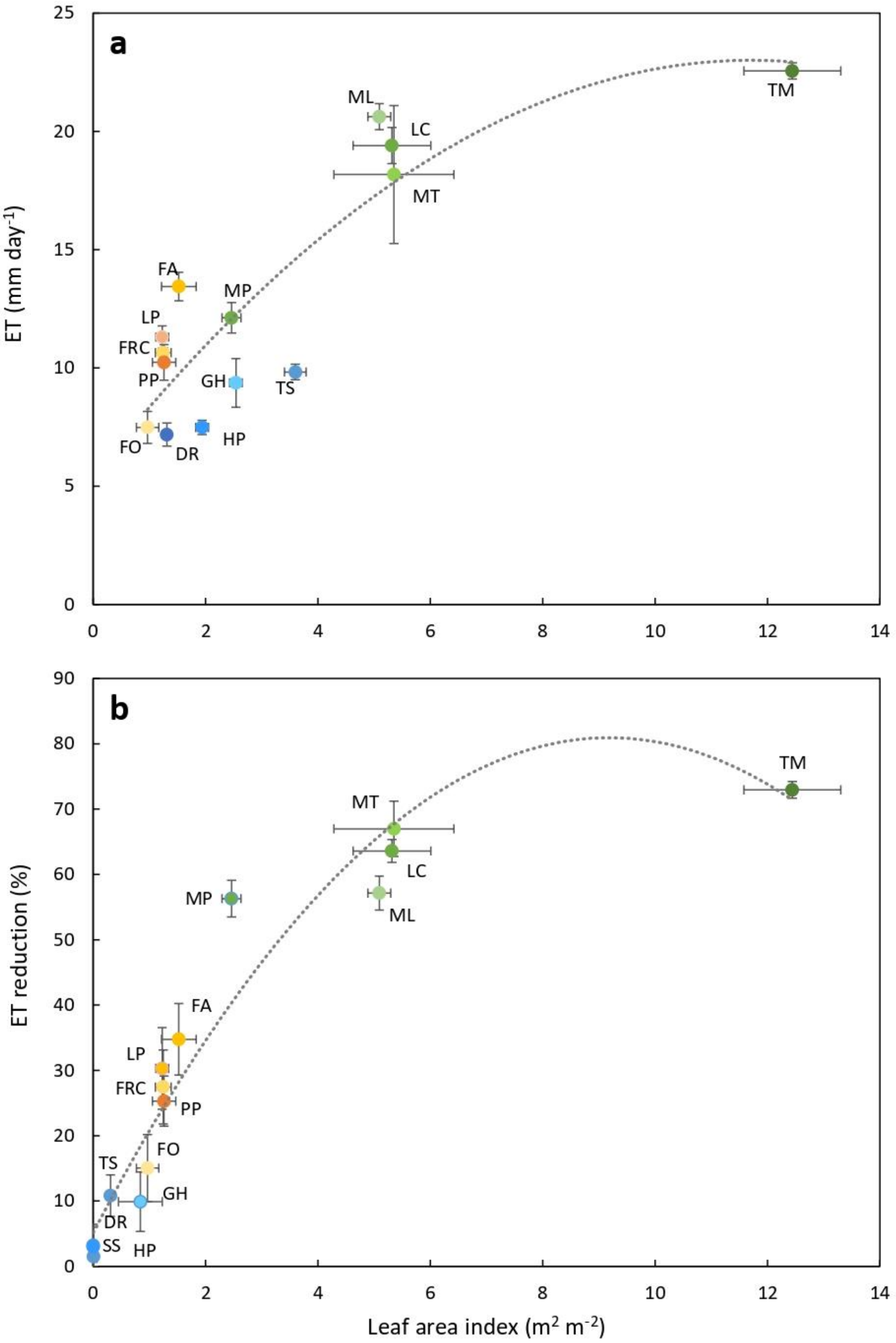


Figure 4.2. Panel A: quadratic regression of leaf area index (LAI, m^2 m^{-2}) vs cover crop evapotranspiration per unit of soil (ET, mm day^{-1}). Each data point is mean value ± SE (n = 4). The quadratic model equation is $y = -0.128x^2 + 2.9968x + 5.4716$, $R^2 = 0.76$. Panel B: the quadratic regression between LAI corresponding to the clipped biomass (m^2 m^{-2}) and cover crop ET reduction (%). Each data point is mean value ± SE (n = 4). Quadratic model equation is $y = -0.8985x^2 + 16.503x + 5.1491$, $R^2 = 0.94$.

The highest values of diameter class length (DCL, mm cm^{-3}) for very fine roots (DCL_VF, <0.075 mm) in the first 10 cm soil were recorded in GR, ranging between 9.75 (*F. ovina,* FO) and 23.35 (*P. pratensis,* PP) cm cm^{-3} (Table 4.4). All remaining species recorded quite low values, comprised within the 0-4 cm cm^{-3} range. A similar pattern was observed in the same soil layer for the fine root class (DCL_F, 0.075-0.2 mm), although *F. arundinacea* (FA)and *F. rubra commutata* (FRC) scored the highest values (25.74 and 26.10 cm cm^{-3}, respectively). For the same diameter class length, none among LE and CR exceeded the 9 cm cm^{-3} except for *G. hederacea*, assessed to be at 16.32 cm cm^{-3}. A more uniform behaviour among species was found for medium (DCL_M, 0.2-1.0 mm) and coarse (DCL_C, > 1.0 mm) roots although, most notably, *L. corniculatus* roots showed the highest abundance for both DCL_M (23.08 cm cm^{-3}) and DCL_C (0.54 cm cm^{-3}).

At the 10-20 cm soil depth, GR confirmed the highest values for both very fine and fine roots, with *F. arundinacea* reaching maximum DCL of 2.269 and 5.215 cm cm^{-3}, respectively (Table 4.4). *L. corniculatus* largely outscored any other species for both medium and coarse root diameter (6.173 and 0.037 cm cm^{-3}, respectively), with *F. arundinacea* ranking second (3.157 and 0.016 cm cm^{-3}, respectively).

The highest root dry weight (RDW, mg cm^{-3}) within the topsoil layer was reached by *L. corniculatus* (8.7 mg cm^{-3}) and *F. arundinacea* (7.6 mg cm^{-3}). Notably, such values were significantly higher than those recorded on the remaining species, except for the *F. arundinacea* vs *F. rubra commutata* comparison (Table 4.4). At 10-20 depth, scant variation was recorded in RDW measured in grasses, whereas *L. corniculatus* held its supremacy within legumes (4.5 mg cm^{-3}). Within the creeping type, *D. repens* (DR) and *G. hederacea* (GH) scored RDW values as high as those determined for grass species (namely *F. arundinacea*, *P. pratensis* and *F. rubra commutata*), whereas *S. subulata* (SS) essentially had no root development.

Table 4.4. Root length density (RLD) and diameter class length (DCL) for very fine (ø = 0-0.075 mm), fine (ø = 0.075-0.2 mm), medium (ø = 0.2–1 mm) and coarse (ø > 1 mm) root diameters as affected by soil cover.

