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Permanent swards increase soil microbial counts in two Australian vineyards

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ARTICLE INFO

Article history:
Received 14 July 2006
Received in revised form
16 February 2007
Accepted 6 March 2007

Keywords:
Grapevine
Organic carbon
Hot water extractable C
Cellulolytic bacteria

ABSTRACT

This study examined the effect of permanent swards and bare soil (by repeated herbicide treatment) on soil microbial counts in Australian cool and warm climate vineyards. In both climates, hot water extractable soil C (HWC) in the vine row was increased by 73% after 3 years of sward development. Most of the soil bacterial counts were greatly decreased by the herbicide treatment and principal components analysis was able to clearly discriminate between microbial populations from the grassed and bare soil. In both the under-vine and inter-row soil, HWC was positively correlated with fungal counts and with cellulolytic, pseudomonad, copiotrophic and oligotrophic bacterial counts. HWC was also negatively correlated with soil bulk density. The grapevine rhizosphere bacterial population was dominated by cellulolytic bacteria in both climates. Many cellulolytic bacteria were slow growing, requiring up to 84 days of laboratory incubation. This study shows that permanent swards can significantly enhance the microbial community in vineyard soil.

1. Introduction

Conventional vineyard floor management systems in Australia maintain bare weed-free soil under the vine and, in some districts, between the vine rows. However, as organic matter from crop residues is minimal in viticulture, bare soil may become depleted of organic matter. Continuous plant cover from a permanent sward can be an important source of soil organic matter. Living plant roots produce exudate containing soluble organic compounds such as sugars, amino acids and organic acids. These relatively labile substances can contribute from 0.1 to 2.8 t C ha^{-1} to the soil microbial community (Rees et al., 2005). Mown sward plant residues are also gradually degraded by cellulolytic soil microorganisms, providing easily mineralised organic matter.

The effect of continuous plant cover on soil organic C and microbial populations was the focus of this study. As pure cultures were needed for future experimentation, the traditional dilution plate counting technique was used to estimate the size of the microbial populations. Although there have been many reports of discrepancies between the number of bacterial colonies formed on solid agar and the total number of bacterial cells present in the soil, the recent literature has shown that these discrepancies are made much lower when long incubations and dilute nutrient agar are used (Janssen et al., 2002; Joseph et al., 2003).

This paper reports the effects of continuous plant cover on soil microorganisms in two Australian vineyards. Soil fungi plus cellulolytic bacteria, copiotrophic pseudomonads, copiotrophic bacteria and oligotrophic bacteria were monitored for 3 years and data from the third year of the trial are reported here.

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0929-1393/$ – see front matter
doi:10.1016/j.apsoil.2007.03.003

2. Materials and methods

2.1. Vineyard field trial

Three floor management treatments, representing some of the main floor management techniques currently being utilised in Australian viticulture, were applied at two New South Wales vineyard sites. One vineyard was located at Wagga Wagga (warm climate) and another at Tumbarumba (cool climate). Both vineyards were drip-irrigated in the vine row but not in the inter-row. Prior to the commencement of the trial the vineyards had been maintained for 5 years with bare weed-free soil under-vine and volunteer grasses in the inter-rows. The three viticultural treatments for the trial were: completely bare vineyard floor with regular post-emergent herbicide application both under-vine and between the vine rows (i.e. inter-rows); complete site adapted permanent sward with no herbicides applied; post-emergent herbicide applied under-vine only. However, for soil microbiological testing purposes the treatments were simplified to: under-vine bare soil; under-vine grassed (sward) soil; inter-row bare soil; and inter-row grassed (sward) soil. Four replicate experimental plots, randomly selected from 12 field trial replicate plots within a randomised block design, were sampled for soil microbiological testing purposes. The inter-row permanent swards were slashed regularly and the under-vine permanent sward was kept short with a 'whipper-snipper'.

Wagga Wagga is a region with a warm and semi-arid dry climate (mean January temperature = 24.0 °C, 2050 degree-days >10 °C, 522 mm annual rainfall). The annual rainfall throughout the trial period (376 mm in 2002; 439 mm in 2003 and 391 mm in 2004) was below the annual average. Soil moisture in the non-herbicided inter-row (0–10 cm, gravimetric method) was low throughout the trial period (13.1% in February 2002; 3.6% in December 2002; 12.0% in June 2003; 6.4% in November 2003 and 10.9% in July 2004; w/w). An inter-row cover crop of cocksfoot (*Dactylis glomerata*) and subterranean clover (*Trifolium subterraneum*) mix was sown in June 2002 but severe rainfall deficiencies during autumn, winter and spring resulted in poor establishment, so it was re-sown in spring. The permanent sward plots also contained a diversity of plant species regenerating from the seed bank resulting in grasses such as *Cynodon*, *Stellaria*, *Erogrostis*, *Digataria*, *Panicum* and other herbaceous species being present as well.

