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Effects of living mulch on young vine growth and soil in a semi-arid vineyard

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Summary

Although the use of under-trellis plants as weed control (living mulch) in vineyards has been gaining popularity, its effects on soil quality and especially soil biology have not been well studied. Due to functional trait differences, plants may differ in how they compete with vines, and may also change abiotic and biotic soil properties. A living mulch trial was established in the semi-arid Okanagan valley of British Columbia comparing vine growth as well as soil abiotic and biotic outcomes for four living mulch treatments: buffalo grass (*Bouteloua dactyloides*), Chewing's fescue (*Festuca rubra* ssp. *commutata*), birdsfoot trefoil (*Lotus corniculatus*), and shepherd's purse (*Capsella bursa-pastoris*) with two industry standards: herbicide and cultivation. After two seasons, strong vine growth responses were seen that depended on living mulch identity, e.g., reduction in leaf N status with grasses, reduction in leaf water potential with the legume, birdsfoot trefoil. These effects were related to plant-induced changes to soil C:N ratio and soil moisture. Although treatments did not change abundance of the measured fungal guilds in bulk soil, abundance of arbuscular mycorrhizal fungi in vine roots was lowest with birdsfoot trefoil as living mulch. This study may help growers to select living mulch species appropriate for the soil conditions and resource availability of their site.

K e y w o r d s : cover crops; living mulch; vineyards; competition; soil properties; arbuscular mycorrhizal fungi; entomopathogenic fungi; soil borne pathogens.

Introduction

In a typical vineyard, as in many perennial cropping systems, management of non-crop vegetation is a significant consideration. Inter-rows are often planted to cover crops or permanent groundcovers to prevent soil erosion, manage fertility, and provide habitat for beneficial organisms (Hartwig and Ammon 2002). The area directly under the vine row, however, is typically maintained as bare soil through the use of herbicide applications or mechanical cultivation in order to minimize competition between weeds and grapevines (Hembree *et al.* 2013). Each of these approaches has notable drawbacks ranging from the development of herbicide resistant weed populations (Heap 2014) and environmental pollution (Louchart *et al.* 2001) to erosion and grape root and trunk damage (Hembree *et al.* 2013). With growing interest in environmentally sustainable production practices in many grape-growing regions, exploration of alternative strategies is warranted.

Recently the use of living mulch, or actively growing plants, underneath vine rows has emerged as an alternative weed management scheme (Centinari 2016). Although much of the work to date has focused on competitive effects with vines (e.g. Karl *et al.* 2016), and there has been some work on how living mulch might affect soil properties (Karl *et al.* 2016a), only recently has an effect on soil biota been demonstrated (Chou and Vanden Heuvel 2018). The effect on soil biota has the potential to add another suite of ecosystem services to the use of living mulch through conservation biocontrol and resource provisioning to vines through symbioses. Soil biota may be especially sensitive to the identity of plants used as living mulch, and changes below ground may also contribute to vine growth outcomes (Vukicevich *et al.* 2018).

Because plants are known to change soil biota through rhizosphere (Badri and Vivanco 2009) and litter effects (Fanin *et al.* 2014), living mulch may change soil microbial communities in ways that promote or inhibit vine growth and resilience. Fungi are common constituents of plant rhizospheres and show some degree of plant host specificity as endophytes inhabiting roots (De Deyn *et al.* 2011, Agusti-Brisach *et al.* 2011, Behie *et al.* 2015). While there is a risk that living mulch plants could host fungal pathogens of grape, such as *Ilyonectria* spp. (Agusti-Brisach *et al.* 2011, Benitez *et al.* 2016), beneficial microbes may also be enhanced via living mulch plants. For example, plant identity is known to affect the abundance of arbuscular mycorrhizal (AM) fungi (De Deyn *et al.* 2011), which are important for maintaining soil structure (Rillig and Mummey 2006) and improving vine nutrient and water acquisition (Torouvelot *et al.* 2015). Entomopathogenic fungi (EPF), another beneficial group responsible for the regulation of soil dwelling grape insect pest populations (Kirchamair *et al.* 2004), also may be differentially abundant depending on plant identity (Behie *et al.* 2015).

Plant functional group may be important determinants of vine growth responses both through changes to soil abiotic properties and fungal guilds. For example, plants with extensive finely branched root systems such as grasses may hinder vine performance in N-limiting conditions because they are known to increase soil C:N ratios and scavenge

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soil N (Clark 2008). Legumes, on the other hand, may enhance vine growth in N-limiting situations by adding biologically fixed N to the system (Clark 2008) or reduce vine performance if they compete for water and other limiting nutrients such as P (Caradus 1980). Certain plant functional groups may also enhance or suppress AM fungi (Hetrick *et al.* 1988), soil borne pathogens (Benitez *et al.* 2016), or EPF (Behie *et al.* 2015). Notably, plants in the family Brassicaceae can decrease the abundance of soil fungi, including pathogens as well as beneficial AM fungi, due to volatiles released when tissues are damaged or decompose (Schreiner and Koide 1993).

Here we explore how living mulch comprised of plants representing different functional groups affect vine growth as well as soil abiotic factors and the abundance of beneficial and pathogenic soil fungi in a young vineyard in a semi-arid region. We selected plants based on suitability to site and climate, including a warm- and cool-season grass, a legume, and a brassicaceous forb. We pose the question: Does living mulch identity influence a) growth of young vines, b) soil abiotic factors, and c) the abundance of AM fungi, the common EPF *Beauveria bassiana,* or the pathogenic *Ilyonectria* spp.?

Material and Methods

Field site and vine establishment: This project was established in the field at the Summerland Research and Development Centre in Summerland, British Columbia, Canada on Osoyoos series sandy loam. Mean annual precipitation at the site is approximately 320 $mm \cdot yr^{-1}$. The 0.08 ha experimental field that was previously planted to grapevines was left fallow for one year and tilled prior to planting to eliminate weeds. Dormant vines of *Vitis vinifera* 'Sauvignon Blanc' grafted onto *Vitis riapria* x *rupestris* '101-14' were planted at 1.22 by 2.44 m spacing (vine by row) on 23 June, 2015. Vines were trained to a vertically-shoot positioned trellis system, pruning to trunks after the 2015 growing season, cordons after 2016, and spurs following the 2017 growing season. Standard viticulture practices for young vineyards were carried out, including spring shoot thinning, shoot positioning, leafing, and crop removal on young vines.

