Ground cover and organic nutrient management practices altering the denitrifier community in an organic apple orchard soil

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Summary (100 words maximum.)

Molecular analysis complemented investigation of the long-term changes in microbial communities and soil nutrients of an organic apple orchard receiving annual additions of ground cover and fertilizer treatments. Seven years of compost and wood chip applications resulted in the greatest soil organic matter (OM), and richest and most diverse denitrifier communities. However, dissolved organic carbon, OM, microbial biomass, and range-weighted richness of denitrifiers revealed different interactions among fertilizers and ground cover combinations. It is necessary to understand how those interactions affect the soil microbial community to properly manage for decomposition and nutrient cycling and to build soil quality.

Table 1. Initial soil properties measured in 2006.

Year	pН	EC	EC Bulk Density OM N		N	С	P	K
		(µmhos/cm)	(g/cm^3)	(%)		(mg	/kg)	
2006	6.57	73.75	1.34	1.47	0.09	0.95	34	170

Table 2. Summary of ground cover and fertilizer nutrient analysis.

Treatment	С	N	C:N	P	K
	(%)		(%	6)
Compost	20.5	1.6	13.5	0.2	0.5
Wood Chips	29.7	0.7	39.2	0.1	0.3
Paper	36.8	0.2	205.9	0.0	0.0
Mow and Blow	40.0	2.2	15.8	0.3	1.5
Commercial Fertilizer	31.3	4.4	7.8	1.4	2.6
Poultry Litter	29.5	1.7	19.4	1.3	1.4

^aData for each ground cover and fertilizer are averaged across 2006-2011.

Table 3. P-values from analysis of variance (ANOVA) for soil properties in the 0-10 cm depth including dissolved organic carbon (DOC), dissolved organic nitrogen (DON), nitrate-N (NO₃⁻-N), ammonium-N (NH₄⁺-N), microbial biomass carbon (Bio C), microbial biomass nitrogen (Bio N), pH, EC, water content (H₂O), and organic matter (OM).

Soil Property	GC ^a	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert*Year
DOC	<0.0001*	0.2663	0.0351*	<0.0001*	<0.0001*	0.7543	0.2842
DON	0.0026*	0.0036*	0.0820	<0.0001*	0.0206*	0.5338	0.8424
NO ₃ -N	0.0256*	0.0350	0.1083	<0.0001*	0.1642	0.1240	0.2563
NH4 ⁺ -N	0.5325	0.1168	0.1440	<0.0001*	0.2194	0.2199	0.9857
Bio C	0.6768	0.2421	0.0395*	0.0021*	0.0396*	0.0545	0.1684
Bio N	0.7494	0.0899	0.0152*	0.0010*	0.3121	0.3170	0.1243
pH	0.0007*	0.0711	0.1981	0.4248	0.0745	0.6749	0.9856
EC	0.3043	0.4268	0.3492	0.0001*	0.3055	0.3952	0.4011
H ₂ O	0.2032	0.9727	0.6848	0.0001*	0.4323	0.5941	0.4878
OM	0.0036*	0.0975	0.0026*	<0.0001*	0.0196*	0.1895	0.0513

^aGC is ground cover and Fert is fertilizer source.

Table 4. Soil pH and nitrate-N (NO_3^--N) in the 0-10 cm depth under different ground cover treatments.

		NO ₃ -N
Ground Cover	рН	$(\mu g N g^{-1})$
Compost	7.01b	4.61a
Mow and Blow	6.83c	2.53bc
Paper	7.32a	1.17c
Wood Chips	6.84c	3.54ab

Means within a column followed by similar letters are not significantly different (P < 0.05).

Table 5. Soil electrical conductivity (EC), water content (H₂O), microbial biomass nitrogen (Bio N), ammonium-N (NH₄⁺-N), and nitrate-N (NO₃⁻-N) in 2007 and 2013 in the 0-10 cm depth.

Year	EC	H ₂ O	Bio N	NH4 ⁺	NO ₃ -
	$(dS m^{-1})$		(µg N g ⁻¹)	(µg N g ⁻¹)	$(\mu g N g^{-1})$
2007	46.63b	0.17b	12.19b	1.78b	0.92b
2013	333.0a	0.22a	16.68a	7.93a	5.01a

Means followed by different letters within a column are significantly different (P < 0.05).

Table 6. Soil chemical and biological properties affected by ground cover and fertilizer treatments.

