Soil organic carbon and nitrogen pools are increased by mixed grass and legume cover crops in vineyard agroecosystems: detecting short-term management effects using infrared spectroscopy

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Abstract

The incorporation of cover crops in orchards and vineyards can increase soil organic carbon (OC) and improve nitrogen (N) availability. This study compared how three herbaceous under-vine cover crop assemblages affected OC and N pools in four edaphically distinct vineyard agroecosystems. Using physical fractionation and soil spectral analysis we: 1) compared effects of grass and legume mono- and poly-cultures on total, coarse (≥50 µm) and fine (<50 µm) pools of OC and total N (TN), as well as extractable N (ExN), and 2) assessed predictions of OC and TN pools by infrared spectroscopy (IRS) and partial least squares regression analyses (PLSR). Compared with the control treatment, total, coarse and fine fraction OC were greater in the presence of grasses and legumes; ExN was increased 38% by legumes, and 78% in legume-grass mixture. With initial calibration, we used one soil spectral analysis to successfully derive models predicting contents of OC in the whole soil, and the allocation of OC to coarse and fine fractions. In addition to demonstrating the efficacy of incorporating grass and legume cover crops into vineyard cropping systems to improve OC and the storage and availability of N across diverse soil types, this study confirms the ability of IRS/PLSR to predict changes in OC concentrations related to

differential ground cover management. IRS/PLSR is an important and practical approach for the rapid quantification of short-term management impacts on SOM pools, contributing significantly towards improved understanding of soil C and N dynamics in vineyard agroecosystems.

1. Introduction

Vineyards are intensively managed agroecosystems and are particularly depleted in soil organic carbon (OC) and vulnerable to soil nitrogen (N) loss (García-Díaz et al. 2017). Typically, deep intensive tillage during vine establishment destroys aggregate structures and increases liberation of OC (Álvaro-Fuentes et al. 2008; Luo et al. 2010), while under-vine removal of natural vegetation using herbicide reduces soil organic matter (SOM) accumulation and protection (Eldon & Gershenson 2015; Whitelaw-Weckert et al. 2007). The direct effects of management practices on SOM retention render soils under perennial fruit crops especially vulnerable to degradation (Cheddadi et al. 2001; Lal 2004) and heightened contribution to global greenhouse gas emissions (Aguilera et al. 2015). Further, very few studies have accounted for the probability of future accelerated degradation of critical soil quality parameters predicted to occur in vineyards (Baldock et al. 2012; Treeby 2018). As important viticultural regions begin to transition towards lower input, organic vineyard management practices to improve soil health (Penfold et al. 2015; Wheeler & Crisp 2011), empirical research quantifying the impacts of differential management on critical soil parameters is of vital importance to the development of an environmentally sustainable wine growing industry.

Incorporating residue retention practices in perennial fruit cropping systems can increase the accumulation of SOM, which improves OC sequestration and soil nutrient availability (Lal & Bruce 1999; Montanaro et al. 2010; Roldán et al. 2003). Further, SOM provides a substrate for the crucial soil biota which mediate soil C sequestration and mineralise organic N to plant-accessible inorganic N (Allison et al. 2010; Cookson et al. 2007; Keiblinger et al. 2010). Growing plants also influence SOM accumulation through their active root systems which contribute significantly to OC and N and improve aggregate stability (Kätterer et al. 2011;

Ovalle et al. 2010a; Rasse et al. 2005). A reduction of soil tillage is also recommended to protect SOM in aggregates from microbial decomposition, although it has been suggested that the agricultural benefits of no-till may be smaller than previously thought (Luo et al. 2010; Powlson et al. 2014). If no-till benefits are indeed low, then increasing plant inputs to agricultural systems is of particular importance for maintaining or enhancing stocks of SOM.

Introducing herbaceous communities between or under vine rows in vineyards - termed cover cropping - has been shown to increase SOM inputs and, depending on the crop-type, to improve nutrient availability in viticultural systems (Gómez et al. 2011; Peregrina et al. 2010; Steenwerth & Belina 2008b). However, the inclusion of ground cover on the normally bare soil under vines is a contentious management technique. This is largely owing to the high requirement for N in fruit development (Gabriella et al. 2019), combined with the concern that some cover crops, especially grasses, may compete with vines for nutrients, negatively affecting yield and fruit quality (Celette et al. 2009; Muscas et al. 2017). Legume cover crops can reduce the need for N fertiliser applications by returning biologically fixed N to the soil potentially facilitating the growth of agricultural crops and other cover crop species such as grasses (Baumgartner et al. 2008; Mitchell et al. 2017; Peoples et al. 2009). In vineyard cropping systems, legumes have been demonstrated to provide the equivalent of 40 kg N ha^{-1} to grapevines (Ovalle et al. 2010b) and, in other cropping systems, to facilitate grass root growth and N uptake in legume-grass polycultures (Ramirez-Garcia et al. 2014). This is particularly important for SOM accumulation as grasses have a fine, dense root structure that contributes significantly to soil OC (Fisher et al. 1994; Ramirez-Garcia et al. 2014); in cover crop species specifically, as much as 44% of plant biomass C has been attributed to roots (Guzmán et al. 2014). In other vineyard ground cover cropping trials, grass and legumes have increased soil OC and water-soluble carbon, improved N availability and increased microbial biomass (Karl et al. 2016; Steenwerth & Belina 2008a; Steenwerth & Belina 2008b). Importantly, whether a cover crop makes a significant contribution to SOM accumulation and nutrient retention is largely dependent on the plant functional type (Pendall et al. 2011; Peoples et al. 2009; Shennan 1992).

Several studies have successfully examined and modelled OC dynamics in agricultural systems under differential management, using carbon pool data obtained from the physical separation of OC into its component fractions (Blair et al. 1995; Jagadamma & Lal 2010; Skjemstad et al. 2004; Zimmermann et al. 2007). The coarse (particulate) organic matter fraction consists of recently decomposed plant inputs, is considered to have a turnover time of years to decades, and is most likely to respond quickly to changes in land management (Cambardella & Elliott 1992). Fine fraction (mineral associated) OC and N pools are generally considered to be less susceptible to alteration by differences in ground cover management, are more strongly influenced by the percentages of silt and clay in the bulk soil and can be vulnerable to destruction of aggregates by mechanical disturbance (Feng et al. 2016; McNally et al. 2017). Changes to bulk OC following different management practices can be small and incremental compared to the large background OC stock, so several studies, including this one, have focussed on examining changes to SOM fractions that serve as early indicators of long-term changes to bulk SOM (Cambardella & Elliott 1992; Cozzolino & Morón 2006; Ojeda et al. 2018).

However simple, measuring SOM in fractions by physical separation is time consuming and for this reason may be prohibitive for routine analyses of agricultural soils (Poeplau et al. 2013). Therefore, quantification of changes in SOM stocks can be challenging at the farm scale, and so techniques to measure these changes using simple and rapid spectral analyses are becoming increasingly popular (Baldock et al. 2018; Barthès et al. 2008; Bellon-Maurel & McBratney 2011; Malley et al. 2000). Infrared spectroscopy (IRS) combined with chemometric analyses to quantify soil chemical and physical properties is a continually developing but robust technique for the analysis of soil parameters, and with sufficient calibration has the potential to replace at least some traditional techniques of soil analysis (Bellon-Maurel et al. 2010; Cozzolino & Morón 2006). The potential of coupled IRS and partial least-squares regression analysis (IRS/PLSR) to predict OC content in bulk soil and particle fractions has proven useful for quantifying changes to OC relating to agricultural management (Baldock et al. 2018), especially when models are developed and validated within a particular ecosystem of interest (Baldock et al. 2013a).

