**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 | pH | EC (μmhos/cm) | Bulk Density (g/cm$^3$) | OM (%) | N | C | P | K |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | | | | | | (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 | C | N | C:N | P | K |
|---|---|---|---|---|---|
| | (%) | | | (%) | |
| 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 |

[a]Data 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 ($NO_3^-$-N), ammonium-N ($NH_4^+$-N), microbial biomass carbon (Bio C), microbial biomass nitrogen (Bio N), pH, EC, water content ($H_2O$), 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 |
| $NH_4^+$-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 |
| $H_2O$ | 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 |

[a]GC 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.

| Ground Cover | pH | $NO_3^-$-N ($\mu g\ N\ g^{-1}$) |
|---|---|---|
| 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 ($H_2O$), microbial biomass nitrogen (Bio N), ammonium-N ($NH_4^+$-N), and nitrate-N ($NO_3^-$-N) in 2007 and 2013 in the 0-10 cm depth.

| Year | EC (dS m$^{-1}$) | $H_2O$ | Bio N ($\mu$g N g$^{-1}$) | $NH_4^+$ ($\mu$g N g$^{-1}$) | $NO_3^-$ ($\mu$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] ($\mu g\ C\ g^{-1}$) | Bio C ($\mu g\ C\ g^{-1}$) | Bio N ($\mu g\ N\ g^{-1}$) | OM (%) |
|---|---|---|---|---|---|
| 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 |
| | Poultry litter | 89.4c | 59.3abcd | 15.6ab | 2.84bc |
| | Commercial | 88.0c | 83.7a | 18.1a | 3.20b |

[a]DOC 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 Index | GC | Fert | GC*Fert | Year | GC*Year | Fert*Year | GC*Fert*Year |
|---|---|---|---|---|---|---|---|
| R[a] | 0.0024* | 0.0371* | 0.0028* | <0.0001* | <0.0001* | 0.0473* | 0.1271 |
| H | 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 |

[a]R 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 | R[a] | H | 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 |

[a]R 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 ($\mu$g C g$^{-1}$) | DOC ($\mu$g C g$^{-1}$) | DON ($\mu$g N g$^{-1}$) | OM (%) |
|---|---|---|---|---|---|
| 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 | R[a] | H | J | D | E | 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 Blow | 2007 | 26.7c | 2.95ef | 0.90bc | 16.59cd | 0.62cd | 0.43bcd |
|  | 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 |

[a]R 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.

| Ground Cover | 2007 | | | 2013 | | |
|---|---|---|---|---|---|---|
| | 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 | GC[a] | 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 |
| pH | 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 |

[a]GC is ground cover and Fert is fertilizer source.
[b]DOC is dissolved organic carbon, DON is dissolved organic nitrogen, $NO_3^-$-N is nitrate-N, $NH_4^+$-N is ammonium-N, Bio C is microbial biomass carbon, Bio N is microbial biomass nitrogen, EC is electrical conductivity, $H_2O$ 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 | $H_2O$ | OM (%) | Bio C ($\mu g\ C\ g^{-1}$) | Bio N ($\mu g\ N\ g^{-1}$) | $NH_4^+$-N ($\mu g\ N\ g^{-1}$) |
|------|--------|--------|--------|--------|--------|
| 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 ($NO_3^-$-N).

| Ground Cover | Year | EC | pH | DOC ($\mu g\ C\ g^{-1}$) | DON ($\mu g\ N\ g^{-1}$) | $NO_3^-$-N ($\mu g\ N\ g^{-1}$) |
|---|---|---|---|---|---|---|
| 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] ($\mu g\ N\ g^{-1}$) | pH |
|---|---|---|---|
| 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 |

[a]DON 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] ($\mu$g N g$^{-1}$) | NO$_3^-$-N ($\mu$g 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 |

[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 |
| H | 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 |

[a]R 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] | H | J | D | E | 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 Blow | 2007 | 32.29 | 3.05 | 0.90 | 18.25 | 0.57 | 319.66 |
| | 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 |

[a]There are no differences in means or treatment effects on indices; thus, table is for reference only.
[b]R 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.