Ground Cover	Fertilizer	DOC ^a	Bio C	Bio N	OM
		$(\mu g C g^{-1})$	$(\mu g C g^{-1})$	$(\mu g N g^{-1})$	(%)
Compost	None	157.5a	74.0ab	17.7a	3.98a
	Poultry litter	140.6ab	47.7cd	12.6bc	2.87bc
	Commercial	130.8b	63.2abcd	16.2ab	3.85a
Mow and Blow	None	36.0e	81.2a	14.5abc	1.96e
	Poultry litter	50.2de	66.3abc	12.7bc	2.24de
	Commercial	38.9e	66.6abc	13.3bc	2.11de
Paper	None	81.4c	39.6d	10.7c	2.27de
_	Poultry litter	86.5c	64.8abcd	15.6ab	2.55cd
	Commercial	83.4c	64.3abcd	15.5ab	2.34cde
Wood Chips	None	60.6d	48.3bcd	10.6c	2.21de
oou empo	Poultry litter	89.4c	59.3abcd	15.6ab	2.84bc
	Commercial	88.0c	83.7a	13.0a0 18.1a	3.20b
	Commercial	00.0C	05.7a	10.14	3.200

^aDOC is dissolved organic carbon, Bio C is microbial biomass carbon, Bio N is microbial biomass nitrogen, and OM is organic matter.

Means within columns followed by similar letters are not significantly different P < 0.05.

Table 7. P-values from analysis of variance (ANOVA) for ecological indices from the 0-

10 cm soil depth.

Diversity	•						GC*Fert*
Index	GC	Fert	GC*Fert	Year	GC*Year	Fert*Year	Year
R^a	0.0024*	0.0371*	0.0028*	<0.0001*	<0.0001*	0.0473*	0.1271
Н	0.0128*	0.0442*	0.0146*	<0.0001*	0.0015*	0.1550	0.6922
J	0.0461*	0.2158	0.0081*	0.0423*	0.0094*	0.2546	0.0903
D	0.0026*	0.1793	0.2499	0.0005*	0.0099*	0.4776	0.4940
E	0.1908	0.9431	0.0953	0.0480*	0.0294*	0.3365	0.4767
Rr	0.0004*	0.1327	0.0019*	<0.0001*	<0.0001*	0.0393*	0.0069*
Fo	0.2323	0.0225*	0.0142*	0.0003*	<0.0001*	0.5431	0.1110

^aR is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; Rr is range-weighted richness; and Fo is functional organization.

Table 8. Ecological diversity indices of ground cover and fertilizer combinations calculated from DGGE profiles of the 0-10 cm depth.

Ground Cover	Fertilizer	Ra	Н	J	Fo
Compost	None	41.0a	3.46a	0.93ab	0.41bc
	Poultry litter	36.1abc	3.37ab	0.94a	0.43abc
	Commercial	35.1bc	3.37ab	0.95a	0.41bc
Paper	None	29.5ef	3.14bcd	0.94a	0.42abc
•	Poultry litter	31.2cde	3.18bcd	0.93ab	0.40c
	Commercial	31.3cde	3.19bcd	0.93ab	0.46a
Wood Chips	None	27.2ef	2.83e	0.89bc	0.42abc
-	Poultry litter	36.7abc	3.26abc	0.92ab	0.45a
	Commercial	37.0ab	3.31ab	0.93ab	0.45a
Mow and Blow	None	25.2f	2.95de	0.92ab	0.44ab
	Poultry litter	26.8ef	3.00cde	0.92ab	0.45a
	Commercial	34.3bcd	3.02cde	0.85c	0.45a

^aR is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; and Fo is functional organization.

Means within columns followed by similar letters are not significantly different (P < 0.05).

Table 9. Species richness (R) in the 0-10 cm depth in 2007 and 2013 in fertilizer treatments.

Year	None	Poultry litter	Commercial
2007	25.5c	26.6c	31.0b
2013	35.9a	38.8a	37.9a

Means followed by different letters are significantly different (P < 0.05).

Table 10. Soil properties measured affected by ground covers in 2007 and 2013 in the 0-10 cm depth including, microbial biomass carbon (Bio C), dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and organic matter (OM).