Vines were irrigated through a drip system with two $2\ L \cdot hr^{-1}$ pressure-compensating emitters delivering water to either side of each vine. Irrigation was delivered as needed in 2015 and 2016. In the summer of 2017 irrigations were made on three and four-day intervals (twice per week), applying 12 L of water to each vine on each event and 16 $L \cdot vine^{-1}$ during the hotter weeks of August. Vines were not fertilized in 2015. In 2016, vines were fertilized using 20-20-20 'Plant-Prod' (Master Plant-Prod, inc., Brampton, ON) applying 8.3 $g \cdot vine^{-1}$ (2 kg total applied) on June 10, 16.6 $g \cdot vine^{-1}$ on June 30 and 16.6 $g \cdot vine^{-1}$ on August 2. In 2017, vines were fertilized with 34-0-0 Urea, applying 8.75 $g \cdot vine^{-1}$ on June 23 and 15 $g \cdot vine^{-1}$ on July 23. Each of these applications was applied through the drip irrigation system with sufficient water to deliver the majority of the solubilized fertilizer to the root zone of the vine.

Experimental design and establishment of living mulch treatments: Treatments were established in a six-by-six Latin square design to account for potential differences among or within vine rows. Each replicate plot was a panel of five vines with at least five vines at each end of each row to eliminate edge effects. In 2015, inter-rows were maintained by cultivation and 1 % glyphosate herbicide was applied to the vine row to minimize weed competition with newly-planted vines. A permanent groundcover of *Festuca* spp. and *Lolium perenne* was seeded in March of 2016 in inter-rows, leaving a 1 m bare strip under vine rows. In preparation for treatment establishment, weeds were removed by hand from the under vine areas in early March of 2016. The cleared area was then raked and seeded on March 16.

Living mulch plant identity: Living mulch treatments were chosen to include one representative of each of four plant functional groups: warm-season grasses, cool-season grasses, legumes, and non-legume forbs as they are expected to vary in their effects on soil properties and vine competition. Specific cultivars were chosen based on successful growth in other groundcover trials at the study site. We also included two industry standard under-vine management practices as comparison.

1) Buffalo grass (*Bouteloua dactyloides* (Nutt.) Columbus) is a warm-season grass native to the Great Plains of North America. It is used as a low-water alternative turf for residential lawns and establishes a virtually weed-free groundcover in inter-rows at the study site. Buffalo grass Columbus '8315' was obtained from OSC seeds (Kitchener, Ontario, Canada) and seeded at a rate of 27 $g\ m^{-2}$ with light raking.

2) Chewing's fescue (*Festuca rubra* subsp. *communtata* Gaudin) is a cool-season fine fescue native to Eurasia. It is commonly used as a residential turfgrass and is noted for shade tolerance, adaptation to poor soils, and drought tolerance (Cook 2011). Chewing's fescue '7117' was obtained from OSC seeds (Kitchener, Ontario, Canada) and seeded at a rate of 27 $g \cdot m^2$ and lightly raked.

3) Shepherd's purse (*Capsella bursa-pastoris* (L.) Medik.) is a brassicaceous forb that is a common winter annual weed in vineyards in Southern Interior British Columbia. It was selected for inclusion in this trial for several reasons: 1) it is a non-legume forb that should be minimally competitive due to spring and fall growth, 2) its presence as a weed in vineyards of the study region is welcome and even encouraged as it acts as cultural control for climbing cutworm pests (Lepidoptera:Noctuidae) (Mostafa *et al.* 2011), and 3) as a brassica, it may have beneficial (Mazzola 2015) or detrimental (Schreiner and Koide 1993) effects on soil fungi. Shepherd's purse was obtained from Richter's seeds (Goodwood, Ontario), mixed with fine sand and broadcast at a rate of 1 $g \cdot m^{-2}$ using saltshakers for more even seeding.

4) Birdsfoot trefoil (*Lotus corniculatus* L.) is native to Eurasia but is well established and commonly used as forage in British Columbia. Of the legumes included in a previous inter-row groundcover trial at the study site, it persisted best under minimal irrigation (Lowery, pers. comm.). Birdsfoot trefoil was obtained from Northstar seeds (Neepawa, Manitoba) and seeded at a rate of 1 $g \cdot m^2$. Pursh's

milkvetch (*Astragalus purshii* M.E. Jones var. tinctus), failed to germinate in the field in 2016, thus we replanted with *L. coniculatus* in 2017.

5) A cultivation treatment was maintained by hand hoeing three times in 2016 and three times in 2017. Timing of hoeing coincided with weeds approaching flowering stage. Weed biomass was left on the surface to decompose as would be the case with most mechanical cultivation tools.

6) A herbicide treatment employed a hand-sprayed application of 1.5 % Crush'R Plus (360 $g \cdot L^{-1}$ glyphosate; AgWest Inc., Calgary, AB) twice yearly in mid-May and early August. Glyphosate was chosen because of its widespread and consistent use in vineyards. Glyphosate was also applied to the shepherd's purse treatment after seed set to eliminate summer weeds and allow for successful re-emergence of the shepherd's purse in the fall and spring of each year. This practice is consistent with management of this weed for cultural control of climbing cutworm in the region (Lowery, pers. comm.).

