FINAL REPORT

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Project title: Influences of organic amendments on microbial aspects of soil fertility

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OBJECTIVES:

1. Determine the effects of selected organic mulches, soil amendments and cover crops on microbial biomass and populations of microfauna that are indicative of microbial turnover and nutrient mineralization.
2. Correlate biological indicators of N and P availability with measured N and P availability, tree growth, and nutrient uptake.
3. Determine the effects of selected organic mulches, soil amendments and cover crops on colonization of apple roots by the root-lesion nematode, *Pratylenchus penetrans*, and mycorrhizal fungi.

SIGNIFICANT FINDINGS:

1. Dairy manure (Naches trial) biosolids (Summerland trial) and chopped alfalfa hay (WVC trial) increased the amount of N flowing from microflora through the soil microfauna (N flux rates), relative to controls. Increased fluxes of N under non-chopped alfalfa hay were not statistically significant at Summerland or Naches.

2. Fluxes of N under shredded paper tended to be greater than in control plots in all experiments, but the differences were seldom statistically significant. The increase in N flux rates, relative to controls, under shredded paper were greater than could be accounted-for by the N that would be released from complete decomposition of the annual paper additions. These results indicate that mulches of shredded paper caused the microbial biomass to immobilize, and cycle, fertilizer N.

3. Among tree-row cover crops evaluated, clover significantly increased fluxes of N in the root zone.

4. The abundance of "enrichment opportunist" nematodes (Enrichment Index, EI), and the nematode community Maturity Index (MI) were both significantly correlated with leaf P (positive and negative correlations for the EI and MI, respectively).

5. Exploration of a novel, simple approach for measuring microbial biomass N was undertaken in 2001. The approach involves lysis of microbial cells with hexanol, extraction with potassium sulphate, and measurement of absorbance of 280 nm light. Although promising and worthy of additional study, data obtained in 2001 seldom revealed significant differences

between treatments. Gross microbial biomass was concluded to be a less sensitive indicator of nutrient status of the soil ecosystem than microfaunal parameters studied.

6. Colonization of roots by mycorrhizal fungi was reduced significantly, relative to the controls, under alfalfa hay at the Wenatchee Valley College trial and at Summerland. Colonization was increased, relative to the control, under the shredded paper mulch at Summerland.

METHODS:

Each of four existing field experiments were sampled twice in the summer of 2001. The field experiments were:
1) Mulch/organic amendment x irrigation experiment at Dave Allan's orchard in Naches. 2) Pacific Agri-Food Research Centre (PARC) mulch/amendment experiment.
3) Wenatchee Valley College (WVC) mulch/cover crop experiment.
4) PARC mulch/cover crop experiment.

At each sample date, ten cores (1 inch diameter x 15 cm deep) were removed from each plot and composited. Population densities of protozoa were determined and nematodes were extracted from each sample using established methods. Nematode communities were characterized at the genus level of resolution. The biomass of each taxon was estimated and the data were consolidated into biomass values for the bacterivorous, fungivorous, omnivorous and predacious trophic groups. Protozoan population densities were also converted to biomass.

A derivation of the trophic structure model of De Ruiter et al. (1993) was used to estimate the annual movement of N from the microbial biomass through the microfauna (N flux). While this value is not net N mineralization in the strict sense, it is a good, easily calculated, approximation of gross N mineralization. Values for N flux in organic mulch-treated plots were subtracted from values in control plots. The difference was assumed to represent the gain in flow of N due to the mulch (ΔNflux). Finally, this value was divided by the N content of the organic mulch to estimate the percentage of mulch N mineralized. For Naches, WVC and the cover crop/mulch experiment at Summerland, N flux data presented are averages of the first three sample dates; data have not yet been analyzed for the final sample date.

