AN ABSTRACT OF THE THESIS OF

Anne Elizabeth Taylor for the degree of Baccalaureate of Science in Bioresource Research with an option in Sustainable Ecosystems presented on June 9, 2000. Title: Suitability of selected enzyme assays as future soil quality indicators.

Abstract approved:_____

Richard P. Dick

Deforestation, intensive agriculture and chemical use all can cause long-lasting negative impacts on soil health. With all the vital functions that soils perform and the potential for further soil degradation, it is important that standards of soil quality or soil health be implemented. Soil aggregation is considered to be a good measure of soil quality because aggregation influences ecosystem function. Erosion, pollution, over-grazing, cultivation, land clearing and compaction negatively impact aggregation. Activities of the microbial biomass, fungi and bacteria, promote aggregation; any measure of soil quality should reflect this relationship. This project used enzyme assays as a surrogate indicator of microbial biomass. It was hypothesized that soil with a higher percentage of macroaggregates would maintain and stabilize soil enzyme activity by increasing the amount of potential habitat for the microbial biomass, and offer greater potential to protect abiontic enzymes. The activity of soil enzymes show a rapid response to changes in management practices which makes them useful indicators of future soil health. This study tested the feasibility of using a microwave stress test (MWES) to gauge a soil's ability to protect enzymes from stressing factors. Two enzyme assays were chosen for this work, arylsulfatase and fluorescein diacetate hydrolysis (FDA). Soils were sampled and tested from agricultural soils under conventional management, clearcut forest sites and undisturbed forest sites. Enzyme activity for both assays was consistently lower for the agricultural soils than for the forest soils. The clearcut forest sites had activity approaching that of the undisturbed forest sites. This suggests that the resident microbial population can recover following a negative impact after a few years. Activity for the stressed forest soils fell to levels that were near those of the unstressed agricultural soils. It appears that enzyme activity in the agricultural soils is largely associated with the mineral fraction of agricultural soils, whereas in forest soils a greater amount is associated with organic matter. It also suggests that the abiontic enzymes associated with organic matter are more vulnerable to stresses such as MWES than are those on the mineral surfaces of the soil. Arylsulfatase activity was found to be too variable under developed MWES protocol to be of use. Testing protocol for FDA is still being developed.

Suitability of Selected Enzyme Assays as Future Soil Quality Indicators

by
Anne Elizabeth Taylor

A PROJECT

submitted to

Oregon State University

College of Agriculture

In partial fulfillment of
the requirements for the
degree of

Baccalaureate of Science in Bioresource Research

Presented June 9, 2000
Commencement June 11, 2000

Table of Contents

Suitability of Selected Enzyme Assays as Future Soil Quality Indicators

INTRODUCTION

All life on the earth relies on four essential resources: air, water, solar energy and soil. The amount of solar energy coming into our biosphere cannot be controlled, but we do have control over the use and management of the other three essential resources. It is important to safeguard these resources and so comprehensive standards of quality have been put in place for both water and air. Soils are inherently variable so it has not been possible to establish similar standards of quality. Soils have complex structure and biological communities and functions which makes it is a challenge to develop standards or soil quality indicators.

Soil quality is defined as, "the capacity of a soil to function within its ecosystem boundaries to sustain biological productivity and diversity, maintain environmental quality, and promote plant and animal health" (Brady and Weil, 1999). Soil quality standards must be capable of evaluating the soil's ability to perform basic functions, and also to determine if current management practices are beneficial or detrimental to future soil health. These standards also should be sensitive enough to evaluate whether newly implemented management practices are improving or degrading soil health.

All members of the food web are dependent on the soil as a source of nutrients and for degradation and cycling complex organic compounds. The organisms living in the soil decompose organic debris into inorganic nutrients making them available for uptake by plants and animals. Plants rely on soil not only for the uptake of nutrients but also as their medium for growth. Soil characteristics influence the mix of gasses and water in the root zone and contribute to determination of what types of plants will grow in a given area. Soil also plays an important part in the hydrologic cycle. All water has either moved through or over the soil. Water moving through the soil horizons leaches to groundwater where it eventually enters rivers. Pathogens and pollutants added directly to soil or with water can be degraded by soil organisms.

Soil functions can be disrupted by a number of natural events such as flooding and volcanic activity. But it is human activities that have a huge potential for causing

soil degradation. Deforestation, intensive agriculture and dumping of chemicals all can cause long-lasting negative impacts on soil health. With all the vital functions that soils perform and the potential for further soil degradation, it is important that standards of soil quality or soil health be implemented.

Soil Quality Indicator

A large number of chemical, biological, and physical properties have been measured on soils. These have included pH, bulk density, cation exchange capacity (CEC), aggregation, nutrient levels, microbial properties and percent soil organic matter. Most of these give only the present status or fertility of the soil, but not future soil fitness. Some soil properties change very slowly (decades to thousands of years) such as CEC and texture. The addition of organic matter to a soil is considered to be beneficial, but measurable increases of soil organic matter takes decades if not centuries. Further, Chesters et al. (1957) showed that additions of organic residues to sterile soils resulted in little improvement in soil structure. These properties that change so slowly characterize differences between soil types but are not sensitive to short-term soil management activities.

Aggregation (soil structure) is a better measure of soil quality because aggregation influences ecosystem function. Soils with relatively large structural aggregates have lower bulk density and a high proportion of macropores, which is desirable for many soil functions (Brady and Weil, 1999). Increased aggregation increases the soil's porosity and improves the movement of water and gases through soils. This is beneficial for resident microbial populations, plant root penetration and nutrient uptake. Erosion, pollution, over-grazing, cultivation, land clearing and compaction negatively impact aggregation. The activities of the microbial biomass, fungi and bacteria, promote aggregation. Bacteria and fungal hyphae exude sugar-like polysaccharides and other organic compounds, forming sticky networks that bind together individual soil particles and tiny microaggregates into larger ones. Root and microbial glues resist dissolution by water helping to ensure future stability (Brady and Weil, 1999).

Any measure of soil quality should reflect the relationship between aggregation and the soil microbial biomass. It is possible to assess populations of the fungi and bacteria in the soil but this process is tedious and perhaps not very accurate because of the huge diversity of organisms and vast number that still have not been described (Morton, 1999). Identifying the microorganisms in the soil is a difficult task but measuring their activities is a possible indicator of the microbial community. To carry out cycling and decomposition activities, microbial organisms produce enzymes to hydrolyze proteins, cellulose, lignin, etc., in order to utilize the carbon and nutrients (Casida, 1977; Hersman and Temple, 1979; Frankenberger and Dick, 1983). Therefore, it has been suggested that soil enzyme activity could be an integrative and cumulative indicator of the microbial community (Dick et al., 1996). Furthermore, there is evidence that it can be a surrogate measure of soil stability and aggregation (Dick, 1994). Enzyme assays also have the advantage of being rapid and relatively inexpensive in comparison to the labor-intensive procedures of standard soil physical and many biological methods.

Some of the enzymes in the soil are associated with living cells (biotic) while others are abiontic (Skujins, 1976), or exclusive of live cells. There are several sources for the abiontic enzymes. Some are excellular enzymes excreted from living cells into the soil solution to hydrolyze polymers that are too large or insoluble to be taken up directly by cells. Others are intracellular enzymes that leak into the soil solution from dead and lysed cells. These abiontic enzymes can be adsorbed and stabilized on the humic colloids and clay surfaces in the soil, remain catalytic, and reflect the cumulative effects of soil management on soil biology. Soil with a higher percentage of macroaggregates will maintain and stabilize soil enzyme activity by increasing the amount of potential habitat for the microbial biomass, and offer greater potential to protect abiontic enzymes (Miller and Dick, 1995).

Previous studies have shown that enzyme activities are sensitive to various soil management treatments, and respond rapidly to changes in management (Dick et. al., 1988b; Bandick and Dick, 1999). It is this rapid response to stress factors that has led to the proposed use of a microwave enzyme stress test (MWES) as a soil quality indicator. Earlier work by Miller and Dick (1995) provided preliminary evidence that improved soil management systems caused greater thermal stability of certain enzymes. It is

hypothesized that a higher quality soil (a soil with more aggregation and greater microbial biomass activity) will better protect biotic and abiontic enzymes from microwave-induced stress. A ratio of stressed and non-stressed soil enzyme activity should result in a larger value for a higher quality soil than for a less healthy soil. A ratio of stressed and non-stressed enzyme activity in soils may hold potential to provide a relative index that is independent of soil type or ecosystem.

Two enzyme assays have been chosen for this work, arylsulfatase and fluorescein diacetate hydrolysis (FDA). These were chosen because they are responsive to soil management and have been linked to the microbial biomass (Biederbeck, 1978; Schnurer and Rosswall, 1982; Bandick and Dick, 1999). The results from this research are a part of a larger EPA soil quality project being conducted by Dr. Richard Dick and his coworkers. It will include other measures of soil properties (e.g. clay content, microbial biomass, total C), and the enzyme assay for beta-glucosidase, which is another promising candidate for MWES.

Arylsulfatase

Arylsulfatase can exist in the soil solution as both a biotic and an abiontic enzyme (Abelmagid, 1980: Kiss et al., 1975). This enzyme cleaves sulfate (SO_4) from organic compounds making it available for plant and animal uptake. Microbial biomass contains only a small portion (2.3%) of the soil S, but this fraction is the most labile and has the greatest importance in the cycling of S in the soil (Biederbeck, 1978). Work done by Saggar et al. (1981) shows that most of the ester sulfate in the microbial biomass is in the fungal portion. It was determined that fungal material could have up to 42% of S in the form of ester sulfate while bacteria had around 10%. Storage of large quantities of S compounds in ester sulfate forms imposes severe osmotic pressure gradients in bacteria (Fitzgerald, 1973), which may explain why there is such a large difference in ester sulfate accumulation between fungi and bacteria.

Because the majority of ester sulfate in the soil will be in the fungal biomass, it is thought that the level of arylsulfatase activity will be surrogate indicator of this microbial population. High levels of the ester sulfate substrate would likely stimulate production of

sulfatases. Since fungi are known to be important in soil aggregation, this enzyme assay will reflect the relationship between microbial biomass, aggregation and soil health.

Fluorescein Diacetate Hydrolysis

Fluorescein diacetate (FDA) is hydrolyzed by a number of enzymes, such as proteases, lipases, and esterases (Guilbaut and Kramer, 1964; Rotman & Papermaster 1966), which has advantages as a broad indicator of enzymatic activity in soil. The ability to hydrolyze FDA is widespread, especially among the decomposers – bacteria and fungi (Schnurer and Rosswall, 1982). Schnurer and Rosswall (1982) showed that autoclaved soil had no FDA hydrolytic activity and that hydrolysis increased with the addition of fungi to autoclaved soil. Hydrolases also are know to exist in the soil solution as biotic and abiontic enzymes (Abelmagid, 1980: Kiss et al., 1975).