Plot maintenance: Under-vine microsprinklers delivered water to the entire experiment as needed to establish treatments in 2016 and spring of 2017. They were not used after May 2017 until September 27 of that year. Several hand-weeding passes were made to encourage establishment of treatments in the spring of 2016. Weeds were maintained in plots based on the particular needs of the focal plant. For example, Fescue and Buffalo grass treatments were mowed twice in 2016 with a weed eater, as trimming favors grasses over weedy forbs due to ability of grasses to regenerate from the crown. The Birdsfoot trefoil treatment was hand weeded twice in the spring of 2017 to encourage establishment. The other treatments were not weeded in 2017 as they had established sufficient cover in 2016. Percent cover by living mulch plants and weeds along with composition of the weed community was assessed in detail in May 2017 and is given in supplemental materials.

Measures of vine growth responses to living mulch treatments: Vine growth data was collected in the summer of 2017 once all treatments had established. Measurements included: leaf greenness, shoot length at bloom, leaf water potential, and dormant pruning weight.

Leaf greenness: Leaf greenness was determined using a *SPAD* chlorophyll meter (Spectrum Technologies, Aurora, Illinois), calculating the mean chlorophyll density for fifteen randomly selected, mature, mid-canopy leaves per vine for the center three vines in each replicate plot (five leaves per vine). *SPAD* readings are used as a proxy for leaf N content, as leaf tissue N concentration and *SPAD* readings are highly correlated in perennial crops such as grape (Porro *et al.* 2000). *SPAD* readings were taken on two occasions in 2017: June 23 and August 1.

Shoot length at bloom: As a representation of early season shoot growth, bloom shoot length was determined on June 21 by calculating the mean length of eight random shoots per vine for the center three vines of each plot (24 shoots total).

Leaf water potential: Leaf water potential was measured mid-season (July 27) on a warm, cloudless day at the end of an irrigation cycle (immediately before the next scheduled irrigation) for two mature, healthy, un-shaded leaves (one from each of two vines per replicate) using a pressure chamber (PMI Instruments, Corvallis, Oregon). Readings were averaged by replicate.

Dormant pruning weight: Dormant pruning weight as a measure of final vine productivity was collected on November 24, 2017, by calculating the mean of total cane weight above the second bud from the cordon for each of the center three vines per plot. Vines were not hedged during the growing season.

Measures of soil responses to living mulch treatments. Soil moisture: Soil moisture was measured mid-season (July 10) during a warm week at the end of an irrigation cycle. A 2.5 cm corer was used to extract the top 0-30 cm as well as 30-60 cm of soil beneath four emitters between the five vines in each replicate plot. Cores were pooled and sealed in an airtight Ziplock bag, weighed, oven dried at 105 °C for 48 h and weighed again to calculate gravimetric water content.

Soil chemistry: A second set of cores (2.5 cm x 15 cm) was collected from beneath emitters on September 15. Although grapevines can be very deep rooted (Smart *et al.* 2006), this shallower depth for analysis of soil chemistry and biota was used because most of the biological activity is concentrated in the top 10-15 cm of most soils (Lavelle and Spain 2001). Four cores per plot were pooled and used to measure total soil C, total N, Bray-P, pH, and abundance of soil fungi (see below). Soil chemistry was analyzed at the British Columbia Ministry of Environment Laboratory (Victoria, British Columbia). Briefly, pH was measured in water at a 1:1 soil:water ratio. Available P was measured as Bray P-1 of a 1:10 soil:water ratio using a one minute extraction and colorimetric analysis at 882 nm with a phospho-molydenum blue complex. Total C and N were analyzed by combustion elemental analysis with a Thermo Flash 2000 analyzer (Thermo fisher scientific).

Soil fungal responses: Abundances of the common grapevine pathogen, *Ilyonectria* spp., the EPF, *Beauveria bassiana,* and AM fungi were determined in soil samples collected on September 15 (see above) using digital droplet PCR (ddPCR). Soils were first sieved to 2 mm following collection and *Vitis* roots were separated and stored in 35 % ethanol at 4 °C for later extraction. Soils were then dried at 60 °C for 72 h. Whole genomic DNA was extracted from 0.5 g of each soil sample using the FastPrep Spin Kit for Soils (MP Biomedical, Carlsbad, California) according to the manufacturer's directions. Whole genomic DNA was also extracted from 65 mg of fine (first and second order, < 2 mm) *Vitis* roots by crushing the roots in liquid N_2 and then using the above mentioned extraction kit according to the manufacturer's directions.

Both soil and root DNA were analyzed for abundance of *Ilyonectria* spp. using digital droplet PCR (ddPCR) with the internal transcribed spacer (ITS) primers YT2F and CYLR as per Agusti-Brisach *et al.* 2014. This primer set targets several species of the soil borne pathogen *Ilyonectria* (= "*Cylindrocarpon*") known to cause black foot disease of grape (Agusti-Brisach *et al.* 2014). The following recipe was used in a 20 µL final reaction volume: 10 µL QX200 ddPCR EvaGreen supermix (BioRad), 250 nM each primer,

2 µL DNA template, and 7 µL nuclease-free water. Reaction conditions were: initial denaturing at 95 °C for 10 min followed by 40 cycles of denaturing at 95 °C for 30 sec and annealing/extension at 60 °C for 1 min, then 4 °C for 5 min, 90 °C for 5 min.

AM fungi were quantified using a similar protocol with the small subunit rRNA primers AMV4.5F and AMDGR (Sato *et al.* 2005) and an annealing/extension step of 56.5 °C for 1 min. These primers target fungi within the phylum Glomeromycota and, of the AM fungal primers that yield products short enough for use with quantitative PCR methods, show the greatest specificity for this phylum (Lumini *et al.* 2010).

The abundance of the common EPF species *Beauveria bassiana* was determined using the same approach with the ITS primers BB.fw and BB.rv (Landa *et al.* 2013) and an annealing/extension step of 56 °C for 2 min.

After PCR amplification, droplets were read for fluorescence in a QX100 droplet reader compatible with EvaGreen dye (BioRad). Droplets were analyzed for fluorescence amplitude using QuantaSoft version 1.7 (BioRad) and raw amplitude and cluster data from each run were exported for threshold determination using 'ddpcRquant' (Trypsteen *et al.* 2015). All ddPCR assays were optimized by running temperature gradients with positive controls (fungal isolates) and environmental samples and selecting the highest annealing temperature at which there was good separation between positive and negative droplet clouds.