Quantification of the impacts of monoculture and mixed species under-vine cover crops on the improvement of soil quality in vineyards has not yet been attempted across multiple sites, nor across varied soil types. This study evaluated the potential for grass and legume cover crops to increase OC and N accumulation in under-vine soils in four edaphically distinct vineyard agroecosystems. Additionally, in an attempt to reduce the time and financial costs associated with quantifying OC and N contents at the farm scale (MacLeod et al. 2015; Schipanski et al. 2014), we also evaluated the use of IRS/PLSR spectral analysis to detect treatment level changes to OC and N pools, thereby developing a calibration dataset for use in vineyards. The aims of this study were: 1) to compare the effects of grass and legume cover crop mono- and polycultures on contents of OC, TN and ExN in bulk soil and their associated coarse (≥50 µm) and fine (<50 µm) soil fractions; and 2) to assess the potential for using easily-acquired infrared spectra in combination with partial least squares analysis to build models that accurately predict the contents of OC and N in vineyard soils under differential management. We anticipate that by demonstrating the effectiveness of under-vine cover cropping for improving soil carbon accumulation and nitrogen retention using a simple, cost effective technique, we might increase the adoption of sustainable viticulture practices in vineyards that have the benefit of improving soil health.

2. Materials and Methods

2.1 Experimental design and sites

A set of intra-row cover crop experiments were planted in 2014 by the University of Adelaide and Wine Australia on commercial vineyards, in collaboration with local growers at Eden Valley, Nuriootpa, Langhorne Creek and Waikerie, in southern Australia (**Fig 1**). The experimental design consisted of grasses and legumes grown in monoculture and mixture, with a herbicide-treated (plant-free) control established in a fully randomised complete block design (**Fig 2**). Vines were *Vitis vinifera* “Shiraz” cultivar at Eden Valley and Nuriootpa, and “Merlot” cultivar at Langhorne Creek and Waikerie. Plant functional types (grass *vs* legume) were maintained across the sites, though it was necessary to adjust the cover crop varieties sown according to soil type and seasonal rainfall, which varied considerably across the viticultural regions. Details of site characteristics, vineyard management and cover crop species at the individual sites are given in **Table 1.** At each vineyard, there were four replicates of each treatment giving a total of 16 experimental plots (each 10.5 m^2) per site. Effective weed control was maintained in the control (bare ground) treatments across sites with an average of 91% (± 5% SD) bare soil, whereas the grass and legume treatments had >80% vegetation cover, with bare soil averaging only 18.8 % (± 17% SD) across sites. The mixed grass and legume treatments averaged 75:25 legume:grass cover at 3 sites (Eden Valley, Nuriootpa and Waikerie), differing at the Langhorne Creek site where the ratio was 55:45. All vineyards were drip-irrigated in the intra-row zone and, prior to commencement of the trials, all plots had been maintained for four years with bare soil under-vine, using herbicide. Herbicides were applied in the cover crop treatment plots in 2014/15 to maintain treatment integrity and subsequent weed control was achieved using a line trimmer. The mid-row zones contained volunteer mixed swards maintained where required by mowing. The under-vine cover crops were not cut, but were instead left to naturally senesce with all above-ground residues remaining in-situ. In the interest of providing information on important vine performance parameters we refer to a report prepared by (Penfold 2018) for these sites. Briefly, bunch yields were not negatively affected by the cover crop treatments and at some sites were increased under mixed cover crops compared with the control. Yeast assimilable nitrogen, which provides a measure of available N for the fermentation process and significantly determines fruit quality (Neilsen et al. 2010) was increased under legume cover crops (Penfold 2018).

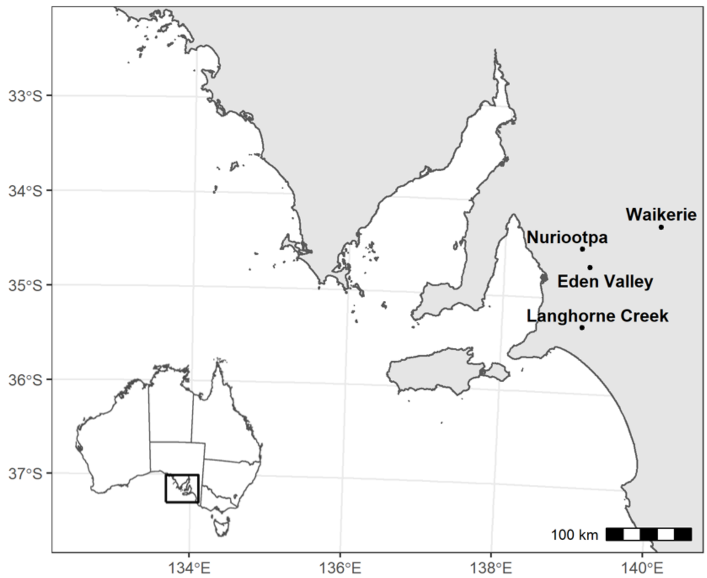

Figure 1: Location map of the studied commercial vineyard sites in South Australia. (Map credit: Johanna Pihlblad 2020)

Figure 2: Plot level experimental design focused on under-vine treatments; **a** Control, **b** Grass only, **c** Legume only, **d** Grass + Legume (mixture). Mid-rows showing volunteer mixed sward. (Photo credit: Chris Penfold, 2015)

Table 1: Site characteristics, vineyard management and plant species within treatments. Variables are reported as means (±se)

Site	Eden Valley	Nuriootpa	Langhorne Creek	Waikerie
Soil Classification*	Black Sodosol	Brown Sodosol	Brown Sodosol	Red Kandosol
pH (H_2O)	7.0	7.0	7.6	6.8
pH ($CaCl_2$)	6.3	6.4	6.9	6.2
Sand %**	62.0 ± 1.91	55.0 ± 2.58	28.0 ± 6.8	84.0 ± 1.91
Silt %**	12.0 ± 1.0	9.0 ± 1.15	38.0 ± 7.37	1.0 ± 4.16
Clay %**	26.0 ± 2	36.0 ± 3.65	34.0 ± 7.74	15.0 ± 3.0
Coarse Soil %	74.8 ± 4.7	58.4 ± 2.7	39.3 ± 2.8	92.2 ± 1.5
Fine Soil %	25.0 ± 4.7	41.5 ± 2.7	60.6 ± 2.8	7.7 ± 1.5
MAT (°C)	21.6	21.6	22.1	25.7
MAP (mm)	620	525	415	255
Fertiliser application*	N – 0.03	N – 0.07	N – 0.02	N - 0.20
(kg ha/year, 2016-18)	P – na	P - na	P - na	P – 0.02
Plot level Irrigation	1986	1125	3312	8496
(L/year, 2016-18)				
Vine Establishment (year)	1998	2001	1999	1998
Vines (ha^{-1})/Spacing (m)	1962 / 1.7	1271 / 2.2	2312 / 1.7	1665 / 2.0
Row width (m)	2.7	3.5	2.5	3.0
Legume Only	*Medicago truncatula*	*Medicago truncatula*	*Medicago truncatula*	*Medicago tornata*
	Medicago littoralis	*Medicago littoralis*	*Medicago littoralis*	
Grass Only	*Dactylis glomerata*	*Dactylis glomerata*	*Dactylis glomerata*	*Dactylis glomerata*
Grass+Legume	*Festuca ovina*	*Festuca ovina*	*Festuca ovina*	*Lolium rigidum*
	Trifolium fragiferum	*Trifolium fragiferum*	*Trifolium fragiferum*	*Medicago polymorpha*

*Soil classification data derived from Department of Environment and Water Soil and Land Program (Hall 2009). **n= 4; # n=16. ***N = nitrogen, P = phosphorus. MAT = Mean annual temperature, MAP = Mean annual precipitation. na = not applied.