Soil layer	Soil cover	RLD (cm cm^{-3})	DCL (cm cm^{-3})				RDW (mg cm^{-3})
			Ø = 0,00 - 0,075 mm	Ø = 0,075 - 0,2 mm	Ø = 0,2 - 1,0 mm	Ø = > 1,0 mm	
0-10 cm	*Trifolium michelianum*	2.4 g	0.619 e	1.190 f	0.537 h	0.006 de	3.7 fg
	Medicago polymorpha	7.4 fg	1.265 de	4.170 ef	1.843 fgh	0.027 de	4.0 efg
	Medicago lupulina	13.6 ef	1.486 de	7.140 de	4.738 def	0.088 bcde	4.8 cdef
	Medicago truncatula	2.8 g	0.290 e	1.350 f	1.078 gh	0.008 de	3.2 g
	Lotus corniculatus	29.7 cd	1.114 de	4.870 def	23.076 a	0.540 a	8.7 a
	Festuca arundinacea	59.0 a	17.637 ab	25.740 a	15.347 b	0.216 b	7.6 ab
	Festuca ovina	30.3 cd	9.749 c	12.770 bcd	7.384 cde	0.068 bcde	5.3 cde
	Festuca rubra commutata	53.7 ab	16.699 ab	26.100 a	10.769 bc	0.071 bcde	6.2 bc
	Poa pratensis	52.5 ab	23.354 a	19.460 ab	9.393 c	0.178 bc	6.1 cd
	Lolium perenne	33.0 bc	11.386 bc	11.700 bcd	9.844 bc	0.032 cde	5.1 cdef
	Dichondra repens	16.1 def	0.341 e	7.550 cde	8.025 cde	0.107 bcd	5.3 cde
	Trifolium subterraneum	7.4 fg	1.112 de	4.080 ef	2.195 fgh	0.059 bcde	4.2 efg
	Sagina subulata	9.8 fg	3.950 d	2.960 ef	2.667 fg	0.001 e	4.3 efg
	Glecoma hederacea	26.9 cde	1.736 de	16.320 abc	8.615 cd	0.033 cde	4.9 cdef
	Hieracium pilosella	10.4 fg	0.508 e	5.540 def	4.235 ef	0.090 bcde	4.7 def
P-value		< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001
10-20 cm	*Trifolium michelianum*	0.2 c	0.029 c	0.074 c	0.056 cd	0.001 c	2.3 ab
	Medicago polymorpha	0.0 c	0.025 c	0.059 c	0.016 d	0.000 c	0.8 ab
	Medicago lupulina	1.6 bc	0.295 c	0.757 bc	0.568 cd	0.003 bc	2.8 ab
	Medicago truncatula	0.0 c	0.000 c	0.002 c	0.002 d	0.000 c	0.0 b
	Lotus corniculatus	7.9 a	0.442 c	1.297 bc	6.173 a	0.037 a	4.5 a
	Festuca arundinacea	10.7 a	2.269 a	5.215 a	3.157 b	0.016 b	4.2 a
	Festuca ovina	0.3 c	0.068 c	0.099 c	0.090 cd	0.000 c	4.2 a
	Festuca rubra commutata	1.3 bc	0.249 c	0.681 bc	0.339 cd	0.006 bc	4.2 a
	Poa pratensis	3.4 b	1.369 b	1.519 b	0.521 cd	0.008 bc	3.7 a
	Lolium perenne	2.4 bc	0.605 bc	0.824 bc	1.008 cd	0.000 c	3.6 a
	Dichondra repens	2.2 bc	0.056 c	0.732 bc	1.415 c	0.002 bc	4.2 a
	Trifolium subterraneum	0.1 c	0.009 c	0.039 c	0.050 cd	0.000 c	1.7 ab
	Sagina subulata	0.0 c	0.000 c	0.000 c	0.000 d	0.000 c	0.0 b
	Glecoma hederacea	0.5 c	0.032 c	0.228 bc	0.220 cd	0.000 c	4.2 a
	Hieracium pilosella	0.1 c	0.004 c	0.026 c	0.068 cd	0.000 c	2.8 ab
P-value		< 0,001	< 0,001	< 0,001	< 0,001	< 0,001	< 0,001

Lowercase letters indicate differences among treatments within the same soil layer. P-values are reported.

4.4.3 Soil aggregates and mean weight diameter

Table 4.5 reports the proportional aggregate weight (g kg^{-1}) for both 0-10 and 10-20 cm soil depths. Compared to bare soil, the largest increase in large macroaggregates (LM, >2000 µm) in the top 10 cm of soil was achieved by *L. corniculatus* with 461 g kg^{-1}. *L. corniculatus* differed from the rest of the LE group, whose grand mean (90 g kg^{-1}) was the lowest of the three tested groups. As a legume, *T. subterraneum* (TS, 122 g kg^{-1}) recorded the lowest values compared to fellow CR species, ranging between 211 (*D. repens,* DR) and 316 g kg^{-1} (*G. hederacea,* GH). GR recorded LM values slightly lower than those of CR, with a mean value of 217 vs 224 g kg^{-1}.

The highest small macroaggregates (sM; 250-2000 µm) in the topsoil layer were found in the bare soil and similarly high values were found in *M. polymorpha* (MP), *M. lupulina* (ML), and *M. truncatula* (MT), while *L. perenne* (LP), with 298 g kg^{-1} had the lowest amount. Within the 0-10 cm soil layer, GR scored the lowest mean sM (340 g kg^{-1}), while CR species ranged between 343 (*G. hederacea,* GH) and 439 (*T. subterraneum*, TS) g kg^{-1}. The overall range of variation among species within the sM fraction at 0-10 cm was 66% (bare soil vs *L. perenne*) vs. the 707% variation (*L. corniculatus*vs *T. michelianum*,) recorded for the LM fraction (Table 4.5). Within the upper soil layer, *T. michelianum* (TM) stands out for the highest values for both microaggregates (m, 53-250 µm) and silt and clay fractions (s+c, <53 µm) recording 346 and 173 g kg^{-1}, respectively. Even though belonging to the same group, *L. corniculatus* had the opposite behaviour, recording the lowest values for both m (163 g kg^{-1}) and s+c (63 g kg^{-1}).

At 10-20 cm soil depth, *L. corniculatus* with 319 g kg^{-1} LM again outscored all other CCs. A quite homogeneous situation could be spotted within GR; measured LM fractions ranging between 65 and 136 g kg^{-1} highlighted GR as the most efficient group in LM production in the lower 10-20 cm depth. *T. michelianum* (TM) is the only one showing an LM value as low as the one of bare soil (36 g kg^{-1}).

Table 4.5. Proportional aggregate weight (g kg^{-1}) of sand-free aggregate-size fractions acquired from wet sieving as affected by soil cover and mean weight diameter (MWD). Aggregate-size fraction divided as macroaggregates with large size (> 2 mm, LM) and small size (2 mm - 250 µm, sM), microaggregates (250 µm - 53 µm, m), and silt and clay (< 53 µm, s + c).