Data generated in this study will be made available on the Open Science Framework upon acceptance for publication.

Statistical analysis

Effects of living mulch identity on vine growth: To determine the effect of living mulch on vine growth responses (bloom shoot length, dormant pruning weight, *SPAD,* and pre-irrigation leaf water potential) a multivariate analysis of variance (MANOVA) was used comparing log-transformed values for each measurement. Univariate analysis of variance (ANOVA) and Tukey's honest significant difference (Tukey 1949) were employed as *post-hoc* tests to parse out differences among treatments for each parameter and growth stage measured. The R packages 'lme4' (Bates *et al.* 2015) and 'lmerTest' (Kuznetsova *et al.* 2017) with Satterwaite approximation of degrees of freedom were used to perform ANOVA, allowing inclusion of row and block as random factors to account for the Latin square design in the field.

Effects of living mulch identity on soil abiotic factors: In order to test if living mulch treatments altered soil factors, MANOVA was first used including log-transformed response variables pH, C:N ratio, C:P ratio, and soil moisture 0-30 cm and 0-60 cm pre-irrigation. Univariate ANOVA and Tukey's honest significant difference (Tukey 1949) were then used as described above to separate the effect of treatment on individual response variables.

Effects of living mulch identity on soil fungi: To test if living mulch identity altered abundance of AM fungi, *Ilyonectria* spp., or *Beauveria* spp., a MANOVA was also used comparing log-transformed target copy numbers $g \cdot soil^{-1}$. Individual ANOVA for each fungal group and post-hoc Tukey's honest significant difference (Tukey 1949) to determine which treatments differed for which fungal groups. Differences in target copy number of AM fungi $g \cdot root^{-1}$ were assessed using ANOVA as described previously.

Results and Discussion

Effects of living mulch identity on vine growth: The identity of the living mulch led to noticeable differences in vine growth responses in our study (*Wilk's* $\lambda = 0.013$, $F = 8.62$, $P < 0.0001$). Both the grasses and the legume treatments decreased vine growth significantly compared to the industry standard practices of cultivation or herbicides, with the fescue treatment causing the most severe growth depression in young vines at bloom ($F = 17.61$, $P < 0.0001$; Fig. 1a) and at pruning ($F = 21.147$, $P = < 0.0001$; Fig. 1b). The shepherd's purse treatment did not reduce vine growth compared to cultivation or herbicide treatments.

Overall growth depressions were expected given the young age of the vines and previous reports of vine growth suppression when perennial cool-season grasses such as *F. rubra* (Hickey *et al.* 2016) and warm-season grasses (Muscas *et al.* 2017) are used as living mulch. For a two-year old vineyard at this site and with the quantities of water and fertilizer applied in this study, both of the grasses and the birdsfoot trefoil led to unacceptable growth depressions compared to industry standards of cultivation or herbicides in this study. Because fertilizer and water application were applied to target balanced (*i.e.* not overly vigorous) growth in cultivation and herbicide treatments, there is potential for some treatments such as buffalo grass or birdsfoot trefoil to work well if inputs were increased or they were established under mature vines. The winter annual shepherd's purse, which has other benefits such as cultural control of climbing cutworm (Lepidoptera: Noctuidae; Mostafa *et al.* 2011), seems not to inhibit vine growth at this site likely due to temporal asynchrony of growth with vines.

Effects of living mulch identity on vine nutrition: The growth depressions seen in this study can be attributed to competition with the vine over soil resources. As expected, the two grass treatments were more competitive for soil N, as was evident from large differences in leaf greenness in June ($F = 44.249$, $P < 0.0001$) and August ($F = 92.066$, $P < 0.0001$; Fig. 1c). Fescue decreased leaf greenness more than any other treatment on both occassions, while buffalo grass produced vines that were less green compared to cultivation, herbicide, birdsfoot trefoil, and shepherd's purse treatments in June. This same pattern was seen in August, except for the birdsfoot trefoil also produced vines with intermediate greenness (Fig. 1c). Muscas *et al.* (2017) also found a reduction in vine N when a mixture of cool season grasses, *Dactylis glomerata* and *Lolium rigidum,* were used as living mulch in a 17-year old vineyard in a Mediterranean climate. In our study, the Chewing's fescue treatment was notably efficient at taking up fertilizer N,