Soil at the Wagga Wagga site was shallow (approximately 30 cm topsoil, which was quite variable across the vineyard), and consisted of coarse sandy loam overlaying hard-to-penetrate subsoil of granite saprolite. In 2002, before the treatments were applied, average inter-row soil pH was 7.0 (1:5, 0.01 M of $CaCl_2$); $NaHCO_3$-extractable phosphate (available P) was 36 mg kg^{-1} (Colwell, 1963) and total soil organic C (OC) (0–10 cm soil depth, dichromate oxidation method) was 8.1 g kg^{-1} in the under-vine and 9.2 g kg^{-1} in the inter-row soil. The vine rows were 3 m apart and each panel contained 3 grapevines (*Vitis vinifera* cv. Chardonnay) spaced 2 m apart. Each experimental plot was three panels long and three mid-rows wide with a buffer row between each plot.

The cool climate site, located in Tumbarumba, has an annual rainfall of 968 mm although rainfall was below average throughout the trial period (752 mm in 2002; 872 mm in 2003 and 811 mm in 2004). Frost is often experienced in the early part of the growing season. Excessive vine vigour can be a problem and canopy management relies on split canopy systems. Soil moisture in the non-herbicided inter-row (19.9% in February 2002; 6.0% in December 2002; 13.7% in June 2003; 15.7% in November 2003 and 18.6% in July 2004; w/w) was significantly higher at Tumbarumba than at Wagga Wagga. A subterranean clover pasture dominated (23–25% of plant species).

The Tumbarumba vineyard had a shallow A horizon of silty clay loam over a deeper (up to 90 cm) B horizon of light clay. Inter-row average soil pH (1:5, 0.01 M of $CaCl_2$) was 5.9; $NaHCO_3$-extractable P was 20 mg/kg (Colwell, 1963) and oxidisable organic carbon (dichromate oxidation method) was 17 g kg^{-1}. The vine rows were 3 m apart and each panel contained eight grapevines (*V. vinifera* cv. Chardonnay) spaced 1 m apart. The experimental plots were two panels long and four mid-rows wide with a buffer row between each plot.

2.1.1. Herbicide applications

To maintain the bare soil treatments at the Wagga Wagga trial site, a diquat and paraquat mixture (Spray.Seed 250®, Syngenta Crop Protection) was applied at the recommended rate, equivalent to 375 g ha^{-1} paraquat and 320 g ha^{-1} diquat, to the appropriate plots in April, September, October and November of 2002 and in June, November and December of 2003. A mixture of carfentrazone-ethyl (Hammer®, Crop Care Australasia) and glyphosate (Roundup®, Monsanto) was applied at the recommended rate, equivalent to 6 g ha^{-1} carfentrazone-ethyl and 666 g ha^{-1} glyphosate, in October of 2003. At the Tumbarumba trial site, glyphosate was applied at the recommended rate, to the appropriate treatment plots in July 2002. A diquat and paraquat mixture at the recommended rate was applied in October and November of 2002; in January, June, November and December of 2003; and in January of 2004. A mixture of carfentrazone-ethyl and glyphosate herbicide was applied at the recommended rate in September and October of 2003.

2.2. Hot water extractable C (HWC)

Hot water extractable C (HWC), a biodegradable hydrophilic fraction of the total soil soluble organic C pool, can be used as a sensitive indicator of changes in soil organic matter and soil quality (Haynes and Francis, 1993; Ghani et al., 2003; Haynes, 2005). HWC reportedly consists of carbohydrates from root exudates or extracellular polysaccharides from microorganisms and is highly available to soil microorganisms (Fischer, 1993).