The ability to recycle nutrients is one of the major functions of a soil and the residue-decomposing organisms, fungi and bacteria, are the major contributors to soil enzyme activity. Because FDA hydrolysis broadly represents this microbial hydrolytic component an assay of FDA activity may give an index of this biological component of the soil (Perucci, 1992). Soil decomposers are the primary sources of soil enzymes (Capriel et al., 1990), and so it is possible that a correlative relationship exists between soil enzyme activities and soil structural parameters (Dick, 1994).

The objectives of this work were:

1. To measure enzyme activity over a range of ecosystems in order to investigate the potential of using ratios of enzyme activity to other soil properties as a soil quality index. A relative index like this would overcome the problem of variability between soil types. Other members of the research group will do these correlations.
2. To determine the best time of year to sample soils for enzyme assays.
3. To evaluate whether the arylsulfatase and fluorescein diacetate hydrolysis assays reflect management over the ecosystems studied.
4. To determine if MWES testing is feasible for the above enzymes and to develop the test protocol.

MATERIAL AND METHODS

Field sites and sampling

Soils were sampled for the enzyme assays at sites that represent different ecosystems and soil types. At each site, soil management treatments had been in place from six to approximately sixty years and some sites have been used for previous studies; (Bandick and Dick, 1999; Ndaiye et al., 2000). All sites have statistically valid design and replication. There is a consistent trend to have lower biomass C and enzyme activities in disturbed forest and agricultural soils (Dick etal. 1988, Whisler et al. 1965), so agricultural sites with conventional and improved management and forest sites with clearcut and undisturbed management were selected.

The first site sampled was the Residue Utilization Plots (RUP) at the Columbia Research Center, Pendleton, Oregon. Winter wheat is grown in a biennial cropping system on a Walla Walla silt loam (coarse-silty, mixed mesic Typic Haploxeroll). In the spring of each fallow year, one of three treatments is applied and mold-board plowed to a depth of 20 cm. The residue treatments which are applied on a biannual basis are: 1) straw and 90 kg nitrogen (N) ha^{-1} (N_{90}); 2) straw plus 2.24 Mg ha^{-1} pea vine (PV); 3) straw and 22.4 Mg ha^{-1} (MAN). The treatments at this site have been in place since 1931. The climate is semi-arid and the mean annual precipitation is 416mm.

The second site was the Vegetable Farm (VF) in Corvallis, Oregon. Broccoli and corn are grown in rotation on a Chehalis silt loam (fine-silty, mixed mesic Cumulic Ultic Haploxeroll). There are two winter treatments at this site that have been in place since 1994. These are: 1) winter fallow (FA), and 2) winter cover crop (CO) consisting of a legume and cereal mix. This site has a Mediterranean climate with a mean annual precipitation of 1040mm.

The remaining four sites were within a 3.5 square mile area in the Willamette National Forest east of Eugene, Oregon. Each of these sites had a clearcut (CL) adjacent to an undisturbed forest (UN) of Douglas-fir (*Pseudotsuga menziesii*). McKenzie Ambush (MA) waslogged in 1993, McKenzie Millstone (MM) in 1991, McKenzie Ambush V (MF) in 1992, and McKenzie Millstone Three (MT) in 1991. These sites

were replanted within three years of the timber harvest. The soil at the MA site was a Bohannon gravelly loam (Typic Haplumbrept). The other three sites were on a Klickitat stony loam (Typic Haplumbrept). All sites had a southern exposure and were at approximately the same altitude. The undisturbed sites were dominated by fir, and there were various shrub species and replanted Douglas-fir growing on the clearcut sites.

Sampling occurred in May, June, and August of 1999. Four samples were taken for each treatment, with the exception of the RUP where only two were taken. At the agricultural sites, 30 soil cores were taken 0-15 cm deep. For the forest sites, 30 samples of A horizon 0-15 cm deep were taken, with the O horizon excluded. Samples were mixed and sieved to 5 mm. Half of each sample was stored field-moist in a cooler at 4° C for biological assays and the rest was air-dried and then stored at 4° C.

Arylsulfatase

Arylsulfatase activity is measured by adding the substrate *p*-nitrophenyl sulfate to a soil in a buffered solution and then incubating under a standard temperature. The reaction is stopped and the amount of *p*-nitrophenol is then measured. One gram of soil is weighed into each of three 50 mL Erlenmeyer flasks. Four mL of acetate buffer [68g sodium trihydrate and 1.7 mL glacial acetic acid in 700 mL of deionized water (DI), mix and bring volume to 1L] and 0.25 mL of toluene was added to each flask. One mL *p*-nitrophenyl sulfate solution (0.312 g *p*-nitrophenyl sulfate mixed with 40 mL acetate buffer, bring to 50 mL volume) (PNS) is added to two of the flasks. The third is the control. Flasks are stoppered, swirled and incubated at 37°C for one hour. The reaction is stopped by adding 1 mL 0.5M calcium chloride ($CaCl_2$) and 4 mL 0.5M sodium hydroxide (NaOH) to all flasks and then adding 1 mL PNS to the control. The solution is filtered through Whatman #42 filter paper and then the absorbance measured on a spectrophotometer set at 410nm. The arylsulfatase activity is calculated by the formula:

*u*g *p*-nitrophenol/g soil/hr =

[(average sample abs - control abs)* (dilution rate)]/(g soil*abs/μg *p*-nitrophenol)

A reference standard was made from a 50 *ug p*-nitrophenol/mL solution as shown in the Appendix.

Fluorescein Diacetate Hydrolysis

This assay measures the rate of hydrolysis of fluorescein diacetate lipase substrate, $C_{24}H_{16}O_7$, by using a spectrophotometer to measure the amount of mg L^{-1} of fluorescein released. Three grams of soil is weighed into each of three 125 mL Erlenmeyer flasks. Fifty mL of 60 mM sodium phosphate buffer (Appendix) is added to the control. The substrate is made by dissolving 30 mg fluorescein diacetate, lipase substrate $C_{24}H_{16}O_7$ in 6 mL hot ethanol and adding to 1.0 L of sodium phosphate buffer solution. This solution hydrolyzes quickly, so it is important to make up substrate solution just before use and to add substrate to the buffer solution while mixing on a stirring plate. Fifty mL is added to each flask with soil, stoppered, and placed on a platform shaker (160 strokes per minute) for 3 hours at room temperature (25° C). When the incubation period is complete, the reaction is stopped by adding 2 mL of pure acetone to all flasks, swirling and then transferring approximately 30 mL of the soil solution to a 50 mL centrifuge tube. The suspension is centrifuged at 16,000 rpm for five minutes in a refrigerated centrifuge and then the supernatant was filtered through a Whatman no. 42 filter paper and the absorbance read on a spectrophotometer at 490 nm.
The activity is calculated with the following equation:

mg L^{-1} of fluorescein= ((absorbance *slope)*dilution)-constant)/(sample weight* 3 hrs.))

Calculate the standard curve as shown in the Appendix.

The fluorescein in the standards, and evolved in this assay, sorbs to the glassware used during the procedure. Therefore glassware was rinsed with in 1 N HCl bath for 15 minutes or soaking overnight in a solution of Conta70 to remove sorbed fluorescein.

MWES Testing

A standard microwave oven is used for this test. If multiple batches of soil are to be stressed, care needs to be taken because with each subsequent use the microwave will get "hotter". Eight watch glasses, each with 10 g of soil, were arranged in a circle in the oven. A beaker with 65 mL of DI water was placed in the center of the oven to absorb some of the radiant energy. The soil samples were then microwaved at 100% power for 10 minutes followed by the arylsulfatase assay, or 6 minutes followed by the FDA hydrolysis assay. The soil was immediately transferred off the hot watch glasses prior to assaying to avoid further stressing the soil.

The MWES ratios were calculated as follows:

$$\text{MWES ratio} = \frac{\text{Activity of microwave treated soil}}{\text{Activity of unstressed (control) soil}} \times 100$$

RESULTS AND DISCUSSION

Arylsulfatase

Tables 2, 3 and 4 show the results from the arylsulfatase assays at the agricultural sites over the three sampling dates. Several expected trends are shown with these data. At the RUP, soils treated with MAN consistently showed the highest enzyme activity, followed by PV and N_{90}. Dick et al. (1988b) found the same trend. This can be attributed to the greater organic inputs for MAN (22.4 Mg) and PV (2.24 Mg). Adding organic material to the soil can only increase the carbon levels in the soil slowly but it provides the microbial biomass with the energy needed to live and grow, which in turn increases the enzyme activity. A similar trend of more enzyme activity with increased organic inputs was shown at VF, where CO had more activity than FA. Most of the coefficient of variability (CV) values for these soils are within an acceptable range (<20%) and suggest this assay will give reproducible results.

Figure 1 shows graphically the data from Tables 2, 3 and 4. The treatments at the two sites show little change over the three sampling dates and have the same ranking between treatments. ANOVA statistical analysis shows that for the May and June samples that there was not statistical difference ($P<0.1$) between the CO and FA treatments.

Tables 5, 6 and 7 show the results from the arylsulfatase assays for the forest soils. Arylsulfatase activity in the forest soils was approximately 100-fold greater than for the agricultural soils. This increased activity was expected since forest soils are fungal dominated and the arylsulfatase assay disproportionately represents the fungal population, and because the forest soils have high soil organic levels. Soils high in organic matter have greater arylsulfatase base activity (Tabatabai and Bremner, 1970). The CV values for the forest soils ranged between 5 and 40%. This can be attributed in part to high spatial variabilities of organic matter in the soil. Also, there may be variability in fungal mycelium which are not distributed evenly in forest soil (Morton, 1999). This variability may make it necessary to take a greater number of samples in forest soils than in agricultural soils to overcome the high spatial variability.

The arylsulfatase activity at the forest sites did not consistently reflect the treatments (Fig. 2). It was assumed that the UN forest sites would have greater arylsulfatase activity than the CL sites because of the compaction and loss of biomass due to logging activities. The combination of both physical changes in soil properties, and the reduced root growth in compacted soils, has been shown to have a negative impact on microbial properties (Dick et al. 1988). Only the MM site showed a large decrease in activity for the CL samples, while there was a small decrease in activity at MA. The other two sites, MF and MT appear to have greater activity at the CL sites. ANOVA analysis shows that for these two sites there was no significant difference ($P<0.1$) between CL and UN.