Ground Cover	Year	Bio C	DOC	DON	OM
		$(\mu g C g^{-1})$	$(\mu g C g^{-1})$	$(\mu g N g^{-1})$	(%)
Compost	2007	64.5abc	73.9c	9.20a	1.84d
	2013	58.8bc	212.0a	9.06a	5.29a
Mow and Blow	2007	93.4a	32.5d	4.72b	1.12e
	2013	49.3bc	50.9d	0.55d	3.08c
Paper	2007	75.1ab	38.3d	5.01b	1.19de
	2013	37.3c	129.3b	1.45c	3.58c
Wood Chips	2007	63.2abc	44.2d	5.41b	1.23de
	2013	64.3abc	114.4b	0.61d	4.26b

Means within columns followed by similar letters are not significantly different P < 0.05.

Table 11. DGGE ecological indices by ground cover in the 0-10 cm depth in 2007 and 2013.

Ground Cover	Year	Rª	Н	J	D	Е	Fo
Compost	2007	31.9b	3.30abc	0.95a	23.34ab	0.74a	0.37e
	2013	43.0a	3.50a	0.93ab	25.21a	0.60d	0.46ab
Mow and	2007	26.7c	2.95ef	0.90bc	16.59cd	0.62cd	0.43bcd
Blow	2013	30.9b	3.04def	0.89c	19.32c	0.62cd	0.47a
Paper	2007	30.0bc	3.13cde	0.92abc	18.99cd	0.63cd	0.45abc
	2013	31.3b	3.21bcd	0.94a	21.21bc	0.67bc	0.40de
Wood Chips	2007	22.2d	2.89f	0.94a	15.43d	0.70ab	0.42cd
	2013	45.0a	3.37ab	0.89c	27.06a	0.60d	0.46ab

^aR is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; and Fo is functional organization.

Means within a column followed by similar letters are not significantly different (P < 0.05)

Table 12. Range-weighted richness (Rr) calculated from DGGE profiles of communities receiving ground cover and fertilizer treatment combinations in 2007 and 2013 in the 0-10 cm soil depth.

- I		2007			2013	
Ground Cover	None	Poultry litter	Commercial	None	Poultry litter	Commercial
Compost	223.7defg	223.1defg	194.0efgh	587.7a	381.4bc	360.8bc
Mow and Blow	126.2ghi	103.6ghi	214.4defgh	120.1ghi	197.0defgh	293.3cde
Paper	139.9fghi	166.6efghi	230.9defg	191.1efgh	257.5cdef	203.9defgh
Wood Chips	41.2i	90.9hi	151.3fghi	322.3bcd	593.9a	450.8b

Means followed by similar letters are not significantly different P < 0.05.

Table 13. P-values from analysis of variance (ANOVA) for properties at the 10-30 cm soil depth.

Soil Property	GCa	Fert	GC*Fert	Year	GC*Year	Fert*Year	GC*Fert *Year
DOC^b	0.0017*	0.0747	0.5700	0.0860	0.0002*	0.1191	0.9817
DON	0.0179*	<0.0001*	0.0042*	<0.0001*	0.0010*	<0.0001*	0.0778
NO ₃ -N	0.0007*	0.0066*	0.1783	<0.0001*	<0.0001*	0.0478*	0.4249
NH_4^+ -N	0.2855	0.6620	0.9722	<0.0001*	0.1003	0.5463	0.9877
Bio C	0.2057	0.3396	0.4185	<0.0001*	0.2010	0.5933	0.8801
Bio N	0.7795	0.1091	0.0552	<0.0001*	0.2990	0.7842	0.9561
pН	0.1163	0.1511	0.0027*	<0.0001*	<0.0001*	0.9331	0.8619
EC	0.0011*	0.0558	0.9007	<0.0001*	<0.0001*	0.0693	0.9133
H_2O	0.0716	0.7834	0.5187	0.0001*	0.0679	0.9474	0.6543
OM	0.0445*	0.8291	0.4186	<0.0001*	0.2399	0.9043	0.5339

^aGC is ground cover and Fert is fertilizer source.

^bDOC is dissolved organic carbon, DON is dissolved organic nitrogen, NO₃⁻-N is nitrate-N, NH₄⁺-N is ammonium-N, Bio C is microbial biomass carbon, Bio N is microbial biomass nitrogen, EC is electrical conductivity, H₂O is gravimetric soil water content, and OM is soil organic matter.

Table 14. Soil water content (H_2O), organic matter (OM), microbial biomass carbon (Bio C), microbial biomass nitrogen (Bio N), and ammonium-N (NH_4^+ -N) in 2007 and 2013 in the 10-30 cm depth.