In 2004 only, hot water extractable C (HWC) was analysed by a modified method of Ghani et al. (2003) (from method of Haynes and Francis, 1993). Five shallow soil cores (0–2 cm depth) from each plot were bulked and sieved (10 mm). Representative 4.0 g (field moist weight) soil samples were ground by mortar and pestle until fine enough to pass a 0.5 mm screen, inverted three times and vortex mixed for 10 s in 30 ml deionised water, capped and incubated for 16 h in a 80 °C water bath. The tubes were then shaken for 10 s, centrifuged for 20 min at 3500 rpm and the supernatant was filtered through a moist washed 25 mm 0.45 μm Nucleopore polycarbonate membrane filter. A modified method of Burford

and Bremner (1975) and Heanes (1984) was used for total C content of the soil extract. The soil extracts (5 ml) were placed in 50 ml pyrex tubes, 5 ml 1N $K_2Cr_2O_7$, concentrated H_2SO_4 (5 ml) was gradually added and the tubes were capped loosely and agitated for 30 s before placing in boiling water bath for 30 min. Absorbance at 600 nm was determined after dilution with 35 ml deionised water.

2.3. Isolation of microorganisms from soil

Composite samples from six soil cores (depth 10 cm, diameter 60 mm, collected in May 2002, 2003 and 2004) were obtained from the vine row and inter-row (50 cm from the grapevine trunk) of 12 replicate plots. Gravimetric soil moisture (oven dried at 105 °C for 24 h) was determined for each composite soil sample. The soil was sieved (0.5 cm) and 10 g (moist weight) representative samples vortex mixed with 90 ml phosphate-buffered saline (pH 7.2) (PBS) for 10 s, sonicated at 260 W cm^{-2} for 15 s and orbitally shaken at 290 rpm for 30 min on ice. The grapevine roots retrieved from each soil sample were washed under tap water, blotted dry and 1 g (fw) was vortex mixed with 9 ml PBS for 10 s, sonicated at 260 W cm^{-2} for 15 s and shaken at 290 rpm for 30 min on ice. Aliquots (0.1 ml) were spread onto solid media and incubated in darkness at 25 °C.

2.4. Microbial counts

Copiotrophic pseudomonads (PS) were selectively cultured on Pseudomonas agar CCF (Oxoid). Cellulolytic bacteria were cultured on cellulose bacterial agar (CBA) (Tuitert et al., 1998) containing carboxy methyl cellulose as the sole source of C plus the antibiotic cycloheximide. Colonies were counted after 4 days (fast growing cellulolytic bacteria, FCB). In 2004 only, colonies were also counted after 84 days (total cellulolytic bacteria, TCB). The slow growing cellulolytic bacterial count (SCB) was calculated from TCB–FCB. Nutrient Benomyl Agar (NBA) (Oxoid Nutrient agar 30 μg ml^{-1} benomyl fungicide) was used to isolate copiotrophic bacteria (CB) which are able to grow quickly (4 days) on a high C medium containing high salt concentrations. Dilute Nutrient Benomyl Agar (DNBA) (100-fold diluted Oxoid Nutrient Agar with 30 μg ml^{-1} benomyl) was used to isolate bacteria able to grow on a low C medium (Hattori and Hattori, 2000). Colonies were counted after 4 days (fast growing low nutrient bacteria, FLNB) and 84 days (total low nutrient bacteria, TLNB). As some copiotrophic bacteria grow on DNBA (Hattori and Hattori, 2000) those that grew on DNBA but not on NBA were considered to be oligotrophic bacteria (OB) (Mitsui et al., 1997). The OB count was calculated from TLNB-CB.

DRBC fungi were isolated on dichloran rose Bengal chloramphenicol (DRBC) agar. Cellulolytic fungi were isolated on cellulose Czapek chloramphenicol agar (CCC) which was modified from cellulose Czapek agar (Omar and Abdel-Sater, 2000) with chloramphenicol (100 μg ml^{-1}) instead of ampicillin.

2.5. Bulk density, pH and OC

Soil bulk density was determined for each replicate plot using the core method (Graecen et al., 1981). Soil chemical analyses for total organic C (OC) (dichromate oxidation and titration), pH ($CaCl_2$) and pH (water) were performed by Pivot Limited, Werribee, Victoria.

2.6. Statistical analysis

Data were subjected to analysis of variance (ANOVA) test (one way) using Genstat for Windows, 8th Edition. Least significant differences (l.s.d.), linear regression correlations, and principal component analysis (PCA) were performed by Genstat for Windows, 8th Edition.