That the CL sites could have the same level of activity as the UN might be explained by the fact that these sites have been undisturbed since replanting and the fungal populations already may have been reestablished. Increased sunlight in the clearcut may have increased the activity of photosynthetic nitrogen fixers, and other species that in turn benefited the fungal population.

Figure 3 shows the arylsulfatase activity at all sites for the June sampling dates. The arylsulfatase activity at the RUP always was lower than the activity at the VF even when there were added organic inputs. This is likely due to the inherent differences in soil types in affecting arylsulfatase levels.

The forest soils always had higher arylsulfatase activity than did the agricultural soils. This can be attributed to fungal domination in the forest soils due to fewer disturbances of the fungal hyphae networks. The logged forest sites generally had much greater activities did than the agricultural soils. This suggests that a single but high disturbance event such as logging is less detrimental than the potentially smaller annual disturbances in agricultural soils.

Figures 1 and 2 graphically show arylsulfatase activity over the three sampling dates. The agricultural sites and the forest sites show the same ranking for all sampling dates with relatively small changes over time. This is encouraging for the use of arylsulfatase as a soil quality indicator because it suggests there are not wide fluctuations over short periods. This means that when changes in arylsulfatase activity are measured,

it is more likely to reflect management effects other than seasonal or temporal fluctuations.

Developing test protocol for the MWES testing was a challenge. Several different microwave times were tried with and without a beaker of water in the oven. When 10 minutes in the microwave without a beaker of water was used, the activity in the forest soils went below zero. When it was tried again with fewer minutes then the agricultural soils would not show the effects expected for the treatments. After trial and error, ten minutes in the microwave with 65 mL of water in a beaker seemed to give the best results.

Tables 8 and 9 show the data from the MWES testing for the agricultural soils from the May sampling date. The averages are given in Table 10. Tables 11, 12, 13 and 14 show the data for the forest soils for the May sampling date. Averages are given in Table 15. The values were highly variable and there were high CV values associated with most of the soil samples; Figure 4 shows this graphically. Some of the samples had many extra replications in an effort to determine what the stressed values really were. The high error indicates that the MWES test would not be useful as a soil quality indicator. However, it is interesting to note that β-glucosidase in agricultural soils has shown more promise as a MWES test (N. Pascoe, personal communication., 2000). This indicates that these two enzymes may be protected differently in soils and that arylsulfatase is more susceptible to microwave stress than is β-glucosidase.

MWES ratios are shown in Figures 5 and 6. Though the CV values are high, it illustrates the greater relative decrease in activity in the forest sites over the agricultural sites. The unstressed forest soils have much greater amounts of organic material and much higher arylsulfatase activity than do the agricultural soils, and it could be speculated that the greater relative decrease in the forest soils is due to the enzymes on the organic material being more exposed to microwave radiation than the enzymes on the mineral soil. When the stressed activity levels of the forest sites are compared with the unstressed agricultural soils they are very similar. This may give some important clues to how soils protect abiontic enzymes.

Fluorescein Diacetate Hydrolysis

The procedure followed was adapted (M. Diack, D. Stott and R.P. Dick, personal communication, 2000) from the method described by Schnurer and Rosswall (1982). This was done because the older method had phosphate precipitation in the buffer while trying to make the absorbance readings. Originally for this new method, 10 mg of the FDA substrate was called for. However, the results from Table 16 suggest that on forest soils, substrate levels were limited as shown by low CV values and that all soils had similar levels of activity. The samples were run again with 30 mg of the substrate, which showed an increase in enzyme activity (Table 17, Figure 7). All remaining forest soils were run at 30 mg FDA substrate. The agricultural soils were run at 10 mg FDA substrate because these soils had 10% of the activity of forest soils and substrate was not limiting the reaction

The FDA hydrolytic activity for the agricultural soils is shown in Tables 18, 19 and 20. The CV values were very low. At the VF the CO activity was always higher than the FA. This was expected because the higher organic inputs would stimulate more activity from the decomposers. The results from the RUP are less clear. During the May sampling the N_{90} activity was higher than the PV activity, but for June and September the order was N_{90}<PV<MAN. These results were similar to those obtained by Bandick and Dick (1999).

Figure 8 shows FDA hydrolytic activity for all the agricultural sites. The ANOVA ($P<0.10$) showed little difference on FDA hydrolysis between treatments at many of the sites. Figure 9 shows the FDA hydrolytic activity at the forest sites for the June sampling date. The treatments with the same letters are not significantly different ($P<0.1$). As with the arylsulfatase data, some of the forest sites have the same activity for both the CL and UN treatments. For the MT site, the CL activity is significantly higher than the UN. Since both the arylsulfatase assay and the FDA assay show little difference between the CL and UN treatments, it can be assumed that the activity of the soil decomposers will return to pre-disturbance levels given time. Distinguishing between CL and UN treatments that have been replanted and allowed to grow should not be a qualification because these sites seem to have recovered. But an assay for a soil quality

index should be able to distinguish between agricultural sites that have been place for many years, as is the case at the RUP. Although the FDA assay has good reproducibility (CV's mostly under 20%), it does not seem to be sensitive for detecting soil management and may not be useful as a soil quality index.

Figure 10 is an overview of FDA activity for all sites and treatments during the June collection date. The VF and RUP have very similar activity levels as opposed to arylsulfatase activity at the VF and RUP, which had different ranges. This suggests hydrolysis is less affected by soil type than is arylsulfatase. FDA hydrolysis rate for the CL forest soils is much higher than the agricultural soils indicating that the organisms that produce the hydrolyzing enzymes are able to recover from a major stressing event such as logging, but they are severely depressed by regular tillage that occurred on the agricultural sites.

Similar results to arylsulfatase activity were found for FDA hydrolysis over the season (Fig. 8). Activity levels remained fairly constant over the three sampling periods.

Work is still in progress on MWES test for FDA hydrolysis. Five minutes in the microwave produced no difference in enzyme activity. At six minutes there begins to be some decrease between MWES stressed and non-stressed soil samples. Table 21 shows data from the agricultural soils collected in June. There are higher CVs associated with the stressed data than with the unstressed data. Figure 11 shows the ratios between stressed and unstressed samples. There is some separation due to treatment. Further work with this enzyme at other microwave times needs to be done to evaluate the feasibility of using this enzyme assay as a soil quality index.

SUMMARY

The results showed there was low temporal variability for arylsulfatase activity and FDA hydrolysis. It should be kept in mind that this work was done on air-dried soil samples which may diminish the role of the viable populations in their contribution to enzyme activity. Thus different results might occur with field-moist soils. None-the-less, use of air-dried soils, particularly with arylsulfatase activity, shows a good potential in detecting long-term trajectory of soil management without wide seasonal fluctuations.

Enzyme activity for both assays was consistently lower for the agricultural soils than for the forest soils. The clearcut forest sites had activity approaching that of the undisturbed forest sites. This suggests that the resident microbial population can recover after a negative impact after a few years. While MWES testing using arylsulfatase is not feasible due to high rates of variability, it may give some insight into how the enzymes are protected in the soil matrix. Activity for the stressed forest soils fell to levels that were near those of the unstressed agricultural soils. This might suggest that the enzyme activity in the agricultural soils is largely associated with the mineral fraction of agricultural soils, whereas in forest soils a greater amount is associated with organic matter. It also suggests that the abiontic enzymes associated with organic matter are more vulnerable to stresses such as MWES than are those on the mineral surfaces of the soil.

Neither arylsulfatase nor FDA were always able to distinguish between different treatments in the agricultural soils. This could have been because these assays were not sensitive enough to reflect the differences, or that there really were no substantial differences between treatments.

MWES testing using FDA seems to have less variability than arylsulfatase. More testing needs to be done at increased microwave stress times to ascertain if it will be a useful tool in determining soil quality.

LITERATURE CITED

Abdelmagi, H.M. 1980. Factors affecting nitrogen mineralization and nitrate reduction in soils. Ph.D. diss. Iowas Statte Univ., Ames (Diss. Abstr. 81-03424).

Bandick A.K., and R.P. Dick. 1999. Field management effects on soil enzyme activities. Soil Biol. and Biochem. 31:1471-1479.

Biederbeck V.O. 1978. Soil organic sulfur and fertility. *Soil Organic Matter.* M. Schnitzer and S.U. Khan, Eds, pp. 273-310. Elsevier, NY.

Brady, N.C. and R.R. Weil. 1999. The Nature and Properties of Soils. Prentice Hall Inc., Upper Saddle River, NJ. p. 152, 789.

Capriel, P., T. Beck, H. Borchert, and P. Harter. 1990. Relationship between soil aliphatic fraction extracted with supercritical hexane, soil microbial biomass, and soil aggregate stability. Soil Sci. Soc. Am. J. 54:415-420.

Casida L.E. Jr. 1977. Micorbial metabolic activity in soil as measured by dehydrogenase determinations. Appl. Environ. Microbiol. 43:630-636.

Chesters, G., O.J. Attoe, and O.N. Allen. 1957. Soil aggregation in relation to various soil constituents. Soil Sci. Soc. Am. Proc. 21:272-277.

Dick, R.P.,1994. Soil Enzyme Activities as Indicators of Soil Quality. Soil Science Society of America special publication no. 35:107-124.

Dick, R. P., D. P. Breakwell, R. F. Turco. 1996. Soil Enzyme Activities and Biodiversity Measurements as Integrateive Microbiological Indicators. Methods for Assessing Soil Quality, SSSA Special Publication 49.

Dick R. P., D.D. Myrold and E.A. Kerle. 1988a. Soil Science soc. Am. Journal Vol. 52 no. 2. p. 512-516.

Dick R.P., P.E. Rasmussen, and E.A. Kerle. 1988b. Influence of long-term residue management on soil enzyme activities in relation to soil chemical properties of a wheat-fallow system. Biol. Fertil. Soils 6:159-164.

Fitzgerald J.W. 1973. The formation of choline-o-sulphate by *Pseudomonas* $C_{12}B$ and other *Pseudomonas* species. Biochemistry Journal. 136, 361-369.

Frankenberger W.T. and W.A. Dick. 1983. Relationships between enzyme activites and microbial growth and activity indices in soil. Soil Sci. Soc. Am. J. 47:945-951.

Guilbaut, G.G., and D.N. Kramer. 1964. Fluoremetric determination of lipase, acrylase, alpha- and gamma-chymotrypsin and inhibitors of these enzymes. Anal. Chem. 36:409-412.