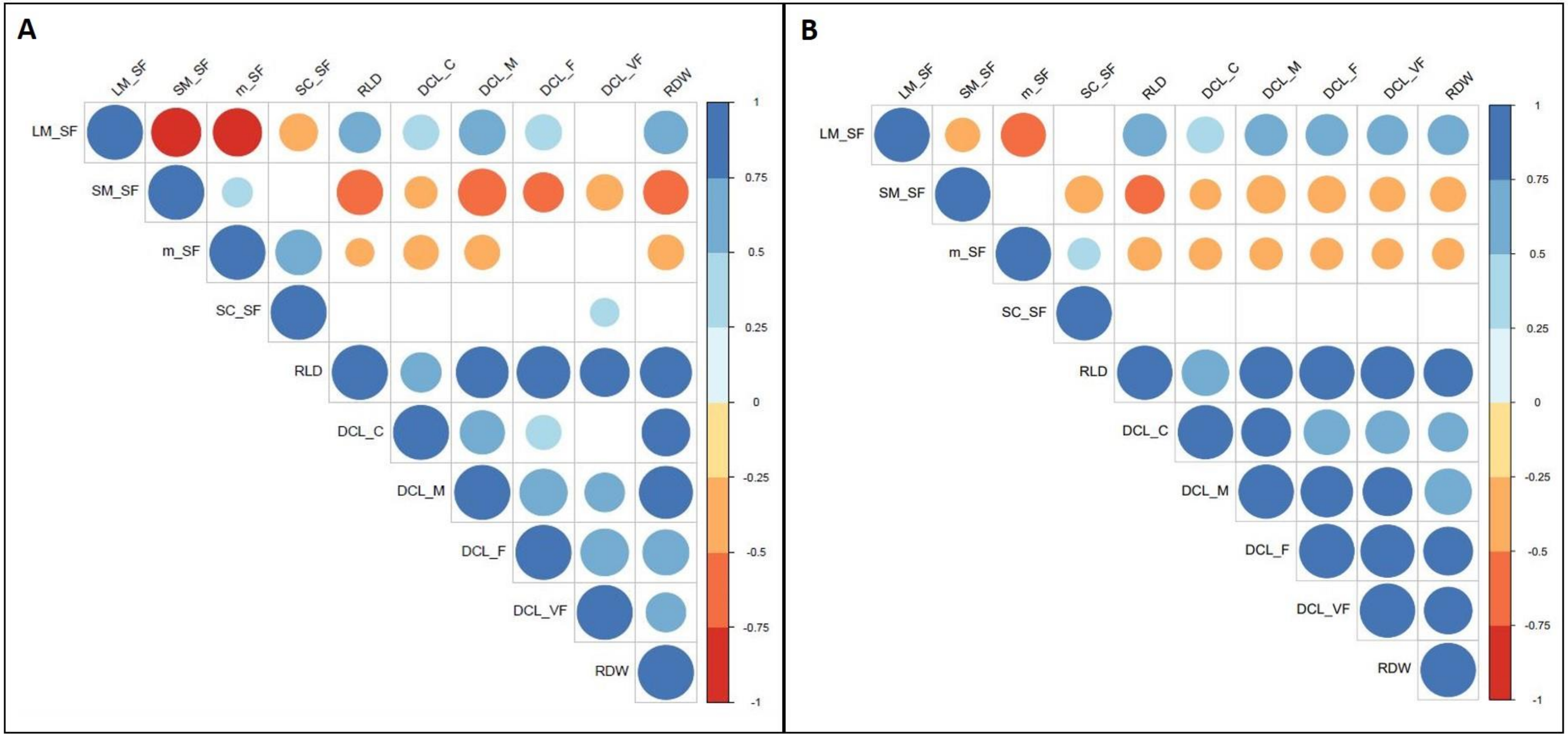
Soil layer	Soil cover	Aggregate-size fraction (g kg^{-1} soil) - Sandfree				MWD (mm)
		LM	sM	m	s + c	
0-10 cm	Bare soil (control)	67 g	495 a	309 abcde	129 bcd	0.94 hi
	Trifolium michelianum	57 g	423 bc	346 a	173 a	0.82 i
	Medicago polymorpha	89 fg	462 ab	317 abcd	133 abcd	1.02 ghi
	Medicago lupulina	148 ef	464 ab	275 def	114 cd	1.30 defg
	Medicago truncatula	66 g	477 ab	327 abc	130 abcd	0.92 hi
	Lotus corniculatus	461 a	313 de	163 g	63 e	2.68 a
	Festuca arundinacea	251 bc	339 de	263 ef	148 abcd	1.67 bc
	Festuca ovina	241 bcd	347 de	280 cdef	132 abcd	1.63 bcd
	Festuca rubra commutata	163 def	348 de	331 ab	158 ab	1.26 efg
	Poa pratensis	210 cde	369 cd	277 def	144 abcd	1.51 cde
	Lolium perenne	219 cde	298 e	327 abc	156 abc	1.48 cdef
	Dichondra repens	211 cde	357 cde	289 bcde	143 abcd	1.50 cde
	Trifolium subterraneum	122 fg	439 ab	298 bcde	141 abcd	1.15 fgh
	Sagina subulata	215 cde	366 cd	283 cde	137 abcd	1.53 cde
	Glecoma hederacea	316 b	343 de	234 f	107 d	2.00 b
	Hieracium pilosella	255 bc	367 cd	262 ef	116 bcd	1.73 bc
P-value		< 0.001	< 0.001	<0.001	<0.001	< 0.001
10-20 cm	Bare soil (control)	35 e	508 a	327 ab	130 c	0.80 bc
	Trifolium michelianum	36 e	446 abcde	338 a	180 ab	0.74 c
	Medicago polymorpha	57 cde	459 abcde	331 a	153 abc	0.86 bc
	Medicago lupulina	54 cde	431 abcde	343 a	172 abc	0.81 bc
	Medicago truncatula	58 cde	440 abcde	353 a	149 abc	0.84 bc
	Lotus corniculatus	319 a	309 f	230 b	143 bc	1.98 a
	Festuca arundinacea	109 bcd	402 bcde	325 ab	164 abc	1.05 bc
	Festuca ovina	99 bcde	423 abcde	318 ab	160 abc	1.02 bc
	Festuca rubra commutata	65 cde	385 def	360 a	190 a	0.82 bc
	Poa pratensis	94 bcde	491 ab	264 ab	151 abc	1.07 bc
	Lolium perenne	136 b	376 ef	333 a	156 abc	1.16 b
	Dichondra repens	104 bcde	396 cdef	323 ab	177 ab	1.02 bc
	Trifolium subterraneum	48 de	475 abc	330 a	147 abc	0.83 bc
	Sagina subulata	66 cde	468 abcd	308 ab	159 abc	0.91 bc
	Glecoma hederacea	119 bc	389 cdef	326 ab	166 abc	1.09 bc
	Hieracium pilosella	67 cde	442 abcde	345 a	147 abc	0.89 bc
P-value		< 0.001	< 0.001	0.003	0.003	< 0.001

Lowercase letters indicate differences among treatments within the same soil layer. P-values are reported.