3. Results

3.1. Soil microbial populations

Three years after the commencement of the trial the presence of a permanent sward had increased the counts of all soil bacterial groups. At Wagga Wagga, the counts for PS, SCB and FCB were 125%, 64% and 50% greater, respectively, in the grassed inter-row soil; and those for PS, FCB, CB, SCB, FLNB and OB were 267%, 265%, 181%, 106%, 86% and 83% higher in the grassed under-vine soil (Table 1). At Tumbarumba the inter-row soil PS, CB, FCB and FLNB counts were increased by 258%, 102%, 86% and 86%, respectively. The Tumbarumba under-vine oligotrophic bacteria (OB) and FCB counts were also increased by 414% and 87%, respectively (Table 2). Principal components analysis (PCA) of the major microbial groups (cellulolytic fungi, DRBC fungi, PS, TCB, CB and OB) was able to discriminate between bare and grassed under-vine soil at Tumbarumba ('sward' points clustered together at right hand side of biplot) and at Wagga Wagga ('bare' points clustered together at right hand side of biplot) (Fig. 1).

Two grapevine rhizosphere microbial groups were positively correlated with their own populations in the under-vine bulk soil: DRBC fungi at Wagga Wagga and SCB at Tumbarumba ($P < 0.05$, Table 3). All microbial counts were greater in the grapevine rhizosphere than in the under-vine bulk soil. At Wagga Wagga, the populations of PS, SCB, TCB, FLNB, FCB, OB, TLNB, CB and cellulolytic fungi were 668%, 633%, 434%, 399%, 293%, 188%, 181%, 167%, 160% and 160%, respectively, higher in the rhizosphere than in the under-vine bulk soil. At Tumbarumba, the populations of OB, PS, TLNB, FLNB, SCB, TCB, FCB and CB were 1181%, 833%, 712%, 672%, 609%, 504%, 490% and 405%, respectively, higher in the rhizosphere than in the under-vine bulk soil ($P < 0.001$, data not shown).

3.2. Organic C

After 3 years, under-vine HWC at Wagga Wagga was significantly higher in the sward (2.6 mg cm^{-3}) than in bare soil (1.5 mg cm^{-3}). HWC was also higher in the sward (3.8 mg cm^{-3}) than in the bare soil (2.2 mg cm^{-3}) in the Tumbarumba under-vine soil. In the inter-row soil, HWC was higher in the sward (8.1 mg cm^{-3}) than in the bare soil (1.8 mg cm^{-3}) at Tumbarumba but there was no significant difference between the HWC in the sward (2.0 mg cm^{-3}) and bare soil (1.6 mg cm^{-3}) at the much drier site at Wagga Wagga ($P < 0.05$, data not shown).

HWC was positively correlated with the cellulolytic bacteria (FCB and TCB) counts in the under-vine and inter-

Table 1 – Bacteria and fungi isolated from bare or grassed (sward) soil (0–10 cm depth) from under-vine and inter-row positions at Wagga Wagga

	FCB (10^5 cfu cm^{-3} ds)	SCB (10^5 cfu cm^{-3} ds)	CB (10^5 cfu cm^{-3} ds)	FLNB (10^5 cfu cm^{-3} ds)	OB (10^5 cfu cm^{-3} ds)	PS (10^5 cfu cm^{-3} ds)	Cellulolytic fungi (10^3 cfu cm^{-3} ds)	DRBC fungi (10^3 cfu cm^{-3} ds)
Under-vine								
Sward	452 a	274 a	382 a	385 a	628 a	30.5 a	62	129
Bare	124 b	133 b	136 b	207 b	344 b	8.3 b	28	84
l.s.d.	206	78	125	59	128	19.3	46	67
Inter-row								
Sward	318 a	166 a	200	392	409	3.6 a	111	177
Bare	212 b	101 b	197	359	350	1.6 b	112	140
l.s.d.	86	44	71	115	88	1.6	46	52

FCB: fast growing cellulolytic bacteria; SCB: slow growing cellulolytic bacteria; CB: copiotrophic bacteria; FLNB: fast growing low nutrient bacteria; OB: oligotrophic bacteria; PS: copiotrophic pseudomonads; values within a column followed by the same letter are not significantly different based on l.s.d. ($P = 0.05$).