2.2 Cover crop composition and cover crop contributions to below-ground biomass

Cover crop composition was assessed three times per year using the 'Botanal' method (Tothill et al. 1992). Briefly, composition was estimated from percentage cover of all species and bare soil in a 10.5 m^2 quadrat and is an average estimate of percentage cover for the years 2015 and 2016.

For the purposes of discussing C and N inputs as they relate to the cover crops, we compared cover crop root biomass among all treatments (including the control). Four soil cores of 5 cm diameter and a depth of 10 cm were removed from each replicate cover crop and control plot in March of 2017, composited, air dried and sieved to 2 mm, and all root biomass removed and quantified. Soils were air dried and roots carefully removed from dry soil (as opposed to washed) to comply with methods for the subsequent infrared spectral analysis (Baldock et al. 2013a). We separated biomass into fine herbaceous roots (<0.1 mm-0.3 mm), fine vine roots (>0.3 mm-1 mm) and coarse vine roots (>1 mm) (Centinari et al. 2016; Garcia et al. 2018; Roumet et al. 2008). Vine roots were easily distinguishable from cover crop roots due to their larger diameter, darker colour and acute branching angles (Klodd et al. 2016), but we were unable to distinguish between grass and legume root biomass in mixed treatments. On average, 95 – 100% of the root biomass in the treatments was contained in the 0-10 cm depth (data not shown), hence we chose to restrict sampling to this depth for the purpose of quantifying cover crop root contributions to measured variables. Cover crop treatment effects on root biomass were quantified only as they related to fine herbaceous root biomass; vine root biomass did not differ significantly among sites or treatments, including the herbicide control (data not shown).

2.3 Total C, total N, extractable N and texture analyses

To prepare soil for C, N and IRS analyses, 10 g of sieved (< 2 mm) air dried soil was ball milled for 180 s using a Retsch stainless steel ball mill (Baldock et al. 2014). The presence of inorganic C (IC) was evaluated by applying a few drops of 1M HCl to a well-homogenized 1g subsample of soil. Where a positive fizz test was recorded, carbonates were removed before C and N combustion analyses by acid digestion (Baldock et al. 2013a). At only one site within the study (Eden Valley) was carbonate removal necessary. Soil water content was determined gravimetrically by drying at 105° C, and all analyses were corrected for soil moisture content. The remaining air-dried bulk soil was kept aside for bulk density calculations and fractionation analyses.

Texture analysis was undertaken by sedimentation (Shirazi & Boersma 1984) on sieved (<2 mm), air dried soil to determine sand, silt and clay contents (**Table 1**). Extractable N (mg g^{-1}) was determined by shaking 40 ml of 2M potassium chloride (KCl) solution with 4.0 g soil (< 2 mm) at 170 rpm for 1 hour and then filtering with a 2.5 µm ashless filter (Grade 42, Whatman plc, Kent, U.K). Soil extracts were stored at -20 ^{o}C until colorimetric analysis in a discrete analyser (AQ2, SEAL Analytical, Ltd., Milwaukee, WI USA). Total C (mg g^{-1}) and TN (mg g^{-1}) were obtained from combustion analyses using a LECO TruMac carbon and nitrogen analyser (LECO, St. Joseph, MI, USA). Site-level edaphic characteristics were measured and compared using soils obtained from the control treatments (n=16).

2.4 Physical fractionation procedure

The physical fractionation procedure used was modified from Baldock et al. (2014) and Skjemstad et al. (2004). Briefly, 10 g of sieved (<2 mm), air-dried bulk soil was dispersed in 40 ml of a 5 g L^{-1} sodium hexametaphosphate solution by shaking on a flatbed orbital shaker overnight at 180 rpm. The dispersed soil and hexametaphosphate solution was poured on an automated sieving system (Analysette Pro, Fristch, Germany) equipped with a 50 µm sieve (Baldock et al. 2014). The shaker was set to apply DI water at a spray rate of 150 ml per minute, and to shake at an amplitude of 2.5 mm for no less than 3 minutes. Sieving was complete when the water exiting the machine ran clear. If this was not achieved within the allocated time, the process was repeated. Sieves were visually inspected to ensure that the fine particles had passed through and the >50 µm fraction (coarse fraction) and the <50 µm fraction (fine fraction) were separated and captured directly. The samples were then freeze dried and weighed. Coarse fraction samples were homogenised and ground for 60 s using a stainless-steel ball mill. Fine fractions were ground with a mortar and pestle by hand. Organic C (mg g^{-1}) and total N (mg g^{-1}) content of the two fractions was determined on a LECO CNS-2000 analyser using the same methods as for bulk soil. The allocation of soil mass to the coarse and fine fractions was expressed as a percentage of the total mass of soil that was fractionated.

2.5 IRS Analysis

Infrared spectra (IRS) were obtained from air dried and finely ground soil as described by Baldock et al. (2013). Approximately 100 mg of prepared bulk soil was placed into 9 mm stainless steel autosampler cups and levelled. IR spectra were obtained using a Nicolet 6700 FTIR Spectrometer (Thermo Fisher Scientific Inc., Waltham, MA USA) equipped with a KBr beam splitter, a DTGS detector and an AutoDiff automated diffuse reflectance accessory (Pike Technologies, Madison, WI, USA). For the set of 64 soil samples, the background signal intensity was acquired on a silica carbide disk by collecting 240 scans; two standard soils were included for determination of analytical precision. For each sample, 60 scans were collected over a spectral range of 8000-400 cm^{-1} with a resolution of 8 cm^{-1}. Spectral peaks in several regions have been positively correlated with soil organic carbon, such as those between 1500 - 2853 cm^{-1} (Hunt 1977) as well as those related to aromatic carbon structures at 1580, 1390 and 1220cm^{-1} (Baldock et al. 2018; Janik et al. 2007).

2.6 Statistical analysis and model selection

We tested site-level differences on the dependent variables total organic C (OC), total N (TN), coarse and fine fraction OC and TN and extractable N (ExN) within the herbicide control treatments using the “aov” function in base R (R Development Core Team, 2018, version 3.5.1) and performed Tukey HSD tests to obtain multiple comparisons. All assumptions of normality and homogeneity of variance were met, and we report means with standard errors. As the root biomass data displayed unequal variance, we used R to perform Kruskal-Wallis tests and performed posthoc comparisons using the “dunnTest” function from the “FSA” package with Bonferroni adjusted p-values. In this instance we report median values and quartiles.