Hersman L.E., and K.L. Temple. 1979. Comparison of ATP, phosphatase, pectinplyase, and respiration as indicators of microbial activity in reclaimed coal strip mine spoils.
Soil Sci. 127:70-73.

Kiss, S., M.M. and I.L. Klevenskaya. 1985. Effect of soil compaction and moisture content on biological activity, nitrogen fixation, and composition of soil air. Sov. Soil Sci. 16:46-54.

Miller, M. and R.P. Dick. 1995. Thermal stability and activities of soil enzymes as influenced by crop rotations. Soil. Biol. Biochem. Vol.27. No.9. pp.1161-1166.

Morton, J.B. 1999. Principles and Applications of Soil Micorbiology. Prentice Hall Inc., Upper Saddle River, NJ. Pp. 72-93.

Ndiaye E.L., J.M. Sandeno, D. McGrathe, and R.P.Dick. 2000. Integrative biological indicators for detecting change in soil quality. American Journal of Alternative Agriculture. Vol. 12, Num. 1.

Perucci, P. 1992. Enzyme activity and microbial biomass in a field soil amended with

municipal refuse. Biol. Fert. Soils 14:54-60.

Rotman, B., and B.W. Papermaster. 1966. Membrane properties of living mammalian cells as studied by enzymatic hydrolysis of fluorogenic ester. Proc. Natl. Acad. Sci. U.S.. 55:134-141.

Saggar. S., J.R. Bettany and J.W. Stewart. 1981. Measurement of microbial sulfur in soil. Soil boil. Biochem. Vol 13. pp. 493-498.

Schnurer, J. and T. Rosswall. 1982. Fluorescein Diacetate hydrolysis as a measure of total microbial activity in soil and litter. Applied and Environmental Microbiology, June 1982, p. 1256-1261.

Skujins, J. 1976. Extracellular enzymes in soil. CRC Critical Reviews in Micorbiology. 4:383-421.

Tabatabai, M.A. and J.M. Bremner. 1970. Factors Affecting Soil Arylsulfatase Activity. Soil Sci. Soc. Am. Proceedings. 34:427-429.

Whisler, F.D., C.F. Engle, and N.M. Baughman. 1965. The effect of soil compaction on nitrogen transformations in the soil. W. Va. Agric. Exp. Stn. Bull. No. 516. Morgantown.

APPENDIX

Arylsulfatase Reference Standards

A reference standard was made from a 50 *u*g *p*-nitrophenol/mL solution as follows:

Dissolve 5 g of p-nitrophenol (Fisher BP612-1) in 1L of deionized water (5000 *u*g/mL).

Mix 10 mL of 5000 *u*g/mL solution in 90 mL H_2O (500 *u*g/mL).

Mix 10 mL of 500 *u*g/mL solution in 90 mL H_2O (50 *u*g/mL).

The standards are mixed to match the matrix of the samples using Table 1. Measure absorbance at 410 nm.

60 mM Sodium Phosphate Buffer for FDA Assay

Dissolve 22.74 g of sodium phosphate, $Na_3PO_4 \cdot 12H_2O$ in 700 mL deionized water, bring to 1 L volume and then adjust pH to 7.8 with 1 N HCl.

FDA Reference Standards

The standard curve is obtained with standards containing 0, 0.2, 0.4, 1.0, 2.0, 3.0, and 4.0, mg L^{-1} of fluorescein by pipetting 0, .05, .10, .25, .50, .75, and 1.0 mL of the FDA stock solution into 50 mL volumetric flasks. One and nine-tenths mL of acetone is added (to match the matrix of the samples) and brought to 50 mL volume with sodium phosphate buffer (pH 7.8). The absorbance is measured at 490 nm on a spectrophotometer. The slope and the constant are found by calculating a regression of this line.

Table 1. Table for mixing arylsulfatase standards using 50 *u*g *p*-nitrophenol/mL solution.

.5M $CaCl_2$	.5M NaOH	Acetate Buffer (mL)	50 *u*g *p*-nitrophenol solution (mL)	*u*g *p*-nitrophenol in solution	Absorbance at 410 nm
1 mL	4 mL	6	0	0	
1 mL	4 mL	5.8	0.2	10	
1 mL	4 mL	5.6	0.4	20	
1 mL	4 mL	5.4	0.6	30	
1 mL	4 mL	5.2	0.8	40	
1 mL	4 mL	5	1	50	
1 mL	4 mL	4.8	1.2	60	
1 mL	4 mL	4.6	1.4	70	

Table 2. Arylsulfatase activity in agricultural soils for May sampling date.[1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
1	17.27	28.75	48.00	77.57	71.38
2	12.39	28.27	40.12	94.29	87.58
3	.	.	.	75.77	53.39
4	.	.	.	84.36	51.62
MEAN	14.83	28.51	44.06	83.00	65.99
STDS	3.45	0.34	5.57	8.39	16.93
CV	23	1	13	10	26

Table 3. Arylsulfatase activity in agricultural soils for June sampling date.[1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
1	14.89	27.75	43.74	74.03	66.34
2	13.06	26.23	33.27	103.16	61.94
3	.	.	.	53.33	44.86
4	.	.	.	67.06	40.80
MEAN	13.97	26.99	38.51	74.40	53.48
STDS	1.30	1.07	7.40	21.02	12.54
CV	9	4	19	28	23

Table 4. Arylsulfatase activity in agricultural soils for September sampling date.[1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
1	12.48	19.48	43.80	68.94	68.08
2	10.54	22.34	34.57	77.97	59.79
3	.	.	.	69.32	47.56
4	.	.	.	71.03	54.38
MEAN	11.51	20.91	39.18	71.81	57.45
STDS	1.37	2.02	6.53	4.20	8.67
CV	12	10	17	6	15

1 RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendmen MAN = manure ammendment, CO = winter cover crop, FA = winter fallow.

Table 5. Arylaulfatase activity in forest soils for the May sampling date. [1]

	M A		M F		M M		M T	
Replication	CL	UN	CL	UN	CL	UN	CL	UN
1	122.07	196.05	236.08	187.12	118.69	251.19	271.79	386.67
2	97.20	161.83	262.46	250.43	142.65	261.47	278.45	310.41
3	89.40	87.84	223.97	228.53	256.27	391.73	216.55	201.09
4	118.41	190.20	205.51	170.05	146.34	181.14	350.38	268.81
MEAN	106.77	158.98	232.00	209.03	165.99	271.38	279.29	291.74
STDS	15.95	49.72	23.88	36.94	61.43	87.81	54.92	77.68
CV	15	31	10	18	37	32	20	27

Table 6. Arylsulfatase activity in forest soils for June sampling date. [1]

	M A		M F		M M		M T	
Replication	CL	UN	CL	UN	CL	UN	CL	UN
1	91.75	211.40	393.87	376.36	130.31	461.06	422.07	415.85
2	95.43	182.68	313.35	272.06	175.75	392.64	324.12	438.43
3	78.60	339.50	212.22	244.30	146.76	654.56	485.11	386.93
4	143.85	181.95	363.36	154.67	162.58	304.60	549.24	402.48
MEAN	102.41	228.88	320.70	261.85	153.85	453.21	445.14	410.92
STDS	28.56	75.01	79.57	91.31	19.67	148.73	95.94	21.82
CV	28	33	25	35	13	33	22	5

Table 7. Arylsulfatase activity in forest soils for September sampling date.[1]

	M A		M F		M M		M T	
Replication	CL	UN	CL	UN	CL	UN	CL	UN
1	115.49	173.30	342.96	317.36	185.90	542.78	324.93	494.71
2	84.50	128.33	200.28	262.63	188.51	423.61	268.12	466.01
3	56.57	221.02	270.53	316.95	290.55	499.88	355.27	291.20
4	68.92	174.85	470.56	284.48	116.66	172.37	439.20	370.75
MEAN	81.37	174.37	321.08	295.36	195.40	409.66	346.88	405.67
STDS	25.46	37.85	115.43	26.71	71.63	165.69	71.37	92.90
CV	31	22	36	9	37	40	21	23

1 MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, Un = undisturbed forest.

Table 8. Arylsulfatase activity for May at RUP when soil samples were stressed for ten minutes with 65 mL of water. [1]

Replication	N90	N90	PV	PV	MAN	MAN
	ug p-nitrophenol/g soil/hr					
1	12.71	11.78	21.67	15.39	10.97	53.60
2	68.45	5.29	44.34	12.96	41.63	36.76
3	11.26	6.53	20.75	15.65	28.99	30.10
4	7.78	.	20.13	.	.	26.96
5	.	.	.	.	.	34.08
6	.	.	.	.	.	26.48
MEAN	25.05	7.87	26.72	14.67	27.20	34.66
STDS	29.01	3.45	11.76	1.48	15.41	10.11
CV	116	44	44	10	57	29

Table 9. Arylsulfatase activity at VF for May when soils were stressed for 10 min with 65 mL of water. [1]

Replication	CO	CO	CO	CO	FA	FA	FA	FA
	ug p-nitrophenol/g soil/hr							
1	16.957	26.291	17.14	35.629	21.648	27.555	18.544	19.598
2	6.704	26.66	21.183	36.034	20.25	27.929	13.286	22.423
3	20.274	32.663	18.554	25.629	27.886	33.293	16.015	21.664
4	20.161		18.208		20.306		18.945	
5			18.336				17.137	
MEAN	16.02	28.54	18.68	32.43	22.52	29.59	16.79	21.23
STDS	6.40	3.58	1.50	5.89	3.63	3.21	2.28	1.46
CV	40	13	8	18	16	11	14	7

Table 10. Summary of stressed data for agricultural soils collected in May. [1]

	RUP			V F	
	N90	PV	MAN	CO	FA
MEAN	16.46	20.69	30.93	23.92	22.53
STDS	16.23	6.62	12.76	4.34	2.65
CV	80	27	43	20	12

1 RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendmen MAN = manure ammendment, CO = winter cover crop, FA = winter fallow.