Table 2 – Bacteria and fungi isolated from bare or grassed (sward) soil (0–10 cm depth) from under-vine and inter-row positions at Tumbarumba

	FCB (10^5 cfu cm^{-3} ds)	SCB (10^5 cfu cm^{-3} ds)	CB (10^5 cfu cm^{-3} ds)	FLNB (10^5 cfu cm^{-3} ds)	OB (10^5 cfu cm^{-3} ds)	PS (10^5 cfu cm^{-3} ds)	Cellulo-lytic fungi (10^3 cfu cm^{-3} ds)	DRBC fungi (10^3 cfu cm^{-3} ds)
Under-vine								
Sward	506 a	91	295	396	216 a	11.3	189	194
Bare	270 b	33	287	302	42 b	3.7	159	182
l.s.d.	131	62	223	182	99	10.2	136	66
Inter-row								
Sward	564 a	97	442 a	562 a	238	14.3 a	237	266
Bare	303 b	102	219 b	302 b	251	4.0 b	174	199
l.s.d.	260.7	86	255	194	162	9.5	112	88

FCB: fast growing cellulolytic bacteria; SCB: slow growing cellulolytic bacteria; CB: copiotrophic bacteria; FLNB: fast growing low nutrient bacteria; OB: oligotrophic bacteria; PS: copiotrophic pseudomonads; values within a column followed by the same letter are not significantly different based on l.s.d. ($P = 0.05$).

Table 3 – Simple linear regression correlation coefficients between soil organic C, soil pH and soil or rhizosphere microbial populations

	Wagga Wagga		Tumbarumba	
	r[a]	P	r	P
Inter-row soil				
HWC *vs.* FCB	0.63	0.030	0.83	<0.001
HWC *vs.* TCB	0.66	0.016	0.82	<0.001
HWC *vs.* CB	NC	NC	0.78	0.0021
HWC *vs.* FLNB	NC	NC	0.80	0.001
HWC *vs.* TLNB	NC	NC	0.75	0.005
HWC *vs.* PS	0.60	0.030	0.83	<0.001
HWC *vs.* cellulolytic fungi	NC	NC	0.64	0.015
HWC *vs.* DRBC	NC	NC	0.69	0.012
HWC *vs.* OC	NC	NC	0.51	0.050
HWC *vs.* bulk density	NC	NC	-0.89	<0.001
OC *vs.* TLNB	NC	NC	0.58	0.035
OC *vs.* DRBC	0.68	0.009	NC	NC
OC *vs.* bulk density	-0.58	0.027	NC	NC
pH (water) *vs.* SCB	NC	NC	0.53	0.045
pH (water) *vs.* TLNB	NC	NC	0.68	0.013
Under-vine soil				
HWC *vs.* FCB	0.61	0.028	0.74	0.006
HWC *vs.* TCB	0.54	0.049	0.73	0.006
HWC *vs.* CB	0.55	0.047	NC	NC
HWC *vs.* FLNB	NC	NC	NC	NC
HWC *vs.* TLNB	0.55	0.045	NC	NC
HWC *vs.* PS	0.67	0.014	NC	NC
HWC *vs.* cellulolytic fungi	0.67	0.014	NC	NC
HWC *vs.* DRBC	0.91	<0.001	NC	NC
HWC *vs.* OC	NC	NC	NC	NC
HWC *vs.* bulk density	NC	NC	NC	NC
OC *vs.* bulk density	NC	NC	NC	NC
pH (water) *vs.* DRBC fungi	NC	NC	-0.52	0.049
pH (water) *vs.* cellulolytic fungi	NC	NC	-0.80	0.001
Rhizosphere soil				
HWC *vs.* SCB	NC	NC	0.65	0.018
HWC *vs.* CB	NC	NC	0.58	0.037
HWC *vs.* DRBC	0.63	0.023	NC	NC
pH (water) *vs.* PS	NC	NC	0.74	0.003
Soil SCB *vs.* rhizosphere SCB	NC	NC	0.57	0.031
Soil DRBC *vs.* rhizosphere DRBC	0.72	0.005	NC	NC

NC = not correlated, $P < 0.05$. $n = 12$, $P = 0.05$.
[a] r: Linear correlation coefficient.

row soil, both at Wagga Wagga and Tumbarumba. HWC was also positively correlated with CB, TLNB, PS, cellulolytic fungi and DRBC fungi in the under-vine soil at Wagga Wagga; and with CB, FLNB, TLNB, cellulolytic fungi and DRBC fungi in the inter-row soil at Tumbarumba. HWC was also correlated with SCB and CB in the Tumbarumba rhizosphere. OC was positively correlated only with TLNB at Tumbarumba and with DRBC fungi at Wagga Wagga ($P < 0.05$, Table 3).

3.3. *Bulk density*

In 2004, after 3 years of herbicide treatment, Tumbarumba inter-row soil bulk density was significantly higher for the bare soil (1.17 mg cm^{-3}) than for the grassed soil (1.10 mg cm^{-3}) ($P < 0.05$, data not shown). The soil bulk density in the inter-row was negatively correlated with HWC at Tumbarumba and with OC at Wagga Wagga ($P < 0.05$) (Table 3).