Preliminary analyses revealed no interactions between site and cover crop treatments on soil parameters, so we evaluated treatment effects across sites. To test the effect of cover crop treatment on the dependent variables OC, TN, coarse and fine fraction OC and TN and

ExN we used linear mixed effects models (LMEMs) constructed using the “lmer” function from the “lme4” package (Bates et al. 2014) within R (R Development Core Team, 2018, version 3.5.1). We tested the null hypothesis that cover cropping treatments did not affect these variables and included ‘site’ as a random effect. Response variables were sqrt transformed to meet model assumptions and allow direct comparison of the response variables with PLSR predictions which are optimally obtained using sqrt transformation (Baldock et al. 2013b). Examination of residual plots were satisfactory, indicating appropriate model selection. We used the “glht” function from the “multcomp” package (Hothorn et al. 2017) within the R statistical package to perform multiple comparisons using the Tukey’s HSD method. Single step adjusted p-values ($\alpha = 0.05$) are presented and we report all summary statistics as means with standard errors. Unless otherwise noted, significant effects are considered at $p < 0.05$.

2.7 Chemometric Analysis of Spectral Data

Omnic software (Version 8.0, Thermo Scientific Inc.) was used to convert the reflectance spectra to absorbance spectra (**Fig 3**). All IR spectra were truncated to 6000 – 600 cm^{-1}, baseline corrected and mean centred prior to analyses and all PLSR analyses were performed using the Unscrambler 10.3 Software (CAMO Software AS, Oslo, Norway). PCA is applied as a component of the PLSR analysis to identify spectral components most important for sample differentiation, and to identify outliers. A square root transformation (sqrt) of all measured analytical variables (OC, TN, Coarse and Fine OC) was performed to improve linearity and homogeneity of residuals prior to model derivation. PLSR derives predicted values *via* detection of the main multivariate syndromes, in this case the spectral components, that maximise the variance explained in the response variable (Wold et al. 2001). Appropriate model selection was evaluated using the relationship between predicted PLSR values ($\hat{y}$) *vs* the measured (reference) (y_i) values, and we report these fits using the slope, R squared value (R^2), root mean square error (RMSE; equation 1) and the ratio of performance to variation (RPD; equation 2). The R^2 represents the total variance of the residuals in the PLSR model, whereas the RMSE defines the standard deviation of the residuals. The RPD value divides the standard deviation (s) of the measured values in the calibration, validation or cross validation sets by their corresponding RMSE values (Chang et al. 2001; Nocita et al. 2014). RPD values >2 have been used to characterise robust model prediction (Chang et al. 2001). All resultant PLSR models were optimally derived from 4 factors, and where spectral outliers were identified they were removed from model derivation. For OC_{sqrt} Coarse, we removed 3 spectral outliers from the calibration dataset after identifying standard residuals greater than 3 times the standard error of calibration (SEC) which we attributed to equipment failure. Models were linear, and homogeneity of residuals was confirmed.

$$\mathrm{RMSE} = \sqrt{\sum_{i=1}^{n} \frac{(\mathrm{yp}i - \hat{\mathrm{y}}\mathrm{p}i)^2}{\mathrm{n}}} \qquad (1)$$

where yp*i* is the observed (measured) value from the sample *i*, and ŷp*i* is the predicted value.

$$\mathrm{RPD} = \frac{\mathrm{SD}y}{\mathrm{RMSE}} \qquad (2)$$

where SD*y* is the standard deviation attributed to the measured reference values.

As one of the aims of this study was to use IRS/PLSR to detect differences in OC and TN contents between different cover crop treatments, where we detected significant differences in our measured variables using LMEMs, we further tested the ability of the IRS/PLSR predicted values to detect the same. To assess the potential for predicted values to detect treatment level differences, we compared the standard error of prediction (SEP) values from the PLSR models with the measured value differences between treatments. Differences in measured values between treatments that exceeded the model's SEP were more likely to detect treatment level differences. SEP values were obtained from the PLSR models using equation 3, which uses the measured data as a test set against the predicted values (Mevik & Cederkvist 2004).

$$\mathrm{SEP} = \frac{1}{n_M} \sum_{i=1}^{n_M} (f_M(x_i) - y_i) \qquad (3)$$

where the measured data is represented as $M \{(x_i, y_i)\}$, and f_M is the standard deviation of the measured data estimated by $\sqrt{V_M / n_M}$, V_M being the sample variance of *M.*

3. Results

3.1 Among-site comparisons of soil organic carbon and nitrogen concentrations

Total soil OC (mg g^{-1}) ranged from 7 to 17 mg g^{-1} and differed among sites at $\alpha = 0.10$; total OC was highest at Eden Valley and lowest at Waikerie, with the other sites intermediate (**Table S1**). TN ranged from 0.5 to 1.8 mg g^{-1} and followed a similar pattern as OC: TN at Waikerie was significantly lower than at Eden Valley (**Table S1**). Extractable N (ExN, µg g^{-1}) ranged from 2.4 to 7.3 µg g^{-1}, and was 204% greater at Eden Valley than Nuriootpa, with the other sites intermediate ($p \leq 0.01$).

Coarse fraction OC concentration (mg g^{-1}) ranged from 1.5 to 12 mg g^{-1} and was significantly higher at Langhorne Creek than at the Waikerie and Nuriootpa sites, with Eden Valley being intermediate (**Table S1**). Coarse fraction TN (mg g^{-1}) was significantly higher at the Langhorne Creek site than at Eden Valley and Nuriootpa (**Table S1**).

Fine fraction OC concentration ranged from 12 to 47 mg g^{-1}, and was highest at Eden Valley and Waikerie, intermediate at Nuriootpa, and lowest at Langhorne Creek; it was 280% higher at Eden Valley than at the Langhorne Creek site (**Table S1**). Fine fraction TN (mg g^{-1}) followed a similar pattern, with Eden Valley having the highest TN concentration, approximately 300% greater than Langhorne Creek (**Table S1**).

3.2 Fine root biomass comparison between cover-crop treatments

Fine root biomass did not differ significantly between sites ($p=0.09$) but was higher in treatments containing grasses compared to those without ($p \leq 0.001$). No fine root biomass was measured in the herbicide-treated controls; biomass was 2.2 kg m^2 ([0-6.2]) in legume treatments, 10.6 kg m^2 ([3.9-34.3]) in mixed treatments and 69.2 kg m^2 ([32.3-94.1]) in the grass treatments.

3.3 Cover crop effects on soil OC, TN and ExN contents

Preliminary analyses indicated no interaction between cover crop treatments and site effects, so here we examined treatment effects across all sites. Treatments containing grasses increased total OC (mg g^{-1}) across sites, being on average 14% higher in the grass and mixed treatments compared with the legume and control (**Table 2**). Mixed cover crop treatments increased TN by approximately 15% from the control, grass and legume (**Table 2**). ExN (µg g^{-1}) was positively affected by the presence of legumes (**Table 2**), and grasses and legumes grown together resulted in ExN on average 75% greater than in control and grass only treatments, and 17% more than in legume only treatment at $\alpha = 0.10$ ($p=0.09$).

Table 2: Means (+/- standard errors) of the dependent variables total, coarse and fine fraction soil OC and TN and ExN by treatment with results of linear mixed effects models examining the effects cover crop type on the dependent variables, across the four sites (n=16). Different lowercase letters represent significant differences between treatment groups (α= 0.05). Values of OC and TN were measured and reported as concentrations (mg g^{-1}) in bulk soil, and coarse and fine fractions.