Within the 10-20 cm soil layer, a more uniform behaviour was found among species for sM, m and s+c under a range of variation of 64% (bare soil vs *L. corniculatus*), 56% (*F. rubra commutata* vs *L. corniculatus*), and 46% (*F. rubra commutata* vs bare soil) respectively vs. the 811% variation (*L. corniculatus* vs bare soil) recorded for the LM fraction (Table 4.5).

L. corniculatus registered the highest mean weight diameter (MWD, mm) among all CCs in both upper (2.68 mm) and lower (1.98 mm) soil layers (Table 4.5), while *T. michelianum* ranked the lowest (0.92 and 0.74 mm, respectively). Within the first 10 cm, GR showed a more homogeneous pattern with an MWD variability of 32% (*F. rubra commutata* vs *F. arundinacea*), increasing to 73% in CR (*T. subterraneum* vs *G. hederacea*) and 226% in LE (*T. michelianum* vs LC). Similarly, at 10-20 cm depth, the highest variability was registered in LE (167% for *T. michelianum* vs *L. corniculatus* comparison). Conversely, less variability was found within GR (41% for FRC vs *L. perenne*) and CR (26% for *T. subterraneum* vs *G. hederacea*).

Spearman coefficients (ρ) calculated for the correlations between the aggregate-size fractions, RLD, DCL and RDW are shown in figure 4.3 for the 0-10 cm (A) and 10-20 cm (B) soil depths. For the topsoil layer (Fig. 4.3A), LM had a close positive correlation with RLD (ρ = +0.56), DCL_M (ρ = +0.69) and RDW (ρ = +0.62). Conversely, sM was negatively correlated with the same diameter class lengths (ρ = -0.68, -0.74, and -0.65, respectively). Overall, a similar pattern was maintained for the 10-20 cm depth, although correlations were in general less tight (Fig.4.3B).



Figures 4.3. Spearman's correlations for differences in soil aggregate pattern and root traits for both 0-10 cm (A) and 10-20 cm (B) soil depth. Blue colour indicates positive correlation, while red indicates negative correlation.

4.4.4 PCA analysis

The Pearson correlation matrix calculated through the Principal Components Analysis (PCA) (Table 4.6) for the data pool over the 15 CCs showed that evapotranspiration before mowing (UMW_ET) was not correlated to RLD or any DCL; rather, a very close correlation (r = 0.96) was found vs ADW_MW1. Conversely, ET evaluated 25 days after mowing (MW_ET_25) showed a significant positive correlation with several root growth variables including DCL_C, DCL_M, RDW, and total above-ground dry weight (i.e., the sum of first and second cuts, ADW_TOTAL).

Table 4.6. The Pearson correlation matrix calculated through the PCA analysis for data pool over the 15 cover crop tested on 10 representative variables is shown here. Variables are reported as following: root length density (RLD), diameter class length for coarse (DCL_C), medium (DCL_M), fine (DCL_F), and very fine (DCL_VF) roots, root dry weight (RDW), above-ground dry biomass clipped at first mowing event (ADW MW1), total above-ground clipped biomass (ADW_TOTAL) and evapotranspiration rates before (UMW_ET) and after (MW_ET_25) grass trimming.

Variables	RLD	DCL_C	DCL_M	DCL_F	DCL_VF	RDW	UMW_ET	MW_ET_25	ADW_MW1	ADW_TOTAL
RLD	**1**	0.513	**0.665**	**0.917**	**0.860**	**0.802**	-0.123	0.288	-0.224	0.195
DCL_C	0.513	**1**	**0.932**	0.218	0.164	**0.782**	0.274	**0.679**	0.276	**0.693**
DCL_M	**0.665**	**0.932**	**1**	0.423	0.261	**0.884**	0.164	**0.659**	0.150	**0.652**
DCL_F	**0.917**	0.218	0.423	**1**	**0.799**	**0.669**	-0.226	0.174	-0.359	0.005
DCL_VF	**0.860**	0.164	0.261	**0.799**	**1**	0.446	-0.213	-0.075	-0.306	-0.122
RDW	**0.802**	**0.782**	**0.884**	**0.669**	0.446	**1**	0.036	**0.562**	-0.042	0.476
UMW_ET	-0.123	0.274	0.164	-0.226	-0.213	0.036	**1**	**0.545**	**0.960**	**0.729**
MW_ET_25	0.288	**0.679**	**0.659**	0.174	-0.075	**0.562**	**0.545**	**1**	0.447	**0.834**
ADW_MW1	-0.224	0.276	0.150	-0.359	-0.306	-0.042	**0.960**	0.447	**1**	**0.748**
ADW_TOTAL	0.195	**0.693**	**0.652**	0.005	-0.122	0.476	**0.729**	**0.834**	**0.748**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Analysis of the bi-plot (Fig. 4.4) reporting the positioning of each CC and the direction and magnitude of variation of each variable along F1 and F2 components, enables quite a sharp separation of the three family groups, though with some within-group exceptions.

Within LE, *L. corniculatus* (LC) clearly isolated itself from the remaining species. *L. corniculatus* combined a strong and positive correlation with RDW, DCL_C and DCL_M along the F1 component and with UMW_ET and ADW_MW1 along the F2 component. Conversely, the location of *M. truncatula* (MT), *T. michelianum* (TM) and *M. lupulina* (ML) in the biplot was dependent on a close positive correlation along F2 with UMW_ET and ADW_MW1. *M. polymorpha* (MP) displayed a further distinct behaviour, determined by a strong negative correlation with RDW, DCL_C, DCL_M along the F1 component.