Table 11. Arylsulfatase activity for May at MA for stressed soils. [1]

Replication	CL1	CL2	CL3	CL4	UN1	UN2	UN3	UN4
				ug p-nitrophenol/g soil/hr				
1	14.95	11.43	11.02	19.04	36.36	30.46	14.22	45.92
2	1.94	21.75	5.33	40.97	30.29	15.72	20.14	67.51
3	9.02	3.14	14.03	9.55	49.30	39.91	24.90	
4	29.40	26.51	29.35	48.97	47.15	38.87	4.97	
5	40.00	32.32	13.64	30.25	46.40			
6	43.00							
7	20.49							
MEAN	22.69	19.03	14.67	29.76	41.90	31.24	16.06	56.71
STDS	15.49	11.73	8.91	15.95	8.19	11.18	8.59	15.26
CV	68	62	61	54	20	36	53	27

Table 12. Arylsulfatase activity for May at MF for stressed soils. [1]

Replication	CL1	CL2	CL3	CL4	UN1	UN2	UN3	UN4
				ug p-nitrophenol/g soil/hr				
1	14.00	54.07	62.06	61.97	45.73	39.91	58.93	10.38
2	39.52	76.91	13.56	59.70	51.70	65.36	58.51	34.09
MEAN	26.76	65.49	37.81	60.84	48.71	39.91	58.93	10.38
STDS	18.05	16.16	34.29	1.60	4.23	18.00	0.30	16.77
CV	67	25	91	3	9	45	1	161

Table 13. Arylsulfatase activity for May at MM for stressed soils. [1]

Replication	CL1	CL2	CL3	CL4	UN1	UN2	UN3	UN4
				ug p-nitrophenol/g soil/hr				
1	35.27	54.26	83.80	49.11	70.39	50.94	41.60	37.62
2	44.99	47.45	121.17	41.69	78.78	27.69	54.66	40.32
MEAN	35.27	54.26	83.80	49.11	70.39	50.94	41.60	37.62
STDS	6.88	4.81	26.42	5.25	5.93	16.44	9.23	1.91
CV	19	9	32	11	8	32	22	5

Table 14. Arylsulfatase activity for May at MT with stressed soils. [1]

Replication	CL1	CL2	CL3	CL4	UN1	UN2	UN3	UN4
				ug p-nitrophenol/g soil/hr				
1	64.58	64.56	68.02	130.18	45.04	39.18	18.91	20.25
2	54.22	28.97	41.97	106.99	65.74	48.47	37.18	40.47
3	41.35				67.05			
MEAN	53.38	46.77	55.00	118.59	59.28	43.82	28.05	30.36
STDS	11.64	25.17	18.42	16.40	12.35	6.57	12.91	14.30
CV	22	54	33	14	21	15	46	47

[1] MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, Un = undisturbed forest.

Table 15. Summary of arylsulfatase stressd data for forest soils, May sampling period.[1]

	M A		M F		M M		M T	
	CL	UN	CL	UN	CL	UN	CL	UN
MEAN	21.54	36.48	47.72	38.74	55.61	50.14	41.35	30.84
STDS	13.02	10.80	17.52	9.82	10.84	8.38	68.43	40.38
CV	61	34	46	54	18	17	18	12

Table 16. FDA hydrolysis activity in forest soils during June sampling using 10 mg substrate.[1]

	M A		M F		M M		M T	
Replication	CL	UN	CL	UN	CL	UN	CL	UN
				ug fluorescein/L				
1	23.385	26.151	23.093	19.432	22.634	25.070	24.669	23.498
2	27.237	25.379	24.033	22.549	25.478	25.565	24.193	22.734
3	24.177	24.821	23.751	23.708	23.952	25.620	22.829	20.305
4	28.208	22.199	21.999	25.491	24.138	24.727	24.236	23.291
MEAN	25.75	24.64	23.22	22.80	24.05	25.25	23.98	22.46
STDS	2.3	1.7	0.9	2.5	1.2	0.4	0.8	1.5
CV	9	7	4	11	5	2	3	7

Table 17. FDA hydrolysis activity in forest soils during June sampling using 30 mg substrate.[1]

	M A		M F		M M		M T	
Replication	CL	UN	CL	UN	CL	UN	CL	UN
				ug fluorescein/L				
1	46.30	44.40	66.10	69.65	53.63	80.60	68.10	53.60
2	45.50	45.60	70.20	75.40	75.10	85.90	61.10	55.10
3	33.90	62.20	60.30	77.70	73.80	79.70	65.70	54.50
4	67.50	55.20	58.44	73.40	79.50	63.03	70.60	58.00
MEAN	48.30	51.85	63.76	74.04	70.51	77.31	66.38	55.30
STDS	14.0	8.4	5.4	3.4	11.5	9.9	4.0	1.9
CV	29	16	8	5	16	13	6	3

Table 18. FDA hydrolysis activity in agricultural soils during May sampling. [1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
		ug fluorescein/L			
1	13.40	13.20	16.40	11.40	14.05
2	11.90	15.20	15.50	15.11	16.70
3	.	.	.	12.00	12.30
4	.	.	.	17.70	12.40
MEAN	12.65	14.20	15.95	14.05	13.86
STDS	1.1	1.4	0.6	2.9	2.1
CV	8	10	4	21	15

1 MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, Un = undisturbed forest. RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, FA = winter fallow.

Table 19. FDA hydrolysis activity in agricultural soils during June sampling.[1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
	ug fluorescein/L				
1	13.13	11.45	17.70	16.65	14.80
2	12.30	13.60	14.60	23.80	14.20
3				12.70	11.10
4				21.60	9.60
MEAN	12.72	12.53	16.15	18.69	12.43
STDS	0.6	1.5	2.2	5.0	2.5
CV	5	12	14	27	20

Table 20. FDA hydrolysis activity in agricultural soils during September sampling. [1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
	ug fluorescein/L				
1	11.65	13.60	21.18	14.80	14.20
2	12.53	16.51	18.25	14.00	12.10
3	.	.	.	15.10	12.00
4	.	.	.	18.50	11.95
MEAN	12.09	15.06	19.72	15.60	12.56
STDS	0.6	2.1	2.1	2.0	1.1
CV	5	14	11	13	9

Table 21. FDA hydrolysis activity for agricultural soils collected during the June and stressed in the microwave for six minutes. [1]

	RUP			V F	
Replication	N90	PV	MAN	CO	FA
	ug fluorescein/L				
1	3.780	7.646	7.251	4.327	5.287
2	4.187	11.125	8.130	10.512	6.894
3	.	.	.	6.189	4.548
4	.	.	.	7.596	3.705
MEAN	3.98	9.39	7.69	7.16	5.11
STDS	0.3	2.5	0.6	2.6	1.4
CV	7	26	8	36	27

1 RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendmen MAN = manure ammendment, CO = winter cover crop, FA = winter fallow.

Figure 1. Arylsulfatase activity for agricultural soils over the three sampling dates. ANOVA performed for each site. Treatments with the same letter are not significantly different at P<0.10. RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, and FA = winter fallow.

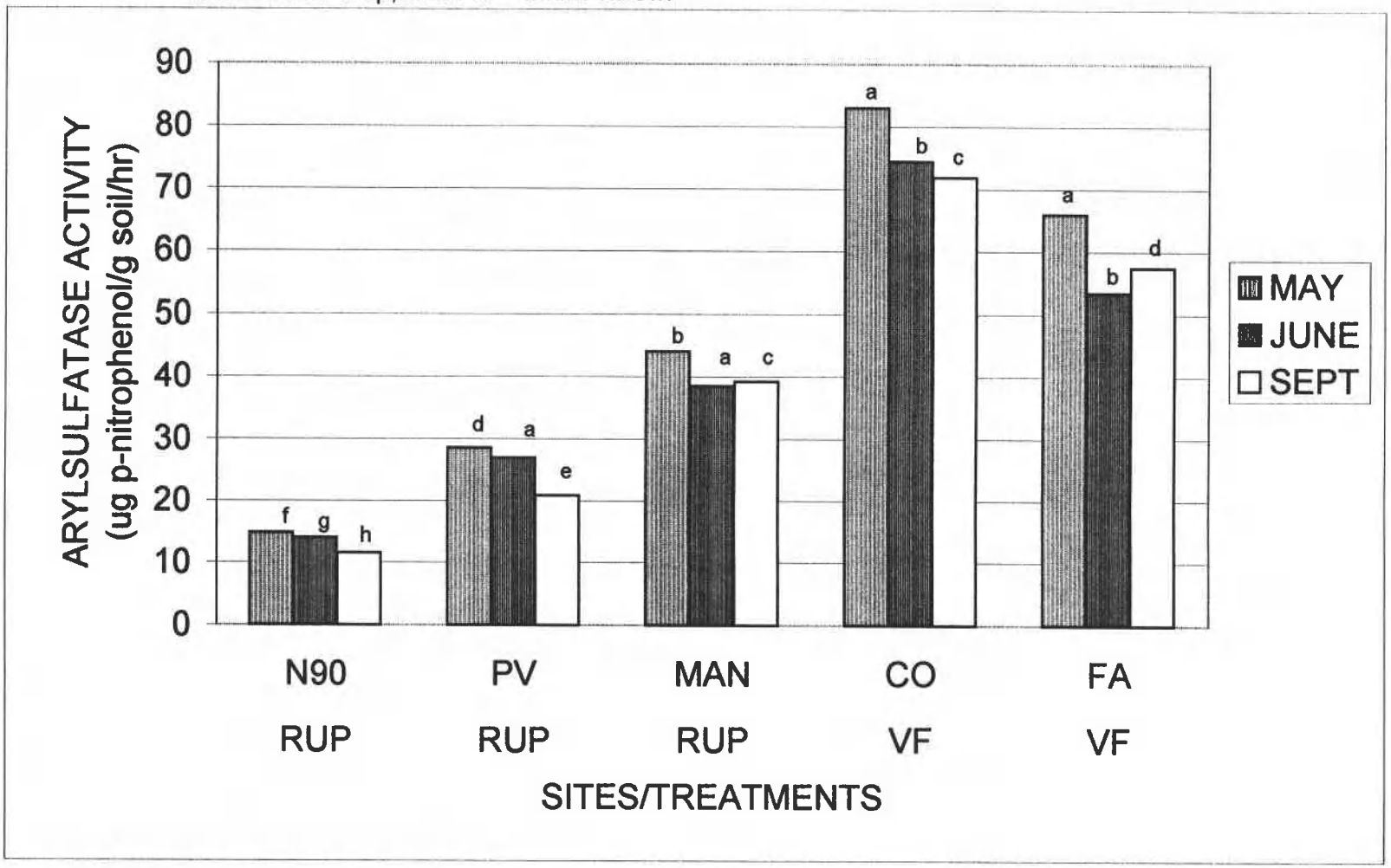

Figure 2. Arylsulfatase activity at forest sites over three sampling periods. ANOVA performed between treatments at each site. Treatments with the same letters are not significantly different (P<0.10). MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest.