Cover Crop	**OC (mg g^{-1})**	**TN (mg g^{-1})**	**Ex N (µg g^{-1})**	**OC (mg g^{-1}) Coarse**	**TN (mg g^{-1}) Coarse**	**OC (mg g^{-1}) Fine**	**TN (mg g^{-1}) Fine**
Grass Only	14.22 ± 1.22 b	1.24 ± 0.16 a	4.77 ± 0.43 a	7.42 ± 1.43 b	0.62 ± 0.06	35.50 ± 4.74 b	3.45 ± 0.38
Legume Only	13.62 ± 1.10 a	1.15 ± 0.10 a	6.82 ± 0.62 b	6.96 ± 1.15 b	0.67 ± 0.11	32.77 ± 4.23 a	3.76 ± 0.35
Mixture	14.64 ± 1.29 b	1.31 ± 0.15 b	8.80 ± 0.10 c	9.36 ± 1.86 b	0.68 ± 0.10	34.56 ± 4.55 b	3.37 ± 0.39
Control	11.41 ± 1.02 a	1.05 ± 0.14 a	4.93 ± 0.56 a	5.36 ± 1.01 a	0.55 ± 0.06	30.57 ± 3.91 a	2.67 ± 0.56
p-value	<0.01	0.01	≤ 0.01	≤ 0.01	0.51	0.02	0.11

Treatment effects on coarse fraction OC (mg g^{-1}) revealed an average of 45% more OC (mg g^{-1}) in grass, legume and mixed treatments compared with the control (**Table 2**). There were no treatment effects on coarse fraction TN (mg g^{-1}) (**Table 2**).

Fine fraction OC (mg g^{-1}) across sites was positively affected by treatments containing grass, which were on average 10% greater than the control and legume (**Table 2**). Fine fraction TN (mg g^{-1}) did not differ among treatments.

3.4 IRS-derived predictions for carbon and nitrogen pools

The obtained spectra defined by site are presented in **Fig 3**. As a component of the PLSR analysis, principle components analysis (PCA) was used to identify differences by site and treatment in the IR spectra. No outliers were removed from the PCA, as potential outlier removal did not improve the explained variance proportions nor alter the spectral loadings. PCA revealed separation among sites (**Fig 4a**), but not among treatments (data not shown); the first four components accounted for 98% of the variation in the spectra (**Fig 4a**). Loading spectra for the first 4 principle components revealed that positive signals around 3700, 3600, 2000, 1950, 1700, 1500, 1200, 1100, 900, 650 and 600 cm^{-1} contributed most to PC1 (**Fig 4b**). In our spectra (**Fig 3**), significant positive peaks in these regions occur at 2000, 1700, 1500 and 1200 cm^{-1}, however peaks at 2000, 1500 and between 3700-3600 are possibly overlapped by mineral signals as these peaks have previously been attributed to the presence of quartz and clay (Hunt 1977; Janik & Skjemstad 1995). Similar positive peaks around 3700-3600 soil spectra have previously been attributed to clay minerals (Janik & Skjemstad 1995). Peaks around 2800, 2500 and 1800 have previously been attributed to the presence of carbonates (Hunt 1977). Although we detected a minimal amount of IC in the Eden Valley samples (data not shown), the spectral signature related to carbonates was not significant. Mineralogy contributed most to the spectral variations across sites, with some important contributions from organic components.

Because many of the spectral peaks overlap in areas that define both mineral and organic characteristics, IRS/PLSR was expected to be less sensitive in its ability to predict treatment level differences than direct combustion analyses. Therefore, in order to measure the ability of IRS/PLSR to quantify treatment level differences, we only tested the PLSR predicted values by treatment where compositional differences were evident from combustion analyses.

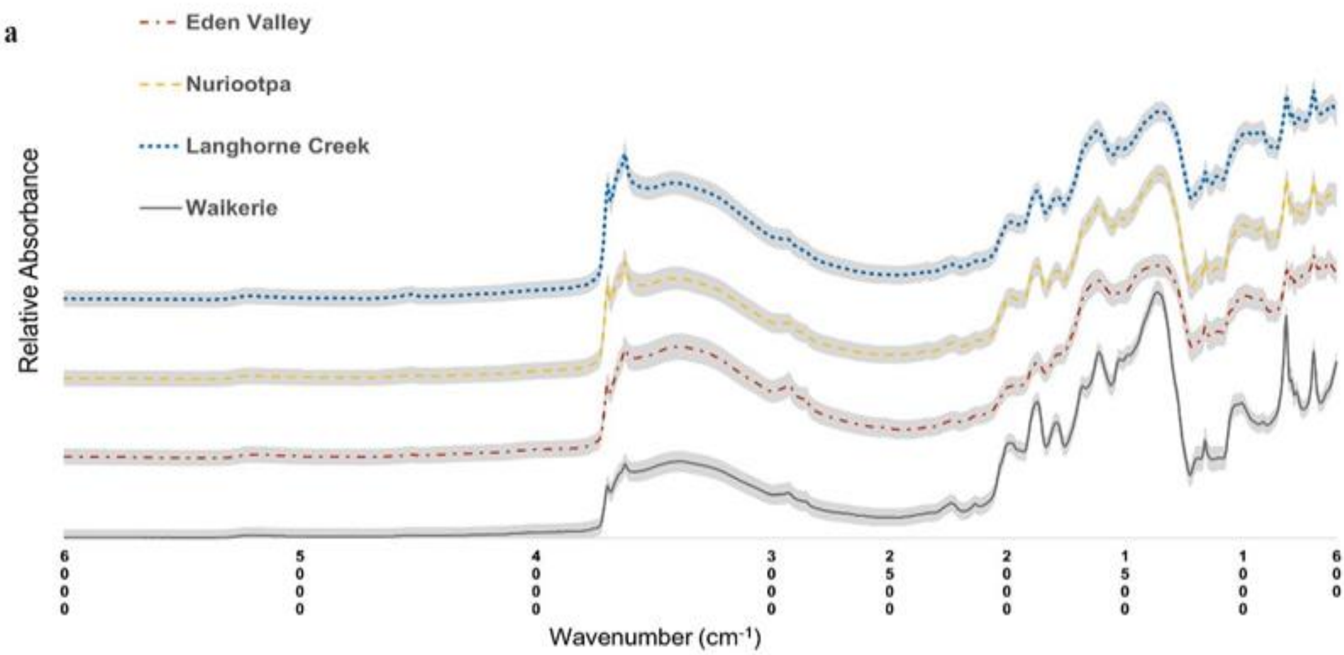

Figure 3: Mean (+95% CI) absorbance spectra by site (baseline corrected 6000-600cm^{-1}) obtained in the control treatments. Values are stacked (+0.5) by site for ease of interpretation. Grey shading indicates the within site 95% confidence interval (n = 4).