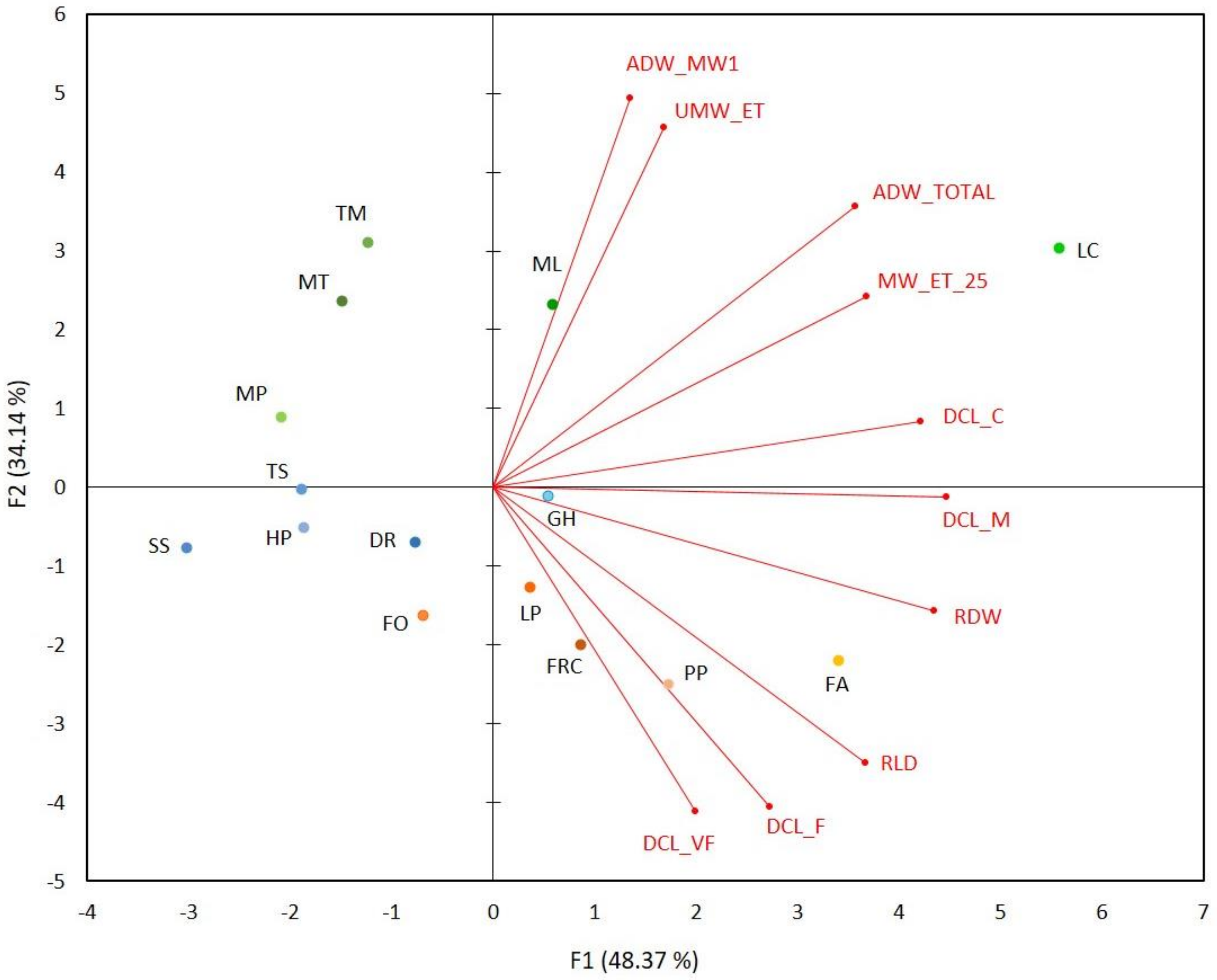


Figure 4.4. Principal component analysis for 15 different cover crop species divided as grasses (orange shades), legumes (green shades) and creeping plants (blue shades). Red lines represent active variables like (i) evapotranspiration before (UNMW_ET) and (ii) 25 days after mowing (MW_ET_25), (iii) above-ground dry clipped biomass at first mowing event (ADW_MW1) and (iv) total (ADW_TOTAL); (v) diameter class length for very fine (DCL_VF), (vi) fine (DCL_F), (vii) medium (DCL_ M) (viii) coarse (DCL_C) roots; (ix) root length density (RLD) and (x) root dry weight (RDW). Root traits are mean values of 0-20 cm soil depth.

GR grouped in the bottom-right quadrant, except for *F. ovina* (FO). Once again, though, a different behaviour between *F. arundinacea* (FA) and *F. ovina* (FO) was apparent, with the remaining grass species having an intermediate behaviour. *F. arundinacea* showed a close positive correlation with RLD and RDW along F1, and a negative correlation with DCL_F and DCL_VF along F2 (Fig. 4.4). Conversely, *F. ovina* (FO) has a negative correlation with UMW_ET and ADW_MW1 (F2) and, albeit lower in magnitude, with DCL_M, DCL_C and RDW (F1). The three remaining grass species (*L.*

perenne, *F. rubra commutata* and *P. pratense*) were essentially grouped together, albeit their behaviour was driven by negative factor scores along the F2 principal components. These CCs set for a negative correlation with UMW_ET and ADW_MW1 and a positive correlation with DCL_VF and DCL_F.

CR had a somewhat more homogeneous behaviour, although *G. hederacea* (GH) too tended to be isolated in the bi-plot distribution. *S. subulata* (SS), *H. pilosella* (HP) and *T. subterraneum* (TS) were almost insensitive to the variables depicted in F2, whereas their behaviour was largely determined by a negative correlation with some F1 variables, viz., DCL_C, DCL _M and RDW.

4.5 Discussion

The results in the present study shed light on a key issue for the ecological transition of modern viticulture under the threat of a changing climate, viz., how and which CC species should be used at the field level to improve the agro-ecosystem performance.

Although our trial was conducted in pots under inherently constrained conditions and a well-watered regime, the detailed and rich nature of the collected data enables the identification of suitable or less suitable CC species. Even if these CCs were here analyzed with the idea of viticultural implementation, the results obtained can be considered valuable information for application in generic orchards.

Concerning ET, measurements taken after 105 days of undisturbed growth indicated very high rates (around 20 mm day^{-1} under well-watered conditions) in all legumes, with the partial exception of *M. polymorpha* (MP). Taking LC as an example, at the same estimated LAI (about 5), ET was higher than those recorded in New Zealand (Grau, 1995), peaking at about 11 mm day^{-1}. The reasons for this discrepancy are probably related to the significantly lower evaporative demand in their experiment than ours since air temperatures during the central hours did not exceed 21 °C, while our daily temperature ranged between 17 and 29 °C (data not shown). Under well-watered conditions and apart from the role played by evaporative demand, daily ET is primarily driven by two factors: the amount of aerial biomass produced and the genetically determined ET_{LEAF}. Our results showed that high ET by the LE group mostly derived from a very fast development after sowing, rather than from higher ET_{LEAF}, the latter being more than halved compared to the average value in the GR group (Table 4.3).

While mowing is known to be a valuable tool to limit water consumption by CCs (Centinari et al., 2013), the availability of previous outcomes quantifying the amount and dynamics of water saving due to the grass cutting is limited. One study found that three weeks after cutting *Medicago sativa* L., daily ET was around 60-70% of that before the cut (Asseng and Hsiao, 2000). Our MW_ET_25 confirms a similar behaviour with ML, while *L. corniculatus* (LC) promptly recovered fully pre-mowing rates (Fig. 4.1).

Here, for the first time, we found that plotting LAI vs ET reduction for data pooled over the 15 species (Fig. 4.2B) yielded a very close fit to the observed data, thus suggesting that (i) a linear decrease in ET is expected anytime the LAI is removed through trimming ranges between 0 and 6; (ii) a saturation effect seems to be reached beyond this limit, probably because with a cover canopy increasing in height and density, the bottom leaf layers become heavily shaded, thereby minimising their contribution to transpiration (Centinari et al., 2013). This has some relevant implications when a temporary winter cover crop, usually containing legumes, is sown for the termination in spring under a green manure purpose. If a desirable feature is obtaining the highest biomass before termination to maximise the N return to the crop, a legume growth above an LAI of 6-7 will not cause luxury water use, based on the mechanism highlighted above.