The primary limitation of this approach is that the data we have used thus far for calculations do not accurately represent the true yearly "steady state" (i.e. average) biomass. Since sampling has been conducted during the growing season, it is reasonable to assume that the biomass values used in calculations are higher than the true average for the year. Additional research will be needed to determine the dynamics of faunal biomass during the entire year and make more accurate estimates of the fluxes of N. The data are, however, a very useful and appropriate means of making comparisons among treatments within a given experiment

Microbial biomass N, protozoan abundance, and several indices of nematode community structure were evaluated for their potential use as bio-indicators of the mineralization process. Microbial biomass was measured in 2001 using an adaptation of the method of Turner et al. (2001). We used hexanol as a lysing agent rather than chloroform. The nematode parameters considered include: abundance of bacterivorous nematodes, the percentage of bacterivorous nematodes classified as enrichment opportunists, overall abundance of enrichment opportunist nematodes, and the nematode Community Maturity Index. To simplify presentation, only data on the abundance of protozoa, the abundance of bacterivorous nematodes, and the nematode Community Maturity Index are presented. Bio-indicator data presented in the tables are from the most recently analyzed set of data from 2001. Ultimate evaluation of these potential bio-indicators involves correlation with leaf P data. Leaf N data are considered inadequate indicators of mineralization-derived N because fertilizer N is being applied at all experiments. At this point in time, these correlation analyses have been performed with data from one of the trials at Summerland only. Consequently, discussion of bio-indicators will be limited primarily to results the Summerland experiment.

RESULTS AND DISCUSSION:

The terms "amendment" and "mulch" are often used to refer to materials applied to the soil surface or incorporated into the soil, respectively. In these studies, all materials were applied to the soil surface. Thus, for clarity, we will use the operational term "mulch" for all materials discussed in this progress report.

Naches experiment:

Dairy manure increased the flux of N through microfauna in the root zone at Naches (Table 1). Alfalfa hay did not significantly increase fluxes of N through the microbial biomass. The increases in N flux due to addition of organic mulches represented 36% and 7% of the total N in the added manure and alfalfa, respectively (Table 1). This percentage of N mineralized will decline in subsequent years. Continued study will enable us to determine whether N fluxes under manure will decline more rapidly than under alfalfa. The combination of alfalfa hay applied over manure resulted in fluxes of N that were 2 times greater than under the treatment of manure alone. The total amount of N in the alfalfa hay/manure combination was approximately 2 times greater than in the treatment of manure alone. Consequently, the flux of N, as a proportion of total added organic N, was similar for the manure only and hay/manure treatments.

Table 1. *Naches experiment:* Estimated annual transfer of N from microbial biomass to microfauna (N flux), increase in N flux due to amendment (ΔNflux), percentage of total amendment N entered into microbial N cycle, and three potential bio-indicators of N mineralization (protozoan abundance, bacterivorous nematode abundance, and the nematode community Maturity Index). Trees were fertigated with 44 kg N/ha for the tree row. Data for N flux were averaged over three sample dates before analyses. Additions of N in the manure (5% N) and alfalfa hay (2.5% N) were estimated to be 1000 and 1100 kg N/ha, respectively. Organic mulches were applied in 2000.

				Potential bio-indicators		
Treatment	N flux (kg/ha/yr)	ΔNflux due to mulch (kg/ha/yr)	% of mulch N	Prot/g soil x 10^3 (June)	Bact Nemas (June)	Nematode MI (June)
Control	377 c			36 c	2308 b	1.17
Alfalfa	456 bc	79	7.2	67 bc	2598 b	1.15
Manure	734 b	357	35.7	143 ab	4180 ab	1.17
Hay/manure	1097 a	720	34.3	235 a	6514 a	1.08

Summerland mulch experiments:

Biosolids and combinations of shredded paper applied over biosolids or compost increased fluxes of N through the microbial biomass at Summerland (Tables 2 & 3). Even though the decomposition of shredded paper contributed relatively little N, the flux of N under shredded paper tended to be greater than in control plots (but not statistically significant). Because the increased flux of N through the microbial biomass in plots of shredded paper was greater than can be accounted-for by complete decomposition of the added paper, we speculate that fertilizer N was incorporated into the microbial biomass and is cycling within the microbial biomass. Because tree N deficiency has not been observed in shredded paper plots at this site, we speculate that shredded paper could improve fertilizer efficiency (reduce loss) by encouraging the incorporation of fertilizer N into the microbial biomass, and subsequent cycling of this N within the microbial biomass.