Year	H ₂ O	OM (%)	Bio C (μg C g ⁻¹)	Bio N (μg N g ⁻¹)	
2007	0.24a	0.96b	74.06a	3.76a	0.05b
2013	0.17b	2.24a	17.42b	1.99b	0.52a

Means within a column followed by different letters are significantly different (P < 0.05).

Table 15. Soil properties affected by ground covers in 2007 and 2013 at the 10-30 cm depth including, dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and Nitrate-N (NO₃⁻-N).

Ground Cover	Year	EC	рН	DOC (µg C g ⁻¹)	DON (μg N g ⁻¹)	NO ₃ -N (μg N g ⁻¹)
Compost	2007	26.80e	6.64c	35.57b	4.39d	1.77c
	2013	215.02a	6.96b	51.10a	23.20a	8.59a
Mow and Blow	2007	16.10e	6.38e	27.24cd*	3.76d	0.64c
	2013	128.71d	6.44de	19.94d*	7.96cd	1.44c
Paper	2007	16.42e	6.56cd	26.64cd	3.10d	0.36c
	2013	158.09c	7.13a	29.52bc	15.05b	1.01c
Wood Chips	2007	16.18e	6.30e	30.94bc	4.16d	0.89c
	2013	182.06b	6.95b	30.71bc	12.38bc	4.61b

Means within a column followed by similar letters are not significantly different P < 0.05.

^{*} Significantly different on the split-plot level.

Table 16. Soil chemical and biological properties affected by ground cover and fertilizer treatments at the 10-30 cm depth.

Ground Cover	Fertilizer	DON ^a (μg N g ⁻¹)	рН
Compost	None	25.13a	6.81c
	Poultry litter	9.27d	6.75d
	Commercial	6.99def	6.84bc
Mow and Blow	None	9.74cd	6.35i
	Poultry litter	4.28ef	6.47g
	Commercial	3.56f	6.41h
Paper	None	14.73b	6.97a
	Poultry litter	7.17def	6.70e
	Commercial	5.33ef	6.88b
Wood Chips	None	13.52bc	6.63f
	Poultry litter	7.66de	6.64f
	Commercial	3.63f	6.60f

^aDON is dissolved organic nitrogen.

Means within a column followed by similar letters are not significantly different P < 0.05.

Table 17. Soil chemical and biological properties affected by fertilizer treatments and year at the 10-30 cm depth.

Year	Fertilizer	DON ^a (μg N g ⁻¹)	NO ₃ ⁻ -N (μg N g ⁻¹)
2007	None	3.93c	0.75c
	Poultry litter	3.86c	1.25c
	Commercial	3.77c	0.74c
2013	None	27.63a	2.53b
	Poultry litter	10.33b	5.13a
	Commercial	5.98c	4.08a

 $^{
m a}$ DON is dissolved organic nitrogen. Means within a column followed by similar letters are not significantly different P < 0.05.

Table 18. P-values from analysis of variance (ANOVA) for ecological indices from

DGGE for the 10-30 cm soil depth.

Diversity Index	GC	Year	GC*Year
R^a	0.8360	0.1445	0.5507
Н	0.4464	0.4689	0.3044
J	0.5698	0.2510	0.2610
D	0.8108	0.2316	0.3967
E	0.9127	0.2058	0.2608
Rr	0.8778	0.1593	0.6957

^aR is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; Rr is range-weighted richness.

Table 19. DGGE ecological indices by ground cover in the 10-30 cm soil depth in 2007 and 2013^a

Ground Cover	Year	R^b	Н	J	D	Е	Rr
Compost	2007	32.17	3.13	0.91	17.92	0.59	251.02
	2013	32.00	3.14	0.90	17.12	0.54	257.34
Mow and	2007	32.29	3.05	0.90	18.25	0.57	319.66
Blow	2013	29.71	3.03	0.91	17.62	0.59	258.20
Paper	2007	30.00	2.69	0.83	16.13	0.53	269.11
	2013	27.00	2.79	0.92	16.43	0.67	217.07
Wood Chips	2007	35.17	3.21	0.91	19.46	0.56	328.69
	2013	24.43	2.74	0.91	13.43	0.61	186.81

^aThere are no differences in means or treatment effects on indices; thus, table is for reference only.

^bR is species richness; H is Shannon Weaver index; J is Shannon Weaver index of equitability; D is Simpson's index; E is Simpson's index of equitability; Rr is range-weighted richness.