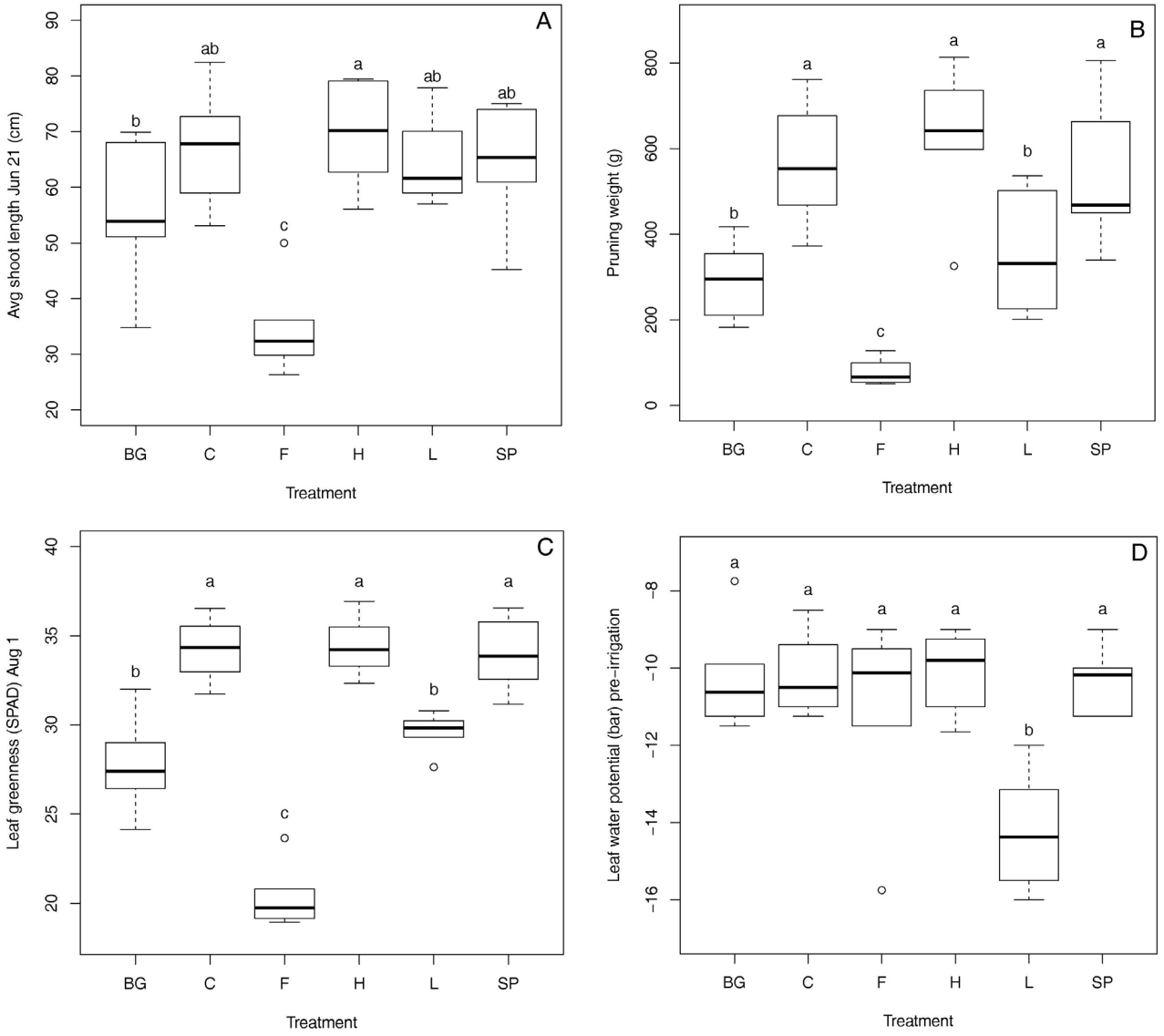

Fig. 1: Vine growth and physiological responses to living mulch treatments. Responses varied by treatment in bloom shoot length (**A**), dormant pruning weight (**B**), leaf greenness (**C**), and leaf water potential (**D**). Treatments are: BG, buffalo grass; C, cultivation; F, Chewing's fescue, H, herbicide, L, birdsfoot trefoil, and SP, shepherd's purse. Letters indicate significant differences assessed at $\alpha = 0.05$.

even producing salt deposits at hydathodes at the tips of leaf blades the day after fertilization events in 2017. This suggests that the Chewing's fescue treatment was strongly competitive with vines for soil N as seen previously with this species of grass (Hickey *et al.* 2016). The buffalo grass treatment did not cause the same degree of yellowing and stunted vine growth seen in the fescue treatment. Because *Bouteloua dactyloides* is native to the Great Plains where it persists in low fertility, droughty soils and N additions to buffalo grass range does not improve growth (Pettit and Fagan 1974), it could be expected that buffalo grass would not be as competitive for soil N.

Effects of living mulch identity on vine water status: Although the birdsfoot trefoil treatment also led to slight decreases in leaf greenness compared to industry standards in August (Fig. 1c), the data suggest that the competitive effects from this plant were largely related to water as birdsfoot trefoil strongly decreased leaf water potential compared to all other treatments ($F = 11.140$, $P < 0.0001$; Fig. 1d). Although this is the first study to use this particular legume as living mulch in vineyards, other perennial legumes such as *Trifolium repens* as living mulch can also compete with vines for water (Karl *et al.* 2016). Birsdsfoot trefoil has been shown to be a strong competitor for water in other systems, e.g. in jujube orchards (Pan *et al.* 2017) and may be more competitive than more drought sensitive perennial legumes such as *T. repens,* as it continues to produce biomass under drought conditions by forming a deep taproot (Peterson *et al.* 1992). This trait may be beneficial for persistence of this legume in dry climates such as in this study, but could lead to unacceptable levels of vine water stress or vineyard water use. Birsdfoot trefoil may be better suited as part of a mixture of plants or as a drought-tolerant inter-row cover, where direct competition with vines for irrigation water would be minimized.

Unsurprisingly, Buffalo grass did not show signs of competing with vines for water as it has relatively low water requirements due to low evapotranspiration (ET) rates even in conditions of low water stress (Qian *et al.* 1997) and the ability to hydraulically redistribute water from lower depths

(Huang *et al.* 1999). Similarly, the fescue living mulch did not induce water stress in vines as measured here. Cool season grasses such as *Festuca* spp., despite having higher ET rates, are known to evade drought stress with the help of foliar endopytes (Malinowski and Belesky 2000) and this could partly explain why little to no water competition has been found between vines and *F. rubra* living mulch in other studies (Giese *et al.* 2014, Hickey *et al.* 2016) and with other cool-season grasses (Bavougian and Read 2018). Although an alternate explanation for the lack of water competition in this treatment could be that the majority of active growth of the fescue is asynchronous with that of the vines, the use of irrigation in our study allows continued growth of fescues though the summer in our area, *i.e.* they are not truly summer dormant as with select species of *Dactylis glomerata* (Volaire and Norton 2006). In the present study, the lack of water competition observed in the fescue treatment could also be explained by the extreme reduction in vine size due to N competition and thus lower total vine water use requirements relative to other treatments.