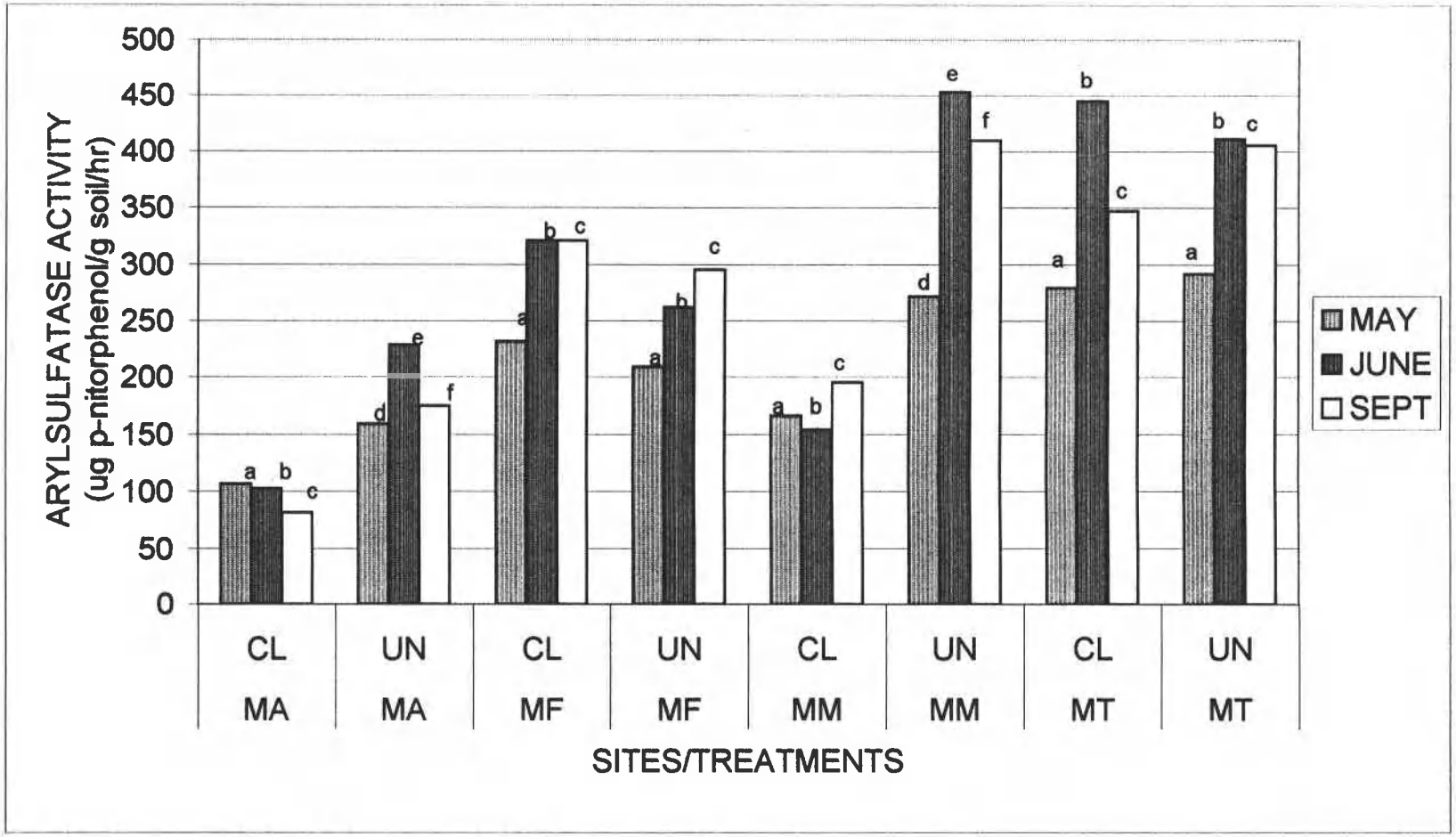

Figure 3. June arylsulfatase activity at agricultural and forest sites for all treatments.
RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, FA = winter fallow, MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest.

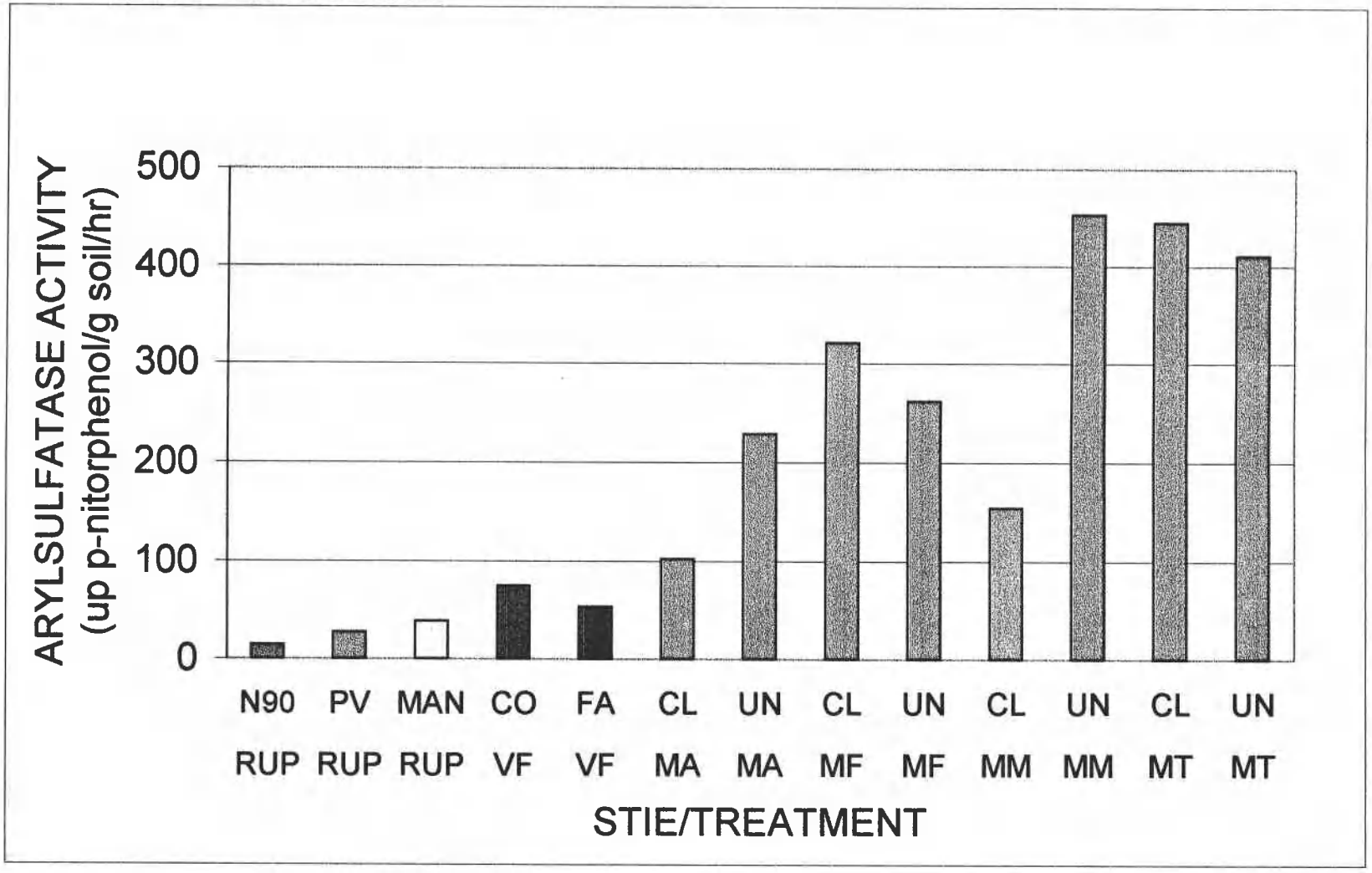

Figure 4. Arylsulfatase activity for agricultural and forest soils when microwave stressed for ten minutes with 65 mL of water. Bars indicate standard error. RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, FA = winter fallow, MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest

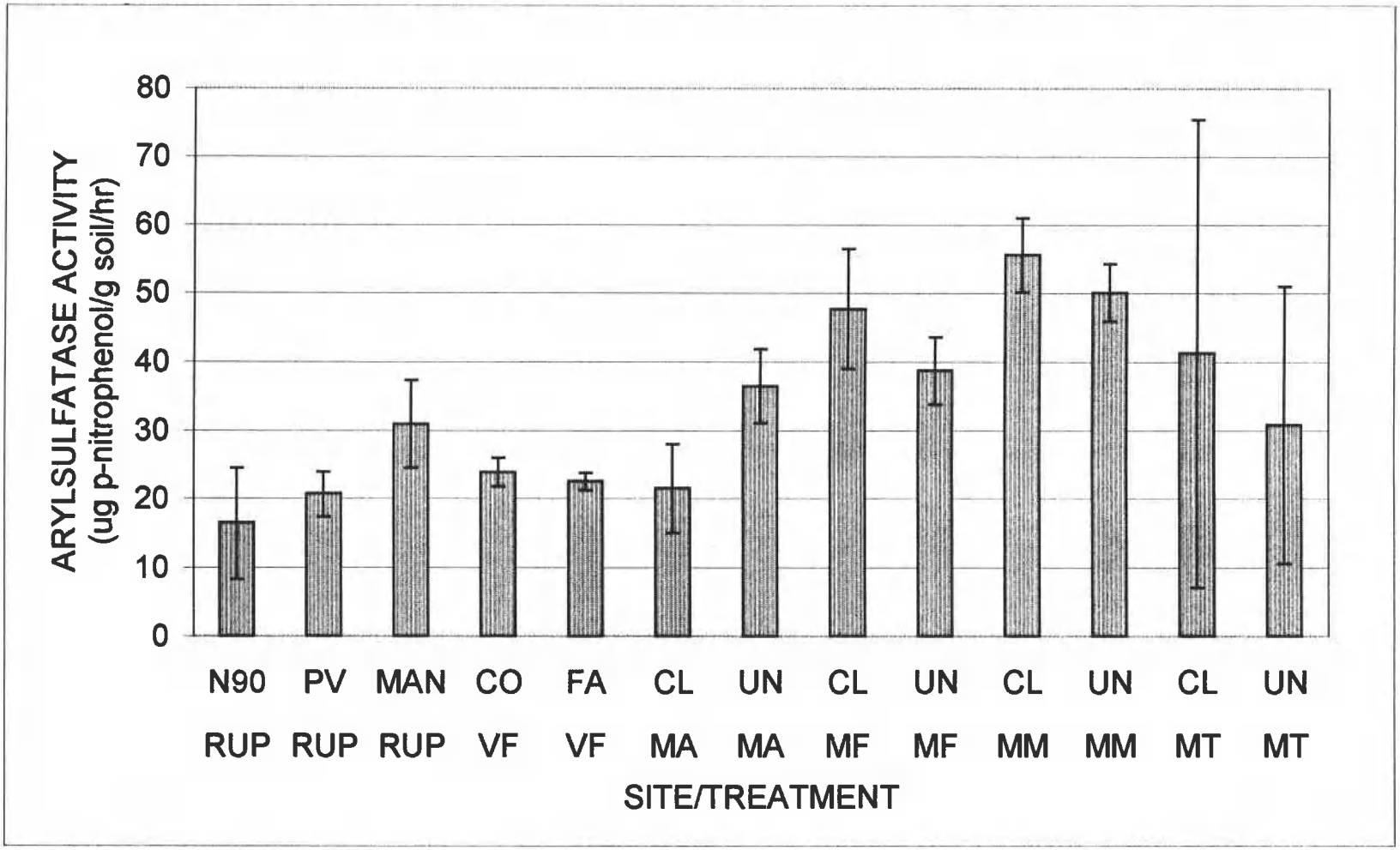

Figure 5. MWES ratios of arylsulfatase activity for agricultural soils collected in May.
RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, and FA = winter fallow.