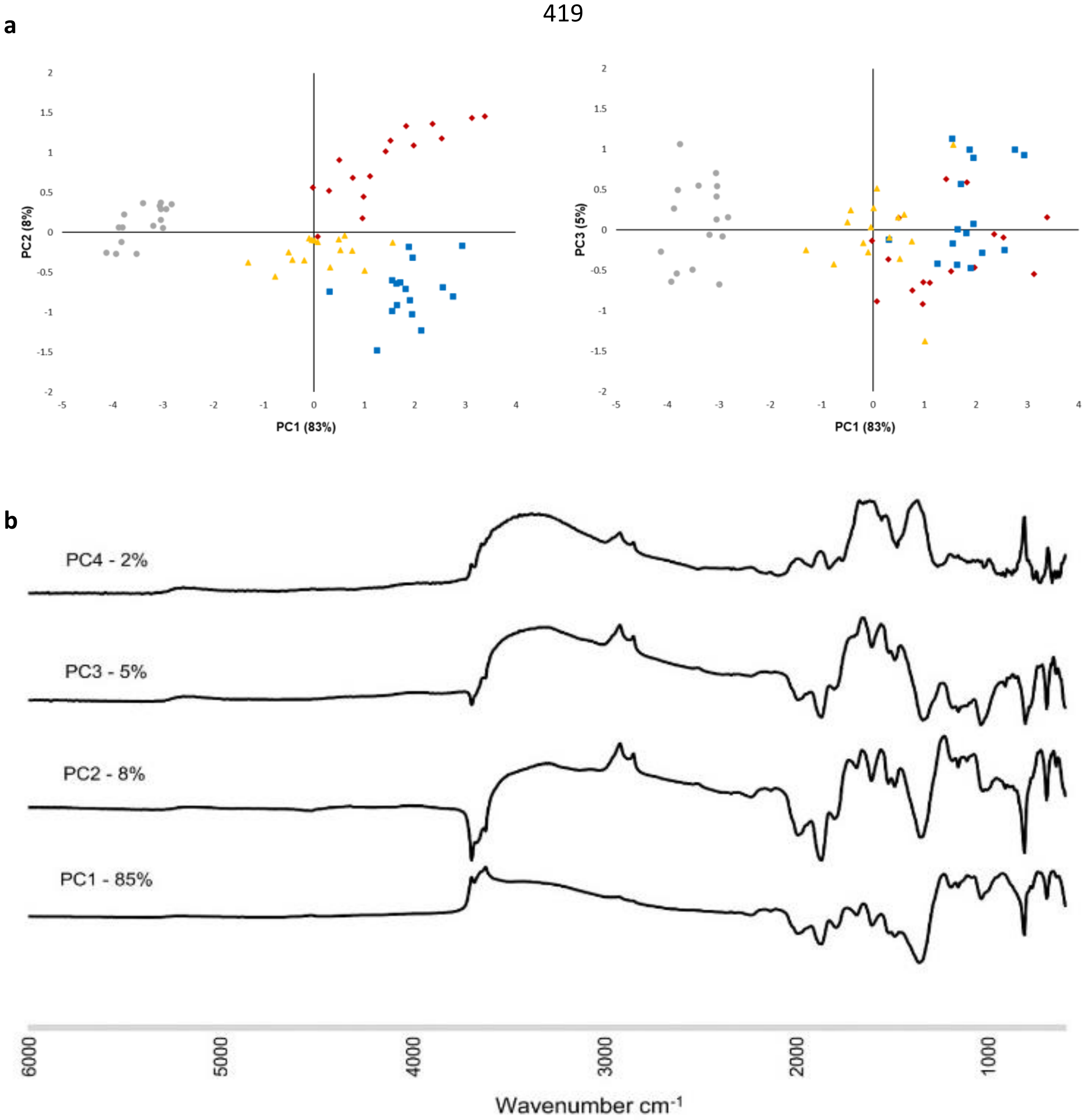

Figure 4 a) PCA plots for the first 3 principle components demonstrating separation between sites. Diamonds = Eden Valley, triangles = Nuriootpa, squares = Langhorne Creek, circles = Waikerie **b)** PCA loadings spectra (cm^{-1}) for each significantly contributing principle component. Individual y axes demonstrate the relative percentage variation explained by each individual principle component.

3.5 Using PLSR to predict OC and TN pools from IRS data

Summary statistics from PLSR models calibrated using spectral data and tested using measured values are presented in **Table 3.** Our derived models, using data from all sites to predict treatment effects on OC_{sqrt}, OC_{sqrt} coarse and OC_{sqrt} fine, predicted a significant amount of variation in the measured variables (**Table 3**). Our derived models predicting TN (mg g^{-1}) in the soil fractions were excluded from the results as the IRS-predicted values explained only 57% (RPD = 1.00) and 67% (RPD = 0.99) of the variation in the coarse and fine fractions, respectively, and were therefore not considered to be reliable (Chang et al. 2001). Additionally, our TN_{sqrt} predicted values were highly correlated with those obtained for OC_{sqrt}, which is not uncommon (McCarty et al. 2002; Reeves & McCarty 2000). Simple linear regression revealed that the PLSR-derived beta coefficients for OC_{sqrt} and TN_{sqrt} were highly correlated ($r^2 = 0.90$, $p<0.0001$) which suggests that OC_{sqrt} and TN_{sqrt} are being predicted with a very similar PLSR algorithm (**Fig 5**). We therefore focus the discussion on the derived PLSR models to predict OC contents, and exclude the IR/PLSR results for TN.

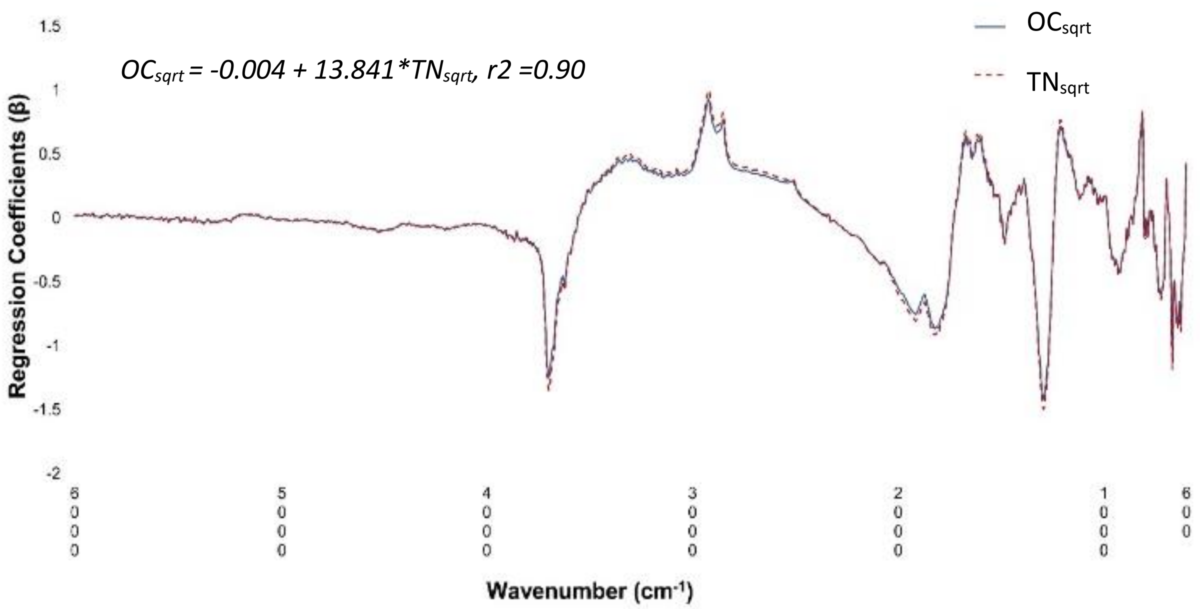

Figure 5: PLSR derived β coefficients for OC_{sqrt} and TN_{sqrt}, as obtained from an optimal number of (4) model factors. Spectral peaks that most influence the models overlap significantly, and simple linear regression between the β coefficients further demonstrates high correlation between the two variables OC_{sqrt} and TN_{sqrt}