Effects of living mulch identity on soil abiotic factors: Living mulch treatments led to changes in measured soil abiotic effects (*Wilk's* $\lambda = 0.137$, $F = 2.25$, $P = 0.003$). Living mulch treatments varied in their effect on C:N ratio ($F = 3.058$, $P = 0.027$), with buffalo grass leading to a higher soil C:N ratio than cultivation where weeds were mechanically removed (Fig. 2a). As soils inhabited by *B. dactyloides* have been shown to have higher microbial biomass C than many other prairie grasses (Bell *et al.* 2014), prolific shallow root production by this species (Derner *et al.* 2006) could have not only helped outcompete weeds, but also increased the soil C:N ratio through prolific root litter and exudation. The fescue treatment increased C:N ratio as well, probably due to efficient uptake of N as discussed previously, but also due to a thick thatch layer that developed over the course of the experiment. This thatch layer was very effective at keeping weeds out, but likely was responsible for the immobilization of any remaining soil N. Because of this, Chewing's fescue as living mulch may only be useful in managing vine vigor and weeds in overly fertile soils (Giese *et al.* 2014). Despite effective weed suppression and production of a healthy, green canopy about 30 cm tall, the birdsfoot trefoil did not reduce soil C:N ratio. This is probably indicative of effective

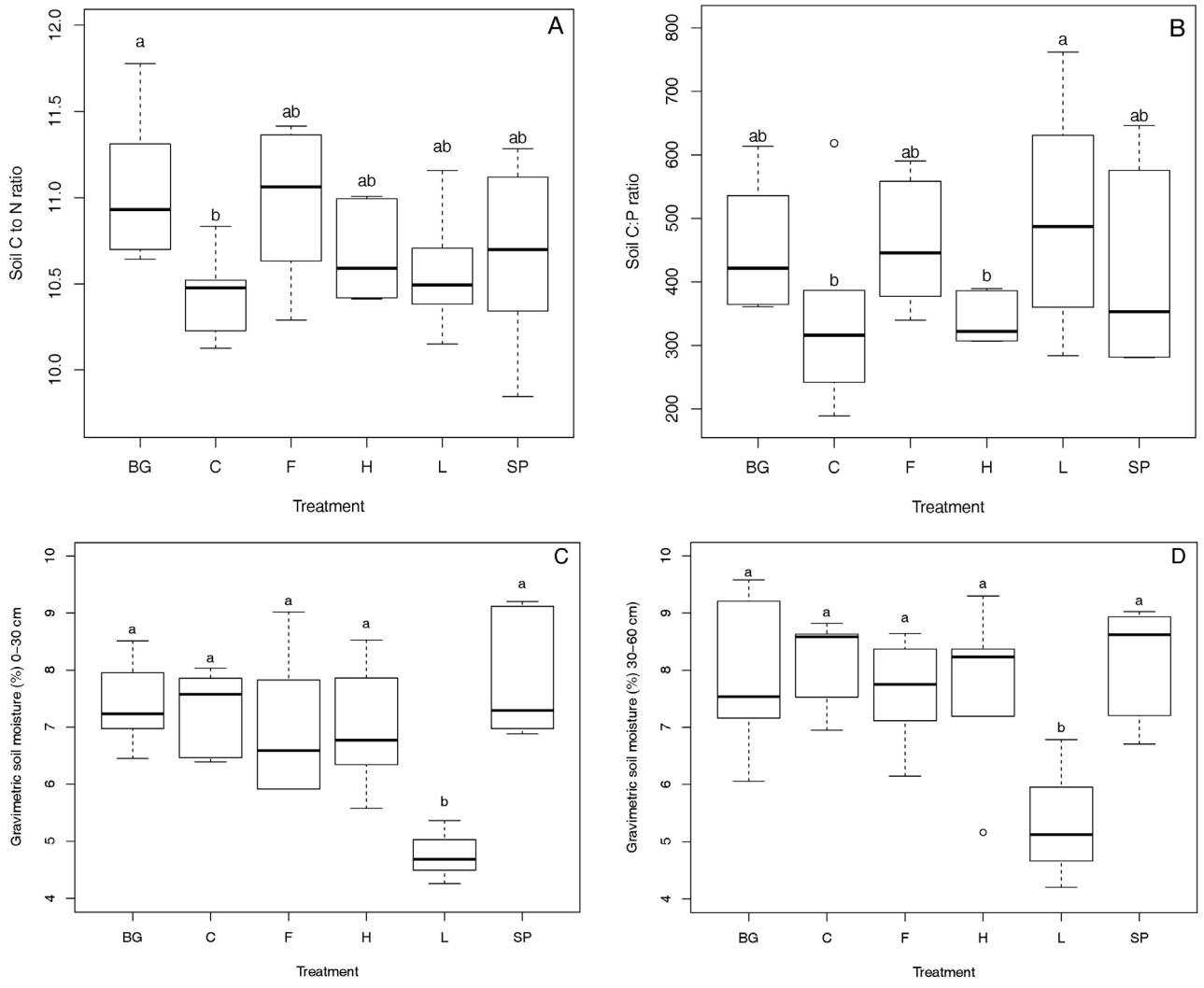

Fig. 2: living mulch effects on soil properties. Treatment effects were seen in soil C:N ratio (**A**), C:P ratio (**B**), soil moisture in the top 30 cm pre-irrigation (**C**), and soil moisture from 30-60 cm pre-irrigation (**D**). Treatments are: BG, buffalo grass; C, cultivation; F, Chewing's fescue, H, herbicide, L, birdsfoot trefoil, and SP, shepherd's purse. Letters indicate significant differences assessed at α=0.05.

symbiotic N_2 fixation and thus little N uptake from the soil solution by this legume. Soil C:P ratio depended on living mulch identity as well (F=3.652, P=0.016) with higher C:P levels in the birdsfoot trefoil treatment than the cultivation and herbicide treatments (Fig. 2b). This can be explained by the fact that legumes tend to have a high P requirement due to the energy required at nodulation sites for symbiotic N_2 fixation (Sa *et al.* 1991) and tend to be more efficient at acquiring soil P compared to grasses (Caradus 1980). Because vine P was not measured in this study, it is impossible to tell if this alteration of soil C:P ratio contributed to the slight, but significant growth depression with birdsfoot trefoil as living mulch.