3.4. *Soil pH*

Soil pH ($CaCl_2$) was not correlated with any microbial group but pH (water) was correlated positively with SCB and TLNB in the Wagga Wagga inter-row soil and with PS in the grapevine rhizosphere; and negatively with cellulolytic fungi and DRBC fungi in the Wagga Wagga under-vine soil ($P < 0.05$, Table 3).

3.5. *Grapevine rhizosphere microbial populations*

After 3 years the Tumbarumba grapevine rhizosphere SCB count was significantly higher in the sward treatment than in the bare soil treatment. A high proportion of the fast growing bacteria in the rhizosphere were cellulolytic (FCB). Similarly, a high proportion of the slow growing bacteria (SCB) and fungi (cellulolytic fungi) in the rhizosphere were also cellulolytic ($P < 0.05$, Table 4).

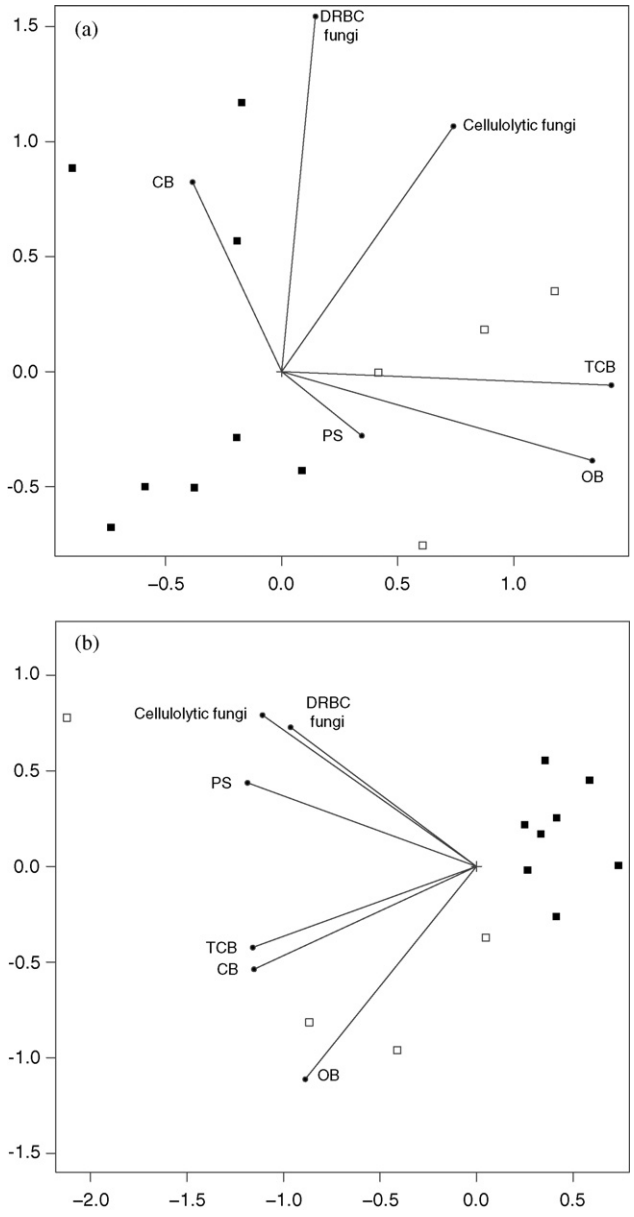

Fig. 1 – Ordination biplot of principal component analysis (PCA) for under-vine soil microbial populations (TCB, CB, OB, PS, cellulolytic fungi and DRBC fungi, 0–10 cm) in 2004. (a) Tumbarumba, first two principal components account for 52% of the variation; (b) Wagga Wagga, first two principal components account for 69% of the variation; permanent sward 2004 (□); bare soil (■).

4. Discussion

4.1. *Organic C*

After 3 years, permanent swards had significantly increased organic C (HWC) and microbial populations in the vineyard soil. This supports the findings of Morlat and Jacquet (2003) who showed that permanent grass cover resulted in higher organic C levels in vineyard inter-row soil. Many non-viticultural studies also support these findings (Voets et al., 1974; Gorlach-Lira et al., 1997; Murata and Goh, 1997).