Table 2. *Summerland mulch trial 1:* Estimated annual transfer of N from microbial biomass to microfauna (N flux), increase in N flux due to amendment (ΔNflux), percentage of total amendment

N entered into microbial N cycle, and four potential bio-indicators of N mineralization (protozoan abundance, bacterivorous nematode abundance, the nematode community Maturity Index, and colonization by arbuscular-mycorrhizal fungi). Data for N flux were averaged over four sample dates before analyses. N contents of biosolids, compost, alfalfa and shredded paper added since 1997 were estimated at 1,485, 855, 1020, and 48 kg N/ha, respectively.

				Potential bio-indicators			
Treatment	N flux (kg/ha/yr)	ΔNflux due to mulch (kg/ha/yr)	ΔNflux as % of mulch N	Prot/g soil x 10^3 (Oct. 2000)	Bact Nemas per 100 g soil	Nema MI	AM coloniz. (Oct. 2000)
Control	216 b			92 b	2080 bc	2.07 ab	18.8 b
Shredded paper	356 b	140	292[A]	137 a	3402 ab	2.13 a	34.4 a
Biosolids	488 ab	272	18.3	190 a	3315 ab	1.87 bc	3.5 c
Paper/biosolids	826 a	610	39.8	217 a	5209 a	1.97 ab	23.2 ab
Paper/compost	514 ab	298	33.0	207 a	3691 ab	2.03 ab	11.5 bc
Alfalfa hay	378 b	162	15.9	123 a	2551 bc	1.67 c	4.8 c
Plastic	130 b	-86	---	35 b	1041 c	2.11 a	22.0 b

[A] Because this quantity is greater than can be accounted-for by complete decomposition of the added paper, it represents immobilization and cycling of N by the microbial biomass.

Table 3. *Summerland mulch trial 2:* Estimated annual transfer of N from microbial biomass to microfauna (N flux), increase in N flux due to amendment (ΔNflux), percentage of total amendment N entered into microbial N cycle, and three potential bio-indicators of N mineralization (protozoan abundance, bacterivorous nematode abundance, and the nematode community Maturity Index). Data for N flux were averaged over two sample dates before analyses. N contents of biosolids and shredded paper were 1,485 and 48 kg N/ha, respectively. Organic materials were applied in 1997.

				Potential bio-indicators		
Treatment	N flux (kg/ha/yr)	ΔNflux due to mulch (kg/ha/yr)	% of mulch N	Prot/g soil x 10^3 (Sept)	Bact Nemas (Sept)	Nema MI (Sept)
Control	239 b			24 b	897 b	2.12 a
Shredded paper	468 a	229	477 [A]	97 a	2114 a	2.17 a
Biosolids	240 b	1	0.1	51 b	1207 b	1.89 b
Paper/biosolids	523 a	284	18.5	162 a	2702 a	1.77 b

[A] Because this quantity is greater than can be accounted-for by decomposition of the added paper, it represents immobilization and cycling of N by the microbial biomass.

Mulch/cover crop trial at Wenatchee Valley College:

Calculated values for flux of N indicate that most of the nitrogen had been mineralized from the chopped alfalfa mulch. These observations are in accordance with general observations of disappearance of the mulch, and high nitrate concentrations in the root zone and leaf color in the first two years, indicating a large flush of nitrogen. Clover resulted in the second-highest estimated N flux values. These data also are in accordance with observations on apparent tree nitrogen (D. Granatstein, personal communication).

Table 4. *Mulch/cover crop trial at Wenatchee Valley College:* Estimated annual transfer of N from microbial biomass to microfauna (N flux), increase in N flux due to amendment (ΔNflux), percentage of total amendment N entered into microbial N cycle, three potential bio-indicators of N mineralization (protozoan abundance, bacterivorous nematode abundance, and the nematode community Maturity Index), and colonization by arbuscular-mycorrhizal fungi. Data for N flux were averaged over three sample dates before analyses. N contents of alfalfa and shredded paper were estimated at 414 and 48 kg N/ha, respectively. Mulches were applied in 1999.