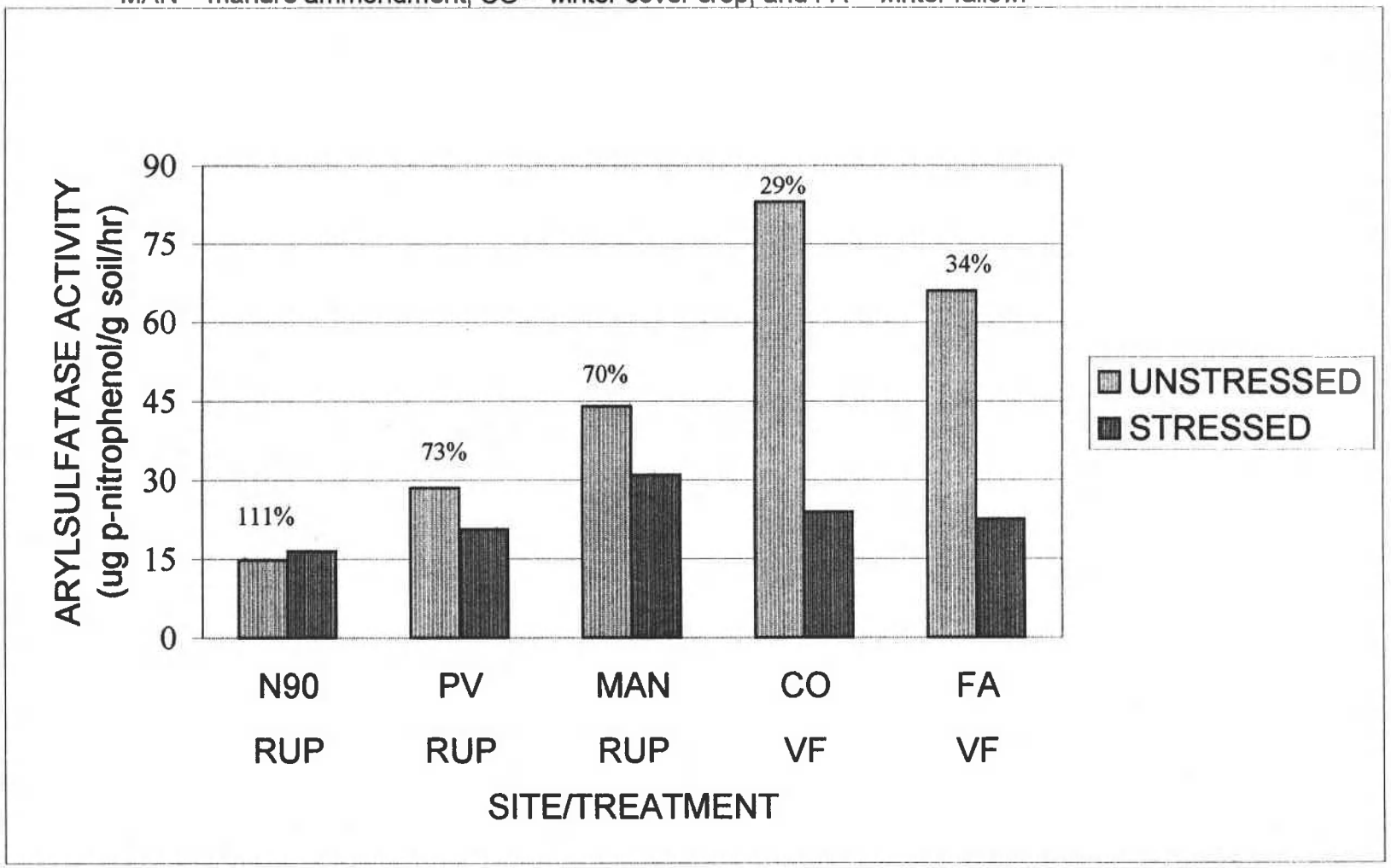

Figure 6. MWES ratios of arylsulfatase activity for forest soils collected in May.
MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone,
MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest.

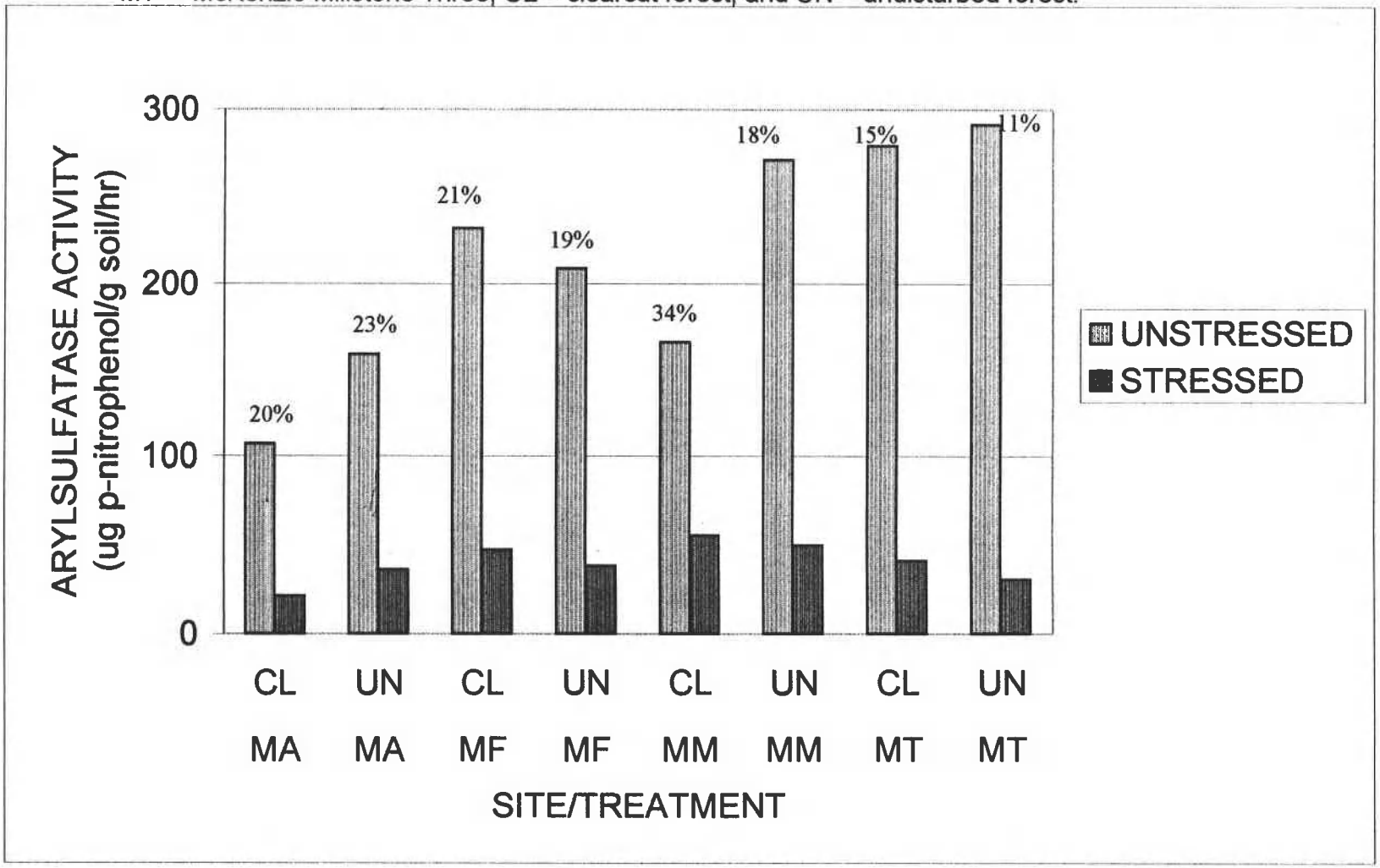

Figure 7. FDA activity in forest soils collected in June tested with both 10 mg and 30 mg substrate. MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest.