Table 4 – Grapevine rhizosphere bacteria and fungi for vineyards at Wagga Wagga and Tumbarumba

	FCB (10^5 cfu g^{-1} rfw)	SCB (10^5 cfu g^{-1} rfw)	CB (10^5 cfu g^{-1} rfw)	FLNB (10^5 cfu g^{-1} rfw)	OB (10^5 cfu g^{-1} rfw)	PS (10^5 cfu g^{-1} rfw)	Cellulo-lytic fungi (10^3 cfu g^{-1} rfw)	DRBC fungi (10^3 cfu g^{-1} rfw)
Wagga Wagga								
Sward	1178	1334	761	1566	1423	132	124	89
Bare[a]	1085	1650	624	1388	1372	166	110	67
l.s.d.	488	708	323	479	467	72	87	52
Tumbarumba								
Sward	2325	708 a	1850	2770	1625	58	152	188
Bare	2250	171 b	1088	2616	1871	82	131	147
l.s.d.	128	30	1246	723	1279	62	48	51

[a] FCB: fast growing cellulolytic bacteria; SCB: slow growing cellulolytic bacteria; CB: copiotrophic bacteria; FLNB: fast growing low nutrient bacteria; OB: oligotrophic bacteria; PS: copiotrophic pseudomonads; sward: grassed, nil herbicide treatment; bare: herbicided treatment; values within a column followed by the same letter are not significantly different based on l.s.d. ($P = 0.05$); rfw: root fresh weight.

HWC (0–2 cm) responded to the sward treatment quickly whereas OC (0–10 cm) was still not significantly higher in the grassed plots after 3 years. In addition most soil microbial populations were positively correlated with HWC whereas only two (TLNB, DRBC) were correlated with OC. This is in agreement with Ghani et al. (2003) who reported that HWC was a more sensitive and consistent indicator for differentiating treatment and land-use effects. However, the lack of response in OC may also be explained in part by the different soil sample depths used for the two methods because accumulated C originating from plant litter is likely to be close to the soil surface. Purnomo et al. (2000) reported that, on a similar Red Kandosol cropping soil situated close to the vineyard site, the organic C content in the 0–2 cm depth was 24% higher than the average for the 0–10 cm depth. They also noted that most of the net N mineralisation occurred at the top 2 cm, indicating that much of the microbial activity also occurred at this depth.

4.2. *pH and bulk density*

The positive correlations between soil pH and SCB or TLNB in the inter-row, and PS in the rhizosphere, are consistent with the inhibition of bacterial growth at low pH, whereas the negative correlations between soil pH and DRBC fungi and cellulolytic fungi in the under-vine soil are consistent with the inhibition of fungal growth at high pH (Alexander, 1977).

After 3 years of grass cover, soil bulk density was significantly lower (6%) in the grassed inter-row plots at Tumbarumba. A French study reported a 24% decrease in bulk density and from 23% to 54% lower soil strength after 17 years of permanent vineyard grass cover (Morlat and Jacquet, 2003). More time may be needed before a decrease in bulk density is observed in the grassed Wagga Wagga inter-row soil. The inter-row sward was less developed at Wagga Wagga than at Tumbarumba because Wagga Wagga was more severely affected by the dry conditions. The negative correlations between organic C (HWC and OC) and bulk density at both sites indicate that soil bulk density is likely to decrease in the grassed plots over time.

4.3. *Soil microbial counts*

After 3 years, the permanent sward (consisting primarily of grasses of low to intermediate fertility) had caused significant increases in nearly all bacterial counts, consistent with the tendency of soil bacteria to act as early indicators of the effects of soil treatments (Wardle et al., 1999; Powlson et al., 1987). The separation of the sward and bare soil microbial response profiles in the PCA biplot is probably attributable to differences in soil organic C levels. These findings support the results of Reuter et al. (2000) who reported that long-term use of herbicides resulted in impoverishment of soil organic nutrient sources and decreased microbial biomass in a vineyard in Burgundy, France. Similarly, Bardgett et al. (1999) showed that the total active microbial biomass (as estimated by PLFA) was increased by grass species of semi-natural grasslands or grasslands of intermediate fertility. The positive correlations between organic C (HWC and OC) and soil microbial populations are in agreement with the strong positive correlations between organic C and microbial biomass in grasslands reported by Haynes (1999) and Ghani et al. (2003).

In agreement with data from a barley study (Højberg et al., 1996) the grapevine rhizosphere contained much higher populations of most microbial groups studied than the surrounding under-vine soil. The fact that the PS population was much higher (668% and 833% for Wagga Wagga and Tumbarumba, respectively) in the grapevine rhizosphere than in bulk soil is also consistent with the results from a wheat study by Smit et al. (2001). Plant roots generally tend to be selective towards members of the *Pseudomonas* genus (Marilley and Aragno, 1999).