High water use by *L. corniculatus* (LC) was corroborated by the highest RLD in the 10-20 cm soil layer (7.9 cm cm^{-3}) and RDW in both the 0-10 cm (8.7 mg cm^{-3}) and 10-20 cm (4.5 mg cm^{-3}) depths. However, our results also clarify that such an effect is primarily due to the very high values of DCL_M and DCL_C, which in turn explain why *L. corniculatus* (LC) was also able to dig into the lower soil layer. Since thicker roots have been reported to be more effective at overcoming issues related to soil mechanical resistance (Blanco-Canqui and Ruis, 2020), our results also suggest the importance of *L. corniculatus* (LC) in improving physical soil quality by decreasing bulk density and preventing soil compaction, even though tillage operations are suspended.

Turning to GR, our results add significant knowledge to previous studies (Bowman and Macaulay, 1991; Kenna M. P. and Horst G. L., 1993; Kim and Beard, 1988) leading to the potential use of grass-based permanent mid-row CCs in orchard floor management. The present trial indicates that *F. arundinacea* (at least as far as the tested cultivar) has to be regarded as quite competitive grass, while *F. ovina* behaves contrarily. The transpiration potential of *F. arundinacea* relies more on high ET_{LEAF} rather than on fostered aerial biomass: hence the ability for higher light interception (Figure 4.1 and Table 4.3). This is substantially different from what was reported above on the LE group (especially *L. corniculatus*) and suggests a relevant implication for the use at the field level: at the same LAI, any of our tested GR species will probably use a significantly higher amount of water than legume species.

Our results on GR ET are confirmed by the literature, where *F. arundinacea* and *F. ovina* are assessed as the most and the least competitive grasses, with values as high as 8.5 and 12.6 mm day^{-1}, respectively (Huang, 2008; Kim and Beard, 1988). Moreover, one study (Bowman and Macaulay, 1991) showed the existing difference between *F. arundinacea* cultivars grown under non-limiting water nutrient conditions whose ET rates ranged between 10 and 13.5 mm day^{-1}, perfectly fitting our data. Inputting our *F. arundinacea* mowed values (161.8 g m^{-2}) in an ET reduction vs dry clipped biomass model made on *Festuca arundinacea* var. Barfelix (Centinari et al., 2013) leads to a 36% ET reduction, which is a very close fit to the 35% reduction registered at an LAI of 1.52 (Table 4.3 and Figure 4.2B).

Notably, while *L. corniculatus* (LC) and *F. arundinacea* (FA) share the capacity to spread their roots into the deeper soil layer, our PCA analysis revealed that values of DCL for any root diameter – thus including very fine and fine roots – under *F. arundinacea* in the 10-20 cm soil layer were several times higher than those under other grasses. It is widely accepted that a well-established and developed root system is essential for the efficient absorption of water (Doussan et al., 2006). Therefore, our results on DCL indicate that *F. arundinacea* can further enhance the absorption of nutrients and water too, by increasing the root hair surface even in the lower soil layers.

All tested GR species, despite large differences in root growth parameters, retained high and similar ET_{LEAF} values. Explanation of such a behaviour is found in ET rates given on a pot basis (Figure 4.5). It is quite striking that for any GR, daily pot ET stayed within 60% of the daily water supply (1.0-1.1 L per pot). Presumably, this allowed optimal leaf function, explaining why ET_{LEAF} did not differ. Consequently, under persisting non-limiting soil water availability, total water use in our tested GR becomes a primary function of LAI (Figure 4.2B).

FO confirms its attitude to low ET due to its "dwarfing" characteristics. A very low ADW_MW1 (Table 4.3) associated with a shallow root system with minimum soil colonization below 10 cm depth renders this CC a quite interesting candidate for a permanent between row establishments. According to the PCA analysis, *F. ovina* (FO) isolated for a negative correlation with ADW_MW1 and UMW_ET. Ideally, in the field, its shallow root system might facilitate temporal and partial drying in summer, with a prompt recovery with incoming precipitation in the fall.