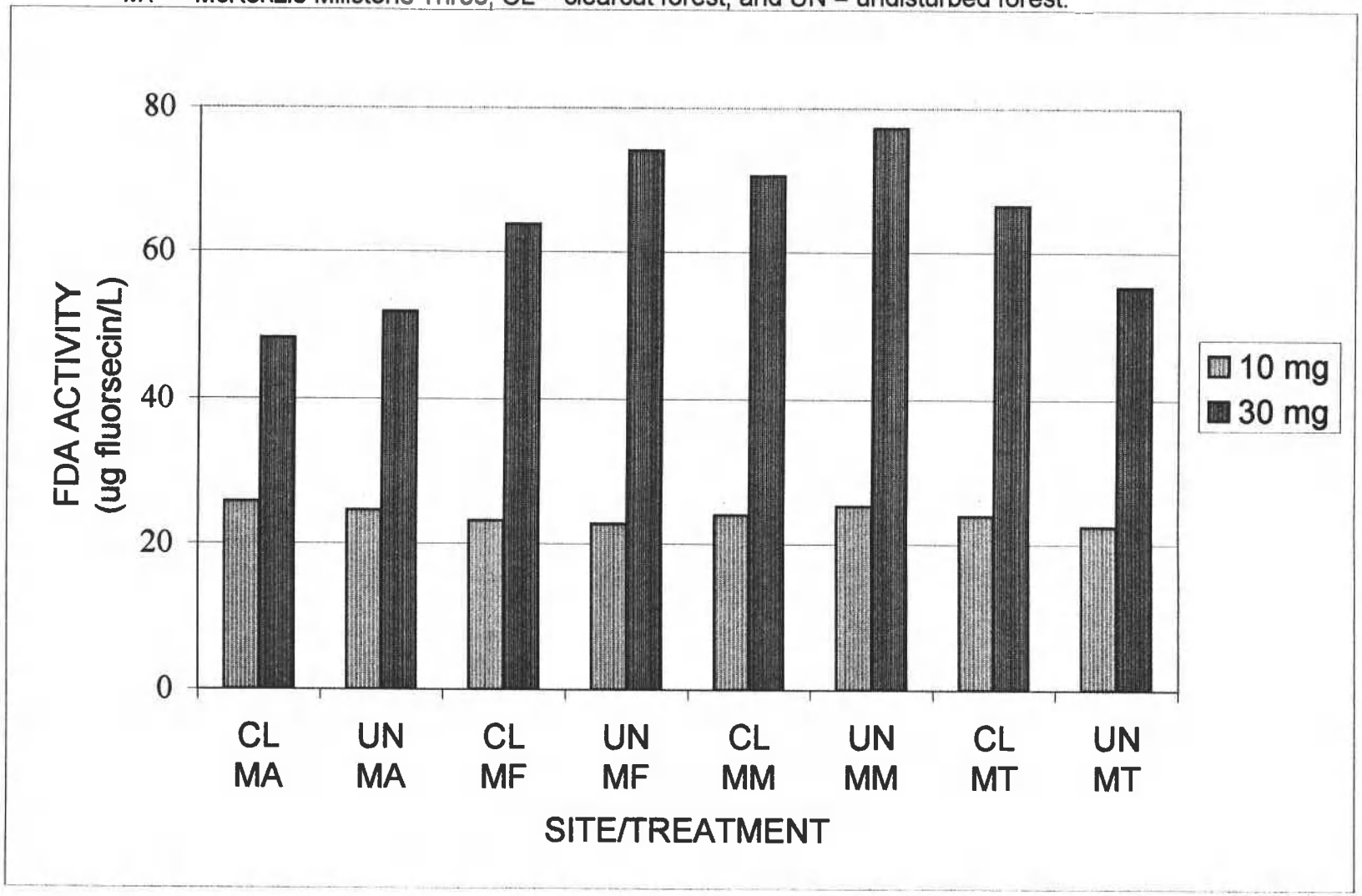

Figure 8. FDA activity for agricultural soils over three sampling dates. ANOVA performed at each site. Treatments with the same letter are not significantly different at P<0.10.
RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, and FA = winter fallow.

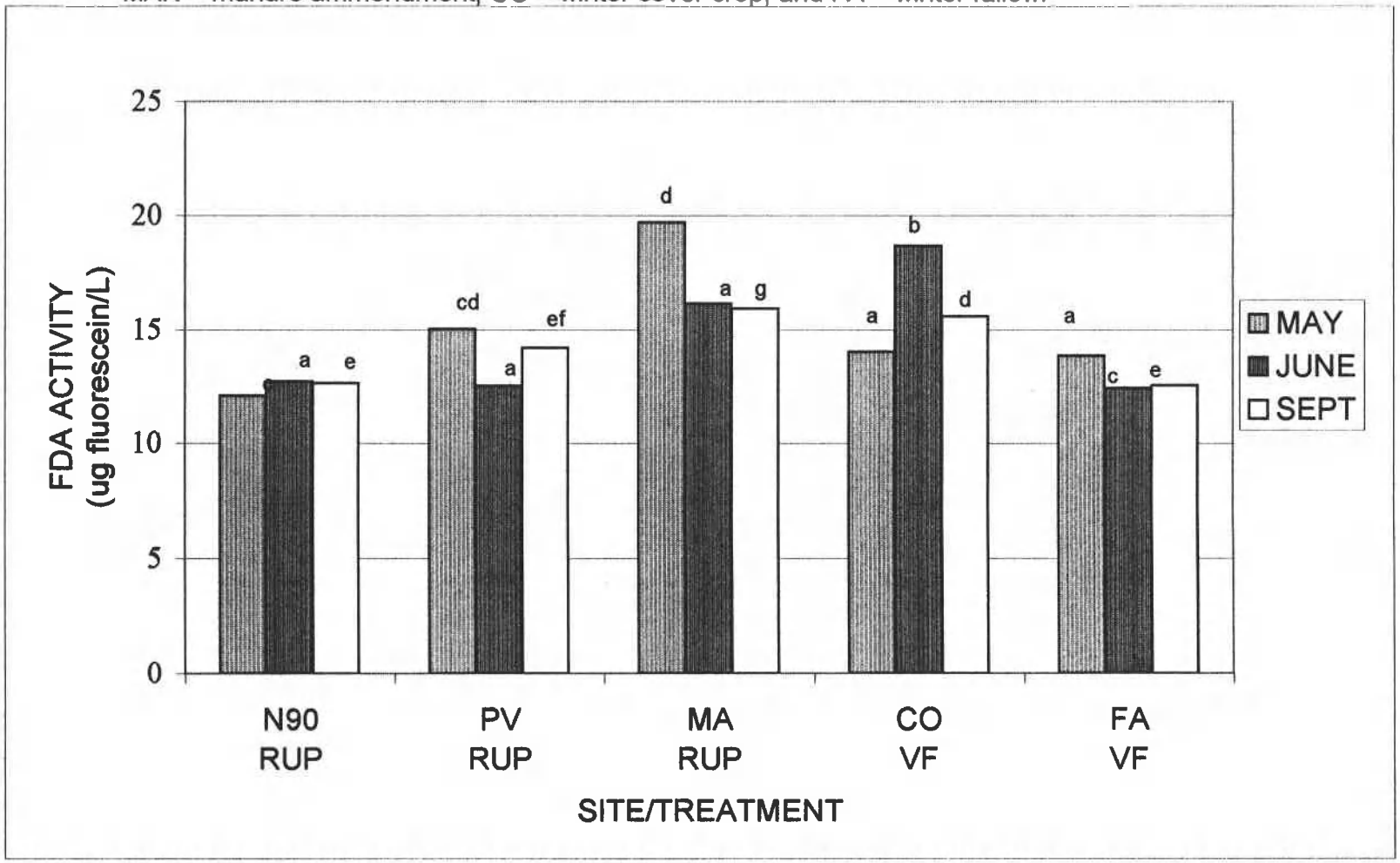

Figure 9. FDA activity at forest sites for June sampling date. ANOVA performed at each site. Treatments with the same letter are not significantly different.
MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest.

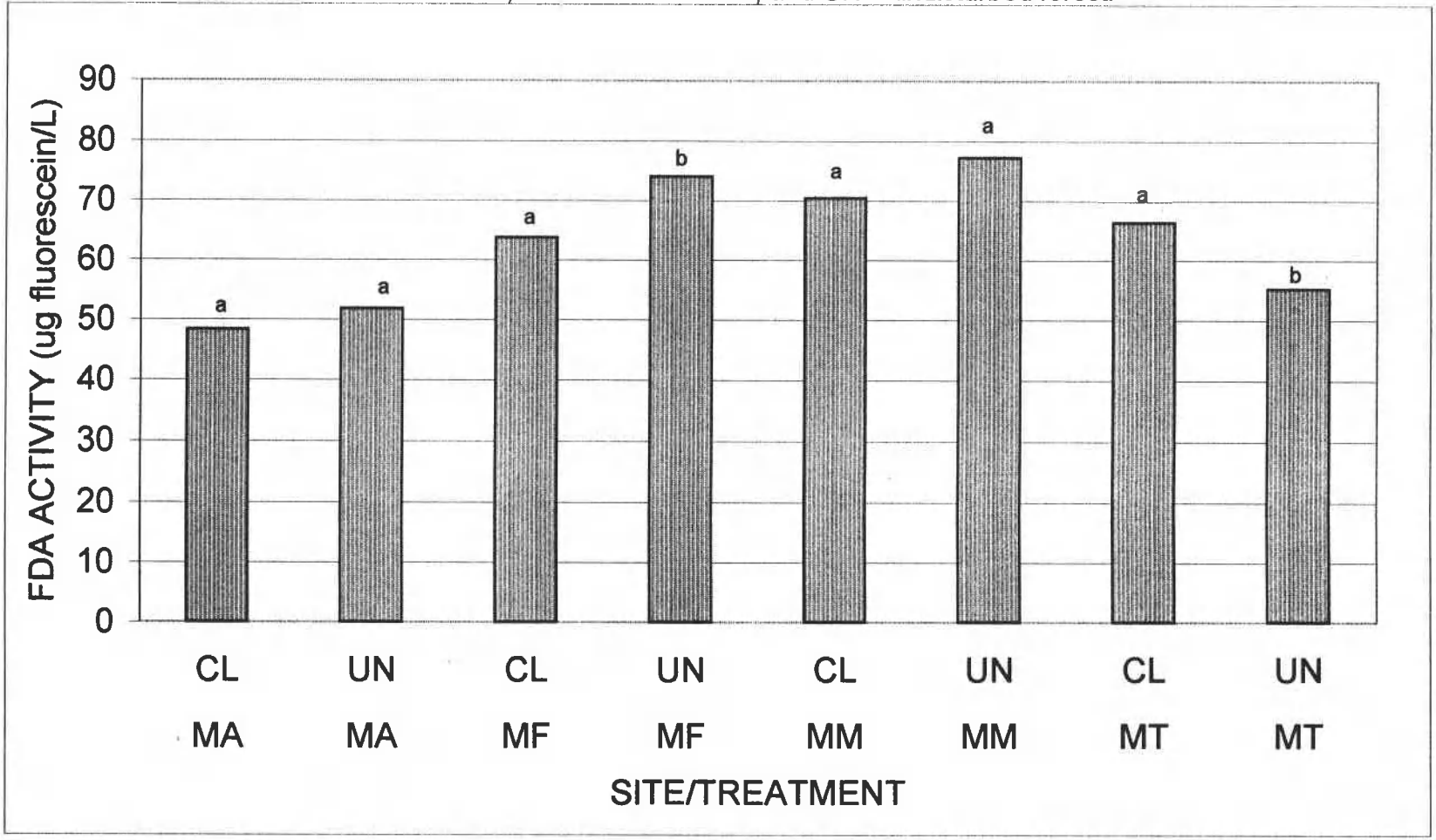

Figure 10. FDA activity for agricultural and forest sites for June sampling date.
RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, FA = winter fallow, MA = McKenzie Ambush, MF = McKenzie Ambush V, MM = McKenzie Millstone, MT = McKenzie Millstone Three, CL = clearcut forest, and UN = undisturbed forest.