				Potential bio-indicators			
Treatment	N flux (kg/ha/yr)	ΔNflux due to mulch (kg/ha/yr)	% of mulch N	Prot/g soil x 10^3 (Aug)	Bact Nemas (Aug)	Nema MI (Aug)	AM coloniz. (Oct. 2000)
Control	119 c			6 c	568 c	2.07 abc	60 a
Alfalfa	529 a	410	99	148 a	1128 bc	1.83 bc	32 b
Wood chips	218 bc	99	Not calc.	12 c	570	2.34 a	66 a
Paper	255 bc	136	283[A]	16 bc	1007 bc	2.05 abc	84 a
Clover	405 ab	286	Not calc.	218 a	2012 a	1.82 c	60 a
Rye	224 bc	105	Not calc.	20 bc	1577 ab	2.12 ab	60 a

[A] Because this quantity is greater than can be accounted-for by decomposition of the added paper, it represents immobilization and cycling of N by the microbial biomass.

Table 5. *Mulch/cover crop trial at Summerland:* Estimated annual transfer of N from microbial biomass to microfauna (N flux), increase in N flux due to amendment (ΔNflux), percentage of total amendment N entered into microbial N cycle, and three potential bio-indicators of N mineralization (protozoan abundance, bacterivorous nematode abundance, and the nematode community Maturity Index). Data for N flux were averaged over three sample dates before analyses.

				Potential bio-indicators		
Treatment	N flux (kg/ha/yr)	ΔNflux due to mulch (kg/ha/yr)	% of mulch N	Prot/g soil x 10^3 (Sept)	Bact Nemas (Sept)	Nema MI (Sept)
Control	318 b			101 b	1330	2.11
Spray-on mulch	377 b	59	Not calc.	121 b	1305	2.05
Clover	753 a	435	Not calc.	327 a	2757	2.00
Rye	430 b	112	Not calc.	271 ab	1472	1.99

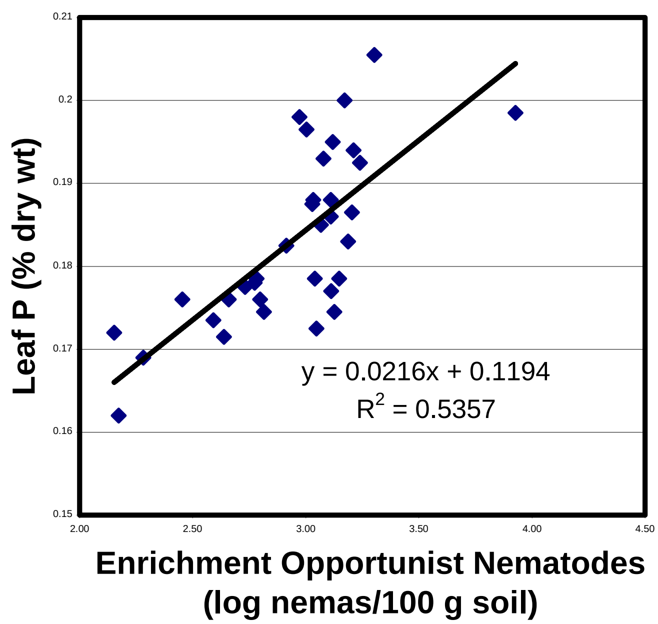
Bio-indicators:

Analyses of trophic structure/dynamics of the soil food web, as described above, require detailed calculations and are not amenable for use as a quick or simple indicators of nutrient mineralization status of the soil ecosystem that could be undertaken by commercial or state extension laboratories. Our approach to identifying bio-indicators is to: 1) identify biotic variables for which there is a functional basis for correlation with mineralization; and 2) correlate these parameters with independent measurements of mineralization.