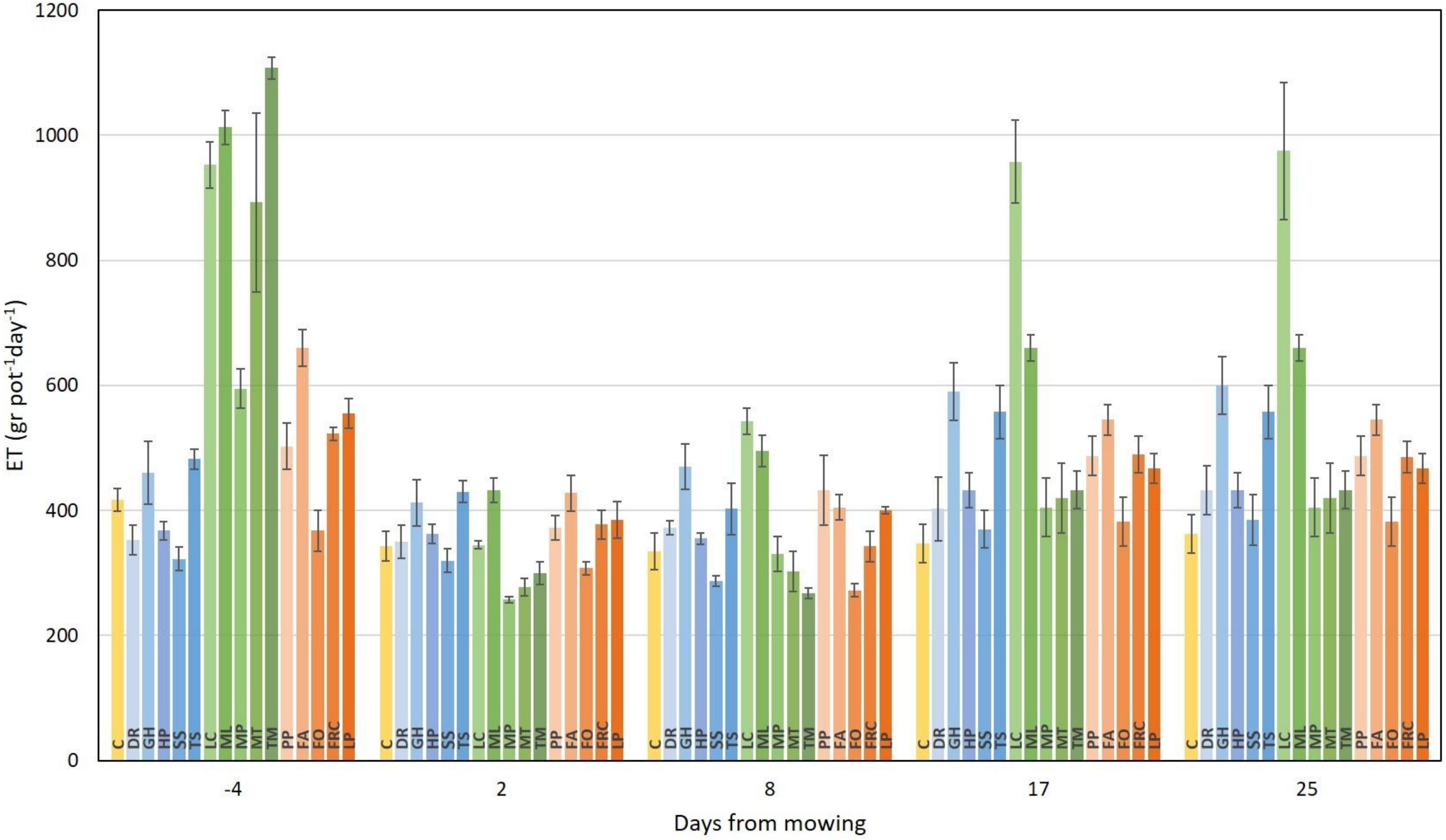


Figure 4.5. Vertical bars represent the daily water use as a referred to the pot surface (ET, g pot^{-1} day^{-1}) for the bare soil (yellow) and all the cover crop species, divided into creeping plants (shades of blue), legumes (shades of green) and grasses (shades of orange). Evapotranspiration was measured through a gravimetric method before (i.e., -4) and 2, 8, 17 and 25 days after mowing. ET data mean values ± SE (n=4).

Turning to the under-vine strip management, a lot of work has been done to investigate how native vs sowing of commercial mixtures might affect the degree of competition towards the root system of the consociated vines (essentially insisting within the same soil volume) with possible effects on the RLD and root distribution (Centinari et al., 2016). Several authors have found that the topsoil layer conquered by a CC will induce the grapevine root system to explore deeper soil horizon or to preferentially spread sideways from the row axis (Atucha et al., 2013; Centinari et al., 2016; Yao et al., 2009). Previously conducted research (Klodd et al., 2016) showed that well-established grapevines with understory *F. rubra* grass grew a deeper root distribution and showed a little evidence of restricted-water uptake. Only at 10 cm soil depth the ^{18}O isotope depletion ($\delta^{18}O$, ‰) was significantly more negative in the soil with the CC relative to the tilled one, but there was no significant treatment effect below that.

Preliminary work conducted in France (Delabays et al., 2006; Delpuech, 2014) has shown that establishing shallow-rooted yet creeping and smothering CCs under the row strip can be quite successful at controlling weeds, thereby reducing the need for tillage or herbicides. At the same time, the grapevine root system will grow underneath the CC, where higher soil moisture is likely to be available. The management of such CCs implies that no mowing is made until the cover outgrows and tends to invade the alley. Therefore, in our trial, we avoided any canopy-shortening cut until the cover started to overflow the pot surface. Such a status was reached by *G. hederacea* (GH) and *T. subterraneum* (TS) only. ET reported in Figure 4.1 strongly supported the assumption that all CR species retain good water-saving characteristics and for three of them (DR, HP and SS), pre-mowing ET rates were slightly lower than those measured on the control and less than 400 g H_20 pot^{-1} day^{-1} were used (Figure 4.5). Despite the actual lack of mowing, data taken on these species at 17 and 25 days after mowing showed a mild increase in ET rates that have to be inherently attributed to a likely thickening of the CC within the pot surface. The second feature which was likewise shared by all CR was that root colonization was essentially restricted to the topsoil layer only (Table 4.4), thus obeying the need of having, under field conditions, two well-separated soil layers, including grass roots on top and grape roots at higher depths.

In our study, a careful assessment of CCs' effects on soil aggregate stability and MWD was performed and associated to root traits. It is well known that soil aggregate size and stability are positively associated with infiltration (and retention) of water and mitigation of soil erosion, due to improved pore size distribution (Prosdocimi et al., 2016). In addition, LM plays a major role in enhancing SOM concentration and stabilization (Fiorini et al., 2020), thus further increasing water and nutrient availability for the cultivated plants. Indeed, it is well known that macro-aggregates provide physical protection to SOM by binding organic compounds to soil minerals and creating a barrier between microorganisms and their substrate (Six et al., 2000). Since Mediterranean vineyards are usually established on steep slopes (Arnaez et al., 2007), our study shows that selected CCs may be considered a promising tool to boost soil aggregation, thus suggesting increased water infiltration, as well as reduced soil erosion and nutrient losses (Wainwright, 1996). As soil water evaporation is mainly affected by soil water content, organic matter, texture and structure (Ma et al., 2020), it is reasonable to assume that CCs may contribute to its change with different magnitude depending on the species characteristics. However, in our trial the evapotranspiration components (i.e., evaporation and transpiration) could not be distinguished, and the different cover crop-induced-soil aggregation effect on soil evaporation was hard to assess as water loss measurements were conducted a few months before the soil sampling and the following aggregate determinations. More in general, little information seems available: increased aggregate stabilization was assessed to increase the amount of water available in soils for plants by reducing losses via evaporation (Hudson B. D., 1994), whereas a more recent work has shown no significant impact on soil particle size on evaporation rate (An et al., 2018).

Our results also show a positive correlation between large macro-aggregates and roots development parameters, such as RLD and RDW, thus suggesting that roots are the main drivers of soil aggregate formation and stabilization in this system. Indeed, roots are known to produce mucilage and other exudates that hold particles together, hence promoting LM formation (Miller and Jastrow, 1990). Similar results were reported in the previous studies (Demenois et al., 2018; Gould et al., 2016; Poirier et al., 2018), which observed a positive correlation between root biomass/length density and aggregate stability. Therefore, CCs with high RLD and RDW should be suggested to promote aggregate stabilization, increasing soil organic carbon (SOC) protection and water infiltration/retention. In our study, GR generally enhanced RLD and RDW, compared to LE and CR plants, except for *L. corniculatus* and *G. hederacea*. In particular, *F. arundinacea* showed the highest RLD (59.0 cm cm^{-3}) among all species and one of the highest LM contents (251 g kg^{-1} soil) in the 0-10 cm soil layer, thus confirming the positive interaction between RLD and LM stabilization. Among legumes, *L. corniculatus* had the highest amount of LM in both soil layers, establishing itself as a promising CC for improving soil structure, while being an external source of N due to N-fixation. This may be explained by the higher DCL (Ø > 1.0 mm) of *L. corniculatus* compared to other species, thus indicating the important role of large roots in soil aggregation levels. The strong influence of *L.*

corniculatus on soil strength, when grown in monocultures compared to other legumes, has been reported. Interestingly, *H. pilosella* had higher LM compared to most of the other species, while having lower RLD and RDW. Previous studies reported lower pH of soil under *H. pilosella* than under other plants (Boswell and Espie, 2010; McIntosh et al., 1995), which was found in turn to be negatively correlated with water stable aggregates (Regelink et al., 2015). The authors explained the negative correlation between the pH increase and the soil aggregation level by the higher loading of humic acids on the mineral surfaces and by a decrease in the electrostatic repulsive forces between negatively charged substances under soil acidic conditions, resulting in higher coagulation of organic and mineral particles. Therefore, for *H. pilosella*, the effect on soil aggregation is more related to changes in soil chemical properties rather than to root characteristics.