3.6 Using IRS/PLSR derived OC predictions to quantify treatment effects among cover cropping treatments

The potential capacity of our IR/PLSR predicted values to detect treatment level differences was assessed by comparing the range of measured OC_{sqrt} values for each model with the model's standard error of prediction (SEP). This information provides the measured OC value increase between treatments that would be required to be detected by the model (**Table S2**). Then, using the same LMEM structure previously described, we tested differences among treatments using model predicted values. On average across sites, the OC_{sqrt} predicted values in mixed treatments (3.91 (mg $g^{-1})^{0.5}$ [3.55-4.27]) were on average 6.5% greater than the control, legume and grass treatments (3.67 (mg $g^{-1})^{0.5}$ [3.46-3.88]), (p=0.04). Predicted OC_{sqrt} coarse values were also significantly different among treatments (p=0.02), where the mixed treatment (2.79 (mg $g^{-1})^{0.5}$ [2.50-3.08] was predicted to be 13% greater than the control, legume and grass treatments (2.46 (mg $g^{-1})^{0.5}$ [2.20-2.72], despite a high model SEP (**Table S2**). Treatment effects on fine fraction OC were not detected. As expected, predicted values were less sensitive at detecting differences than combustion measurements.

Table 3: Summary statistics calculated according to equations 1 and 2 for the derived partial least square regression models for OC_{sqrt}, OC_{sqrt} coarse and OC_{sqrt} fine. Cal = calibration, Val= validation. RMSE = Residual mean square error, RPD = Ratio of performance to deviation.

Variable		Factors	n	Slope	Offset	r	R^2	RMSE	Bias	SE	s	RPD
OC (mg $g^{-1})^{0.5}$	Cal	4	64	0.946	0.200	0.972	0.946	0.176	0.000	0.177	0.739	4.175
	Val		64	0.930	0.250	0.964	0.931	0.201	0.000	0.203	0.760	3.744
OC Coarse (mg $g^{-1})^{0.5}$	Cal	4	61	0.869	0.331	0.932	0.869	0.352	0.000	0.355	0.907	2.555
	Val		61	0.846	0.387	0.910	0.828	0.404	-0.004	0.408	0.975	2.390
OC Fine (mg $g^{-1})^{0.5}$	Cal	4	64	0.935	0.357	0.967	0.935	0.397	0.000	0.400	1.519	3.798
	Val		64	0.925	0.406	0.955	0.915	0.462	-0.006	0.466	1.568	3.365

4. Discussion

4.1 Grass and legume cover crops both contribute positively to soil OC

After accounting for the variability in OC concentration across the sites, grasses consistently increased OC in the total pool, coarse and fine fractions. We attribute the increases in OC contents to greater root biomass in treatments containing grass, with fine root biomass being, on average, 22% higher than in legume-only treatments. Indeed, in cropping systems it has been shown that, on average, 35% more root biomass-derived C is retained in the soils compared with shoot-derived C in a single growing season (Puget & Drinkwater 2001) and root-C has been demonstrated to be a significant contributor to long term soil OC storage (Fisher et al. 1994; Molina et al. 2001; Rasse et al. 2005), contributing an average of 2.4 times the amount of OC compared with senesced shoots (Rasse et al. 2005).

A higher potential for the retention of C in fine soil fractions because of mineral adsorption (Solomon et al. 2012), coupled with higher grass root biomass may explain the observed increase in fine fraction C under grass treatments. Higher root biomass is also likely to have a greater effect on C retention and aggregate stability in clay soils than in sandy soils due to particle binding occurring between high surface area minerals (Six et al. 1998; Six et al. 2006; Tisdall & Oades 1982). In a study comparing the effects of grasses and legumes on soil aggregate structures, grasses were found to positively influence stability, compared to legumes which decreased it (Pérès et al. 2013). However, it has been suggested that in low nutrient, sandier soils more prone to C and N losses (Lobe et al. 2001), legumes may have greater potential to build root biomass and contribute to aggregate stability compared to grasses, as they are more resilient under less favourable conditions (Garcia et al. 2018). Although we did not measure the direct impacts of these crop species on soil matrix structures, we highlight their positive benefits to the system *via* their role in the provision or retention of nutrients and carbon that may lead to increases in overall plant biomass and the subsequent building of SOM.

As changes to bulk SOC resulting from differential management are not easily detected in the short-term, measuring changes in the more sensitive coarse organic matter fraction is becoming increasingly popular and was useful to confirm treatment level effects in our study (Cambardella & Elliott 1994; Ojeda et al. 2018). The positive effects of grass and legume cover crops on OC concentration were, as expected, more strongly observed in the coarse fraction (Ojeda et al. 2018) where legumes were also found to increase OC. It is well understood that N transfer from legumes to grasses can increase growth of the whole plant, including root biomass, root density and rooting depth (Heichel & Henjum 1991; Peoples et al. 2009; Peoples et al. 2015; Ramirez-Garcia et al. 2014) which can, in turn, increase soil carbon accumulation (Fornara & Tilman 2008). Compared with grasses, legumes are also considered to provide a more readily decomposable source of C from root structures owing to lower root C:N ratios and higher root N contents (Fornara et al. 2009). Therefore, we can explain the measured positive impacts on coarse fraction OC resulting from grasses by their dense root biomass (Fisher et al. 1994), from legumes owing to their increased root decomposability (Amato et al. 1984; Fornara et al. 2009), and from mixtures because of potential facilitation and complementarity (Duchene et al. 2017). Despite differences in site management, rainfall, fertilisation and irrigation, the incorporation of legumes into cropping systems has been shown to positively affect the mean residence time of C in soil owing to the deposition of more resistant, aromatic forms of OC (Drinkwater et al. 1998; Gregorich et al. 2001). Moreover, the presence of legumes has been shown to slow the decomposition of grass roots *via* a reduction in microbial priming (Saar et al. 2016), potentially enhancing OC content in mixed swards.

4.2 Grass + legume mixtures increase soil nitrogen to a greater extent than legumes grown alone

Unfortunately, the majority of vineyard-based cover cropping studies have focussed on the potential for resource competition, and were performed in pure grass stands negating exploration of the possible effects of legume-grass complementarity and increased nutrient retention by grass roots (Beslic et al. 2015; Celette et al. 2009; Ripoche et al. 2011). Across our sites, treatments containing legumes had higher concentrations of soil extractable N, likely owing to the presence of N-fixing symbionts which are known to increase available N (Peoples et al. 2009). Unexpectedly, however, the increases in soil available and total N were greatest in our mixed treatments. In many cover cropping systems, N retention has been shown to increase in grass-legume mixtures compared with monocultures (Finney et al. 2016). Therefore, although grasses are rarely seen to be beneficial in vineyard cropping systems, N retention by root structures is a currently undervalued benefit that could be obtained *via* the incorporation of mixed- compared with legume-only cover crops in vineyard systems. Cover crop effects on N retention were not, however, directly tested in our study. In addition to N retention, N fixation in legumes has been shown to be up-regulated by the presence of grasses in mixed stands compared with legumes grown alone

(Nyfeler et al. 2011). Further, despite the perception that grass cover crops may negatively influence N availability to vines (King & Berry 2005a), other studies have found that deeper-rooted, mature grapevines are fairly robust to competition with grasses for both N and water (Klodd et al. 2016). Earlier data from these field sites showed that fruit yield was not affected by the legume-only or mixed sward, and yeast-available N in fruit was higher in treatments containing legumes (Penfold 2018). Therefore, the combined benefits of increased N retention and symbiotic N fixation in grass and legume mixtures demonstrate that mixed cultivations have the potential to contribute significantly to building more resource-efficient viticultural systems.