4.4. *Cellulolytic bacteria*

Cellulolytic bacteria formed a high proportion of the grapevine rhizosphere bacterial count. It is likely that cellulolytic enzymes confer an advantage for colonisation of the grapevine rhizosphere. Compant et al. (2005) reported that colonisation of Chardonnay plantlets by a plant growth-promoting cellulolytic bacterium, *Burkholderia* sp. strain PsJN, appeared to be aided by cell wall-degrading cellulolytic enzymes which would allow the bacterium to gain entry into root internal tissues. The observation that populations of SCB and DRBC fungi in the rhizosphere were positively correlated with their populations in the under-vine bulk soil is consistent with the suggestion by Jjemba and Alexander (1999) that rhizosphere competence of some soil microorganisms may be dependent on their ability to survive in large numbers in soil.

The presence of a permanent sward had no significant effect on most rhizosphere microbial counts within the 3-year time frame of this trial. The exception was the rhizosphere SCB count which was greatly increased in the sward treatment at Tumbarumba and was positively correlated with HWC. Long incubation periods were used for the isolation of SCB. The possibility that the SCB may be part of the previously 'unculturable' bacterial population will be the subject of further investigation.

Soil contains significant populations of microorganisms with the ability to attack or suppress pathogenic fungi. Data obtained soon after the initial herbicide application for this vineyard trial showed that a very high proportion of soil microorganisms isolated from the newly herbicided inter-row had *in vitro* suppressive activity against the growth of the grapevine fungal root pathogens (Whitelaw-Weckert, 2004). It has often been reported that fungistasis is strongest in soils with high organic C content and microbial activity, with cellulolytic bacteria and pseudomonads implicated as major causes of such fungistasis (Sturz et al., 1997; Whipps, 2001). Increasing soil organic C in the vineyard may result in a 'suppressive' soil: one that is able to suppress grapevine fungal root pathogens.

The grapevine rhizosphere also contained large numbers of OB. These oligotrophic bacteria exploit soil ecological niches with low substrate concentrations and are adapted for low nutrient environments (Ohta and Hattori, 1983). This may seem surprising as roots are often seen as high nutrient copiotrophic environments, but parts of the rhizosphere are oligotrophic, with C concentrations ranging from 10 to 100 $\mu g\ g^{-1}$ dry soil (Maloney et al., 1997).

4.5. *Herbicides*

It is possible that herbicide toxicity may have contributed to the reduced soil microbial population in the bare soil plots and this possibility should be further investigated. Results of field and laboratory studies of glyphosate have been contradictory. Long-term laboratory studies have reported reductions in soil microbial abundance (Mekwatanakarn and Sivasithamparam, 1987; Gorlach-Lira et al., 1997) but a long-term (10 years) pine plantation experiment concluded that glyphosate had minimal effects overall (Busse et al., 2001). In a field cropping study, paraquat increased populations of bacteria, fungi and actinomycetes at normal application rates but at higher application rates was toxic to fungi (Camper et al., 1973). Paraquat also caused increased soil urease and invertase activity (Sannino and Gianfreda, 2001) whilst diquat and paraquat increased total fungal populations but decreased the population of some fungal antagonists to 'take-all' in wheat (Mekwatanakarn and Sivasithamparam, 1987).

5. Conclusions

As vineyard soil receives few inputs of organic matter, maintenance of permanent swards can improve the organic C content and thus the microbial abundance of the soil. Where there was sufficient soil moisture (i.e. at the cool climate site, Tumbarumba) 3 years of permanent sward treatment increased soil HWC and decreased soil bulk density in the inter-row. At both the warm and cool climate sites, under-vine and inter-row soil bacterial counts were markedly higher in the grassed soil and were generally positively correlated with HWC. Further work will investigate individual cellulolytic soil bacteria isolated, and their role in the suppression of grapevine fungal pathogens.

Acknowledgments

This study was supported by Australia's grape growers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government. Mark Conyers, NSW Department of Primary Industries (NSW DPI), contributed valuable advice regarding soil chemical aspects of the project. We thank Robert Lamont, Emily Rouse and Andrew Smith for their technical assistance. Greg Gallagher of Charles Sturt University Winery and Stuart Barclay of Tumbarumba Wine Estates provided the experimental vineyard sites. Janet Wild of New South Wales Department of Natural Resources prepared the soil profile report.

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