4.6 Conclusions

The current pot trial is, to the best of our knowledge, the first case of a comparative screening to evaluate the water use, root characterization, and soil-aggregation potential of a large number of herbaceous species and define the most recommended ones for vineyard usage. The highest ET rates recorded for legumes were mainly due to a very fast development after sowing, rather than to a higher ET_{LEAF}. For both legumes and grasses, mowing was confirmed as a valuable practice to limit water use proportionally anytime until a LAI of 5-6, while a saturation effect seems to be reached beyond this limit. Among grasses, *F. ovina* was assessed to be the one with the lowest ET, which renders it an interesting candidate as a permanent between-row living mulch. Moreover, ideally, once used in the field, its shallow root system might facilitate temporal and partial drying in summer, with a prompt recovery with incoming rainfall in the fall. CR confirmed their potential for under-trellis strip management as, while maintaining a full soil coverage (i.e., potentially successful in weed control), they did not need any mowing for height reduction, registered low water use rates and a superficial (i.e., 0-10 cm) root colonization. Lastly, our study showed that CCs with enhanced RLD and RDW such as GR, *G. hederacea* and *L. corniculatus* may be considered promising species to boost soil aggregation, increase SOC protection and water infiltration, as well as reduce soil erosion and nutrient losses.

5. General conclusions

This project provides a better understanding into several research areas regarding vineyard soil management, towards a more sustainable, wise and conscious approach. Vineyard cover cropping (either natural or seeded) is considered a necessity in sustainable viticulture, although it can clash with possible excessive competition towards vines. Thus, underling the importance of knowing how to correctly manage vineyard soil cover cropping in terms of both space and time; through knowledge of evapotranspiration levels and the effects of mowing on this particular parameter, and with the possibility of selecting low-competitive herbaceous species with the desirable characteristics.

The goal of the study presented in Chapter 2 of this thesis was to test and validate floor management treatments capable of minimizing well-known disadvantages of either tillage or native vegetation while focusing on a balance between the two techniques with variations in space and time. As this happened under the same under-the-trellis management (i.e., tillage), observed differences are due to the different inter-row soil management that featured varying combinations in space and time of tillage, native grass, and sown cover crops. The study emphasized the need to reprogram inter-row management in the vineyard in order to favor its adaptation to the stress imposed by climate change. Treatments tested provided different responses, highlighting how the technique can be diversified according to specific environmental and productive needs. The AGT treatment (i.e., alternate tillage and permanent grass every second mid-row) set almost in an intermediate position, between the two extremes, without assuring any significant marginal gain. However, as it resulted in a reduction of the competition of the plot proportional to the degree of soil cover, it makes possible to draw up operational protocols that can modulate the effects of competition according to the proportion of grassed and tilled surface area. Modulating PG (i.e., permanent grass) into TG (i.e., temporary grass) via a temporary removal of the resident vegetation in the fall and AGT into AGC (i.e., alternate permanent grass and temporary cover crop every second mid-row) by growing a winter cover crop terminated in the spring as green manuring, gave the highest yield at adequate technological and phenol ripeness. In particular, TG also assured higher YAN levels for more regular must fermentation, this way being particularly interesting for certain oenological types of wines (e.g., sparkling wines). While, ACG ensured the achievement of adequate technological and phenolic maturation associated with a significant decrease in K^+ accumulation in the must. Even though, in this case, data taken over four years in a non-irrigated vineyard did not show any major limitations in leaf gas exchange and water status across treatments, and most of the observed changes were primarily season-related, the knowledge of different soil cover species need is real. Evapotranspiration fluxes (ET) can be measured with several methods, each with advantages and limitations. In this study, a low-cost, easy-to-set-up and rapidly transport across experimental plots, custom-made, closed portable chamber is suggested as a suitable solution when fast multipoint evaluations of ET fluxes are needed. As reported in Chapter 3, the chamber was tested under controlled and semi-controlled conditions (i.e., laboratory and outdoor pot-lot), and the plot of the gravimetric evaporation data versus the corresponding values obtained using the chamber system yielded a highly significant linear relationship for both the laboratory and the pot system calibration test (R^2 equal to 0.96 and 0.99, respectively). Interestingly, data obtained infer that running calibration under ambient conditions (as opposed to controlled) greatly reduces chamber biases and provides best accuracy. The chamber proved to be a reliable, efficient, and accurate way to measure ET for a range of time scales (i.e., instantaneous and cumulated daily) under bare soil conditions and sown crops of *L. corniculatus and F. arundinacea*.

The chamber was then used, together with a gravimetric method, to assess ET fluxes of fifteen cover crop species potentially targeted for vineyard use. The pot trial described in Chapter 4 is, to the best of our knowledge, the first case of a comparative screening to evaluate water use, root characterization, and soil-aggregation potential of a large number of herbaceous species, aiming at defining the most recommended ones for vineyard usage. Together with some already used cover crops, such as grasses (GR) and legumes (LE), new creeping (CR) ones were included in the study for their potential interest as living mulches under the trellis. Although the trial was conducted in pots under inherently constrained conditions and a well-watered regime, the detailed and rich nature of the collected data enables the identification of suitable or less suitable CC species. Selection of cover crop species suggested to be used in the vineyard was here mainly based on water use rates as well as dynamic and extent of root growth patterns. Among grasses, *F. ovina* was assessed to be the one with the lowest ET, which renders it an interesting candidate as a permanent between-row living mulch. Moreover, ideally, once used in the field, its shallow root system might facilitate temporal and partial drying in summer, with a prompt recovery with incoming rainfall in the fall. Creeping plants confirmed their potential for under-trellis strip management as, while maintaining a full soil coverage (i.e., potentially successful in weed control), they did not need any mowing for height reduction, registered low water use rates and a superficial (i.e., 0-10 cm) root colonization. Legumes, with the exception of *M. polymorpha*, registered highest ET rates, mainly due to a very fast development after sowing, rather than to higher ET rates referred to per square meter of removed leaf area (ET_{LEAF}), the latter being more than halved compared to the average value in the grasses group. For both legumes and grasses, mowing was confirmed as a valuable practice to limit water use proportionally anytime until a LAI of 5-6, while a saturation effect seems to be reached beyond this limit. This has some relevant implications when a temporary winter cover crop (usually containing legumes) is sown for the termination in spring under a green manure purpose. Based on the mechanism highlighted above, if a desirable feature is obtaining the highest biomass before termination to maximize the N return to the crop, a legume growth above 6-7 LAI will not cause luxury water use.

Finally, a careful assessment of cover crops' effects on soil aggregate stability and mean weight diameter (MWD) was performed and associated to root traits. In particular, it showed that cover crops with enhanced root length density (RLD) and root dry weight (RDW) such as grasses, *G. hederacea,* and *L. corniculatus* may be considered promising species to boost soil aggregation, increase soil organic carbon protection, and water infiltration, as well as reduce soil erosion and nutrient losses.

Soil management complexity is well known, and vineyard cover cropping is long to master. This work opens the way for further investigations. Future work could involve the use of the chamber for field evapotranspiration measurements, in different soil/pedological contexts, with various herbaceous species and potentially observing the effect of diverse termination techniques on the water consumption of the herbaceous cover (thus on the competition of the vine associated with it).

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