The abundance of enrichment-opportunist nematodes (Enrichment Index) was correlated with leaf P in the Summerland trial (Figure 1). The Maturity Index (presented in tables) is a related expression of the relative abundance of enrichment-opportunist nematodes, and was also correlated with leaf P at Summerland (data not shown). Several of the potential bio-indicators were correlated with estimated fluxes of N (See all tables), but none of the potential bio-indicators were correlated with leaf N. The lack of correlation with leaf N is not surprising. Fertilizer N applied regularly to all treatments at all experiments, has obscured the expression of mulch-derived N in leaves. The data clearly show that the abundance of enrichment opportunist nematodes, or some index derived from it, could be an indicator of organic mulch-enhanced availability of phosphorus.

Measurement of microbial biomass has been evaluated as a possible simple, “wholistic” measure of microbial activity in the root zone. Because measurement of microbial biomass does not require any biological expertise, it may be more likely adopted for routine analyses. In 2000, microbial biomass C was measured using a microwave-lysis/extraction/dichromate digestion approach. Due to extreme variation, we seldom found statistically significant differences between treatments. In 2001 we began evaluation of an alternative, simpler approach for measuring microbial biomass N. The approach involves lysis of microbial cells with hexanol, extraction with potassium sulphate, and measurement of absorbance of 280 nm light. While the method appears to be less prone to extreme analytical variation than the microwave/dichromate approach, it also seldom revealed differences between treatments (data not shown; see Hogue et al. 2001 progress report). This was partially the result of inadequate analytical replication in 2001, and partially because standing-crop microbial biomass is inherently less sensitive to treatment differences than faunal indicators. However, being a new approach, the method is still under evaluation.

Figure 1. Relationship between the average (over 3 sample dates representing 1999 & 2000) abundance of enrichment opportunist nematodes and leaf P (averaged over 1999 and 2000). Data were taken from the mulch trial at Summerland.



Root-lesion nematodes and mycorrhizae:

Root-lesion nematodes were not sufficiently abundant at either of the experimental sites in Washington to warrant statistical analyses. In the cover crop trial at Summerland, population densities tended to be greatest under clover, but the effects were not statistically significant. In the primary mulch experiment at Summerland, population densities under the paper mulch treatments (with and without biosolids) were significantly lower than in controls (data not shown).

Colonization of roots by mycorrhizal fungi was reduced significantly, relative to the controls, under alfalfa hay at the Wenatchee Valley College trial and alfalfa hay and biosolids at Summerland (Tables 2 and 4). Colonization was increased, relative to the control, under the shredded paper mulch at Summerland. Mycorrhizal colonization of roots is known to be suppressed under conditions of high phosphorus availability, and we consider it likely that high phosphorus availability in the alfalfa and biosolids treatments were responsible for the apparent suppression in mycorrhizae. Mycorrhizae are important for uptake of Zn and Cu, and may also help suppress pathogens. Analyses are currently underway to relate mycorrhizal colonization to Zn and Cu uptake.

Epilogue:

It is important to note that several of the materials considered in this study were recognized at the outset to contain considerable amounts of organic N (e.g. biosolids, manure, alfalfa hay), and the release of N from these materials, when incorporated into soil of annual crops, is well documented. We initiated studies on the mineralization of N from these materials because we recognized that mineralization rates would not likely be the same when applied to the soil surface, in relatively large quantities, to the root zone of a perennial crop. Furthermore, there has been no previous research to document the mineralization dynamics of these materials when co-applied with mulches of shredded paper. The study was successful in demonstrating that even surface-applied materials can have profound influences on cycling of N by the microbial biomass. The study also provided a basis for ranking treatment combinations in terms of their contributions to N mineralization. The sampling was insufficient to allow for firm estimates of *actual* annual mineralization rates. Additional research is necessary to go beyond describing the effects of organic mulches in relative terms, and to measure actual annual mineralization rates. Such information could be used to recommend reduced fertilizer rates when and where organic mulches are used. We would like to thank the WTFRC for supporting this research.

Budget:

Year	**2000**	**2001**
Total	$11,584	$9,150
Wages	$11,584	$8450
Equipment		
Travel		$700

TOTAL: 20,734