Consistent with lower leaf water potential discussed above, birdsfoot trefoil living mulch greatly decreased soil water content in the top 30 cm by the end of the irrigation cycle compared to any other treatment (F = 12.822, P < 0.0001; Fig. 2c). This same trend was seen in the 30-60 cm soil depth as well (F = 3.17, P = 0.03; Fig. 2d), indicating the effect of a deep rooting pattern that allows this legume to persist in semi-arid climates. Soil pH was not affected by living mulch treatment (F = 1.41, P = 0.26).

Effects of living mulch on soil fungi: Living mulch treatment did not affect the abundance of any of the three fungal guilds in bulk soil (*Wilk's* λ = 0.749, F = 0.57, P = 0.89; Fig. 3a, 3b, and 3c). This was surprising given the reports of plant effects on populations of these soil fungi (Agusti-Brisach *et al.* 2011, De Deyn *et al.* 2011, Behie *et al.* 2015). Because each of these assays targeted functionally distinct fungi at various levels of taxonomic resolution, *i.e.* specificity, the reasons for the lack of effects seen may be best explored for each individual guild.

Effects of living mulch on *Ilyonectria* spp.: The lack of treatment effects on *Ilyonectria* abundance may indicate that living mulch plants do not act as a good alternate host for these pathogens as has been seen with some vineyard weeds (Agusti-Brisach *et al.* 2011) and certain vetch cover crops in annual cropping systems (Benitez *et al.* 2016). It was also surprising that shepherd's purse did not decrease *Ilyonectria* spp. abundance due to the anti-fungal volatiles produced by plants in the family Brassicaceae (Fahey *et al.* 2001). However, *Ilyonectria* spp. can survive as durable chlamydospores in soil (Halleen *et al.* 2006) and thus may be more resistant to these biofumigant effects (Stephens *et al.* 1999). More work is needed to see if there are any temporal effects, *i.e.* suppression of soil fungi when shepherd's purse is actively growing in spring or shifts in communities of fungi if some fungi are more tolerant of these volatiles than others. The quantity of *Ilyonectria* detected in this soil was several orders of

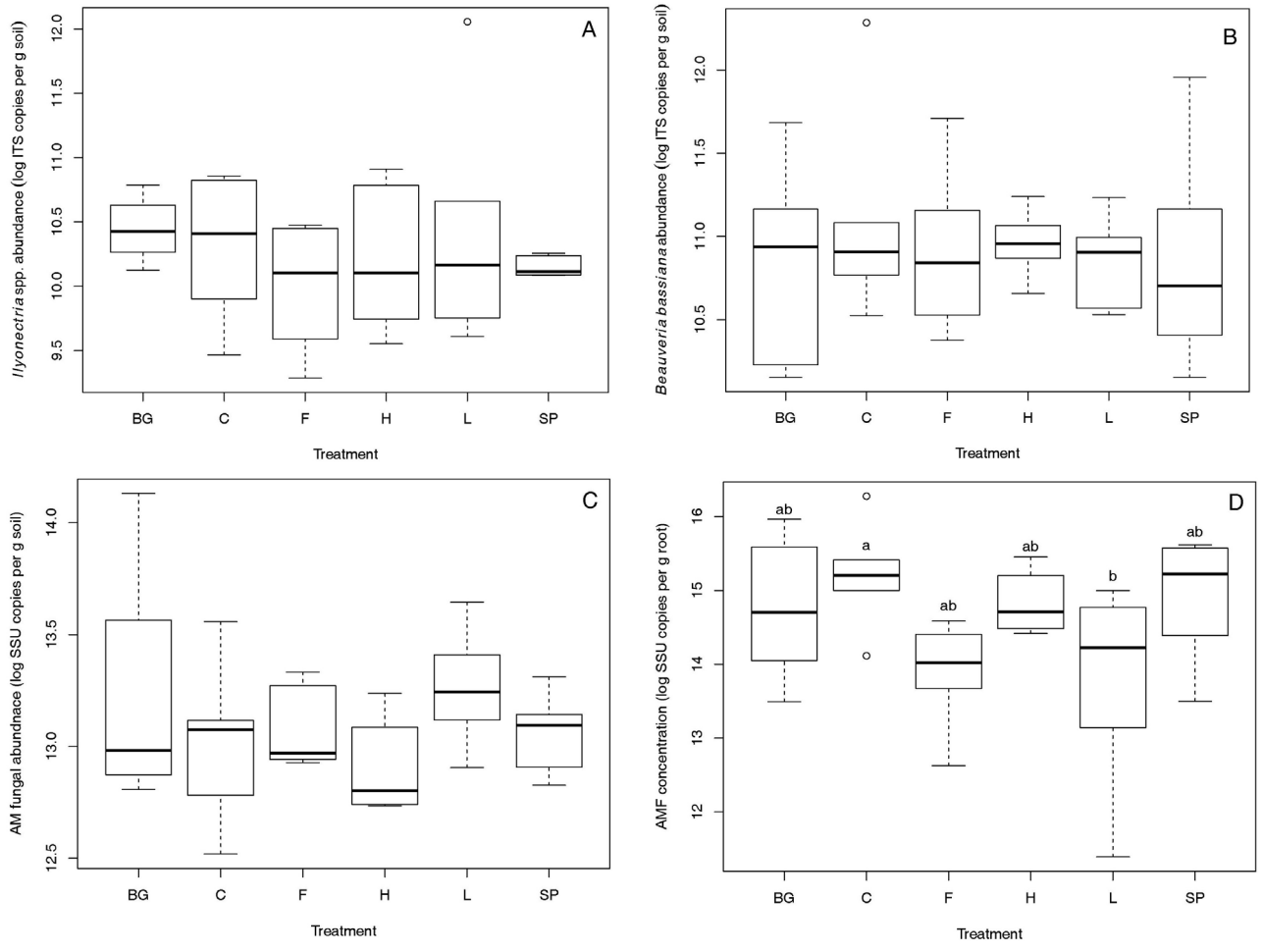

Fig. 3: Effect of living mulch on abundance of *Ilyonectria* spp. (**A**), *Beauveria bassiana* (**B**), and AM fungi (**C**) in soil as well as AM fungi in vine roots (**D**). Treatment effects were only seen for AM fungi in roots. Treatments are: BG, buffalo grass; C, cultivation; F, Chewing's fescue, H, herbicide, L, birdsfoot trefoil, and SP, shepherd's purse. Letters indicate significant differences assessed at α = 0.05.