4.3 IRS/PLSR accurately predicts soil OC pools in vineyard agroecosystems, detecting treatment level differences

Total OC and TN are two of the parameters most accurately predicted using IRS and visible near infrared spectroscopy (Brunet et al. 2007; St. Luce et al. 2014). Both the spectrally derived OC and TN models accurately predicted the measured OC and TN contents ($p<0.001$), however due to correlation between the model β coefficients (Fig. 5), we excluded the PLSR predictions for TN from our analysis. In a few previous studies, IRS determination of N content in soils has been shown to be closely correlated with predictions of C content (Malley et al. 2000; Morra et al. 1991; Reeves & McCarty 2000). Thus, we focus our discussion on the reliability of IRS to predict OC contents and highlight the need for further calibrations of IRS models for soil N contents along a gradient of N availability.

This study confirms the accuracy of IRS/PLSR for predicting OC concentration in edaphically distinct vineyard agroecosystems, as well as the reliability of using IRS/PLSR to detect the effects of differential management (i.e., cover crop types). Between our two most contrasting treatments (control *vs* mixture), treatment-level differences in total OC were accurately predicted using the IR/PLSR derived estimates. Similarly, for the coarse OC fraction, we were able to use the IRS/PLSR predicted values to detect a treatment difference, again only in the mixed treatment. The conservativeness of the predicted values is likely an artefact of the nature of PLSR model derivation, which predicts the response (y) variables based on the independent variables (x) by explaining as much of the covariance as possible between x and y (Zhao et al. 2014). In a dataset where measured variables display a naturally large amount of variation, PLSR 'smooths' the within-treatment variation in the derivation of the y predictions to a greater extent than analysis of variance does using transformed, measured values. Nevertheless, if SEP values are larger, and differences between organic C contents are smaller, a high level of replication will be required to reduce the signal-to-noise ratio and improve predictive capacity (Forouzangohar et al. 2015). In an agricultural study reporting coarse fraction OC contents similar to ours, changes to OC after differential management were also successfully detected with a similar SEP (1.1 mg $g^{-1})^{0.5}$, but over a longer timeframe (9 years) (Baldock et al. 2018). Prediction errors for other OC spectral models of 2.70 mg g^{-1} (McCarty et al. 2002) and 6.70 mg g^{-1} (Grinand et al. 2012) were larger than we observed, but the former models were calibrated across a larger variation in OC contents which likely allowed for strong predictive capacity despite large SEPs. It is important to note that the PLSR-derived estimates of increases in total and coarse fraction OC were ~40% more conservative than those obtained from combustion analyses. By successfully comparing measured pools with spectrally-derived estimates of OC, we have demonstrated the capacity of the calibration dataset to predict carbon pools among different cover cropping treatments and highlight its potential to be used in other vineyard agroecosystems for the same purpose.

4.4 Improving the predictive capacity of IRS/PLSR for application across varied soil types

Organic matter is a complex mixture of chemically diverse, mostly infrared-active compounds which are difficult to differentiate with clearly separated spectral peaks (Janik & Skjemstad 1995). Mineral composition is considered to control predictions of C and N contents in fractions using spectra obtained from whole soil and, in our derived spectra, peaks associated with changes to C contents were strongly associated with mineral peaks. A high degree of correlation between silt+clay fraction C and total OC has been found elsewhere (Brunet et al. 2007), and therefore, it may be argued that using IRS to detect changes in OC content is more to do with the 'relatedness' of OC to other mineral components than to a direct measurement of OC content itself. Nevertheless, as we continue to recognise the roles of different components of the soil matrix in the building and maintenance of OC (Allison 2012; Solomon et al. 2012) we suggest that soil spectral analysis will become an increasingly useful tool to predict changes to OC pools. There is potential to separate spectral diversity relating to mineralogy from diversity in organic compounds *via* larger sample sets spanning a greater range of organic C contents collected in texturally similar soils (Brunet et al. 2007). In contrast, it is possible that spectral diversity, such as occurs in the presence of compounds that correlate positively (clay minerals) and negatively (quartz minerals) with organic C contents, may be reduced in more homogenous soil samples and diminish the predictive capability of IRS/PLSR (Van Groenigen et al. 2003; Wight et al. 2016). These limitations emphasise the need for repeated calibrations across a range of soil types, as in the current study, to improve the accuracy of IR predictions for wider geospatial applicability. In recommending the IRS/PLSR technique for similar applications, we would advise caution using existing calibration models in uncalibrated systems; specific calibration of the IRS/PLSR technique in various soil types to produce robust models is of vital importance to the development of the method. A comprehensive library of spectral indicators that can reliably detect changes in organic matter composition across variable soil types would also help to predict the outcomes of differential management in diverse systems.

4.5 Conclusions

Traditional ground cover management practices in vineyards will require significant re-thinking and improvement to prevent significant soil degradation (Daane et al. 2018). However, expanding industry engagement in vineyard management practices that improve soil health by increasing soil OC and N relies on both proving the efficacy of practices such as cover cropping for this purpose (García-Díaz et al. 2018), and making the results of differential management easily quantifiable and accessible (Askari et al. 2015). This study contributed to achieving both outcomes by demonstrating the positive influence of cover crops on important soil properties, and by demonstrating the capacity of soil spectroscopy to detect management-related changes across varied vineyard soil types. Whilst it is well understood that grass cover crops can increase soil OC, and legumes soil N, it is not common to discover that legumes can also improve soil OC, or that grasses play a role when grown alongside legumes in increasing soil N to a greater extent than legumes grown alone. Despite the potential benefits of cover cropping, there is industry resistance to incorporating grasses in the under-vine region because of perceived cover crop-vine nitrogen competition, which has been indicated in previous studies, but not assessed in mixed grass+legume cultivations (Celette et al. 2009; King & Berry 2005b; Vystavna et al. 2020). Not only were yields and fruit quality unaffected by the cover cropping treatments in our study, we demonstrated that the combination of grass and legume cover crops in the under-vine region represents a more valuable contribution towards the building and maintenance of OC and N in the rooting zone, where vines can access it, than legume cover crops alone.

The ongoing development of soil spectroscopy for the purpose of monitoring soil health is likely to contribute significantly to improving the sustainable management of vineyard agroecosystems internationally (Dunne et al. 2020; Sanderman et al. 2020; Sepahvand et al. 2019). With relatively low-effort sample collection and processing, our acquired IRS/PLSR analyses accurately predicted total and coarse OC (mg g^{-1}) across all sites confirming the usefulness of IRS/PLSR to predict OC pools from easily obtained bulk soil analyses, in different soil types. Additionally, and most importantly, we successfully used IRS/PLSR to predict differences in OC pools related to differential ground cover management in vineyard agroecosystems; this represents an important contribution to validating new approaches for the rapid quantification of short-term impacts of differential management strategies for the viticultural industry.

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