magnitude lower than those reported previously in infested nursery fields using this primer set (Agusti-Brisach *et al.* 2014). Although an accurate threshold of these fungi in soils indicating disease potential has not been established, black foot pathogens such as *Ilyonectria* spp. are perhaps too infrequent at this site to warrant an assessment of the impacts of living mulch vegetation on disease potential. The coarse textured soil here could be a deterrent to the proliferation of these fungi as finer textured soils that hold more water are generally more problematic for development of black foot disease of grape (Berlanas *et al.* 2017).

Effects of living mulch identity on *Beauveria bassiana*: The EPF, *Beauveria bassiana,* was similarly unaffected by living mulch treatment in our study. This was surprising given reports of plant host preference as a root endophyte for this species (Behie *et al.* 2015). However, because *B. bassiana* is a fungus with known intraspecific genetic diversity that varies along environmental gradients (Bidochka *et al.* 2002) and between natural and managed habitats (Meyling *et al.* 2009), it is possible that changes occurred at the population level in response to living mulch treatment that were not detected by measuring total abundance of *B. bassiana* at the species level as done here. Although *B. bassiana* is among the most common EPF isolated from Canadian agricultural soils (Bidochka *et al.* 1998), other EPF such as *Fusarium* spp. have been isolated more frequently at this study site and thus represent a target outside of our *Beauveria* assay that might have been affected by living mulch treatment. The development of a molecular assay targeting all EPF, perhaps using a functional gene, would be highly advantageous for future studies investigating the functional consequences of management practices such as living mulch on the infectivity of soil-dwelling insect pests by these natural enemies.

Effect of living mulch identity on AM fungi: Similarly, no differences were seen in abundance of AM fungi in bulk soil under living mulch treatments. It is surprising that the C_4 buffalo grass did not promote a greater abundance of AM fungi than other treatments as C_4 grasses are known to be strongly mycorrhizal (Hetrick *et al.* 1988) and are therefore expected to increase the quantity of AM fungi in surrounding soils. *Bouteloua* spp., however, are also known to be colonized extensively by dark septate endophytes that may also relieve abiotic stresses (Barrow 2003) while displacing AM fungi. It is possible that these plants may instead enhance a different group of soil fungi that do colonize *Vitis* (Likar *et al.* 2017) but have unknown effects on vines. The cultivar used here, a residential turfgrass selection, may also be adapted to higher resource environments as it stays greener during the heat of summer compared to wild populations (Lowery, pers. comm.), perhaps indicating a trend toward less reliance on AM fungi commonly seen in cultivated plants when resources are provided (Martin-Robles *et al.* 2018).

Surprisingly, the shepherd's purse living mulch did not decrease AM fungal abundance compared to other treatments, which is contrary to reports in the literature of other brassicas having fungicidal properties, e.g., garlic mustard against AM fungi (Stinson *et al.* 2006). There is a wide range of glucosinilate profiles and quantities produced within the Brassicaceae (Fahey *et al.* 2001) and shepherd's purse may have relatively weak biofumigant effects relative to other species. In fact, shepherd's purse can be colonized by AM fungi at rates upwards of 30 % if growing in proximity to good AM fungal host plants (Demars and Boermer 1994). This could further decrease the competitive effects of this living mulch with vines as the shepherd's purse does not gain any benefit from colonization by AM fungi due to the absence of arbuscules (Demars and Boermer 1994).

AM fungal abundance in vine roots, in contrast, varied among living mulch treatments ($F = 2.977$, $P = 0.03$), with more AM fungal target copies $g \cdot root^{-1}$ detected in roots of vines from the cultivation treatment compared to those from the birdsfoot trefoil treatment (Fig. 3d). This is surprising because legumes are typically strongly mycorrhizal (Chalk *et al.* 2006) and might therefore be expected to increase the abundance of these fungi in neighboring host plants. We speculate that the birdsfoot trefoil treatment may have reduced AM fungi in vine roots due to water stress-induced carbon limitation in the vine (Valentine *et al.* 2006). The decrease could also be due to changes in identity of the AM fungi colonizing vine roots with a selection towards drought-tolerant fungal species that do not colonize roots as thoroughly and instead invest more in external soil hyphae (Hart and Reader 2002).

The physical disturbance of topsoil during hoeing in the cultivation treatment also might be expected to reduce AM fungi (Jasper *et al.* 1989), but this was not the case in this study. Although it is possible that the disturbance from hand hoeing was shallower than some other cultivation equipment, preventing overall damage to AM fungal hyphae in the top 20 cm of sampled soil, mechanical means of weed control common in organic vineyards may not be as harmful to AM fungal symbioses as perceived.

Conclusion

Establishment of living mulch beneath two-year old grapevines in this semi-arid habitat led to changes in vine growth, soil characteristics, and AM fungal abundance in vine roots, though no changes in soil fungi were detected in bulk soil within this time frame. The treatment effects were related to relevant functional traits of the living mulch species selected, indicating the importance of this knowledge for living mulch selection as well as for overall management of vineyard floor vegetation, *i.e.* the identity of weeds and inter-row vegetation are likely also to affect soil properties and vine competition. Growth suppression caused by living mulch treatments in this trial was amplified by the use of young vines and further work would be needed to assess the feasibility of these treatments in a mature vineyard. Trade offs between productivity and tightly regulated irrigation and fertilizer applications to make up for living mulch competition should also be considered when choosing appropriate living mulch. This study shows clear differential effects on vine growth and soil properties using plants from different functional groups as living mulch and suggests that plant functional traits can help growers choose appropriate species depending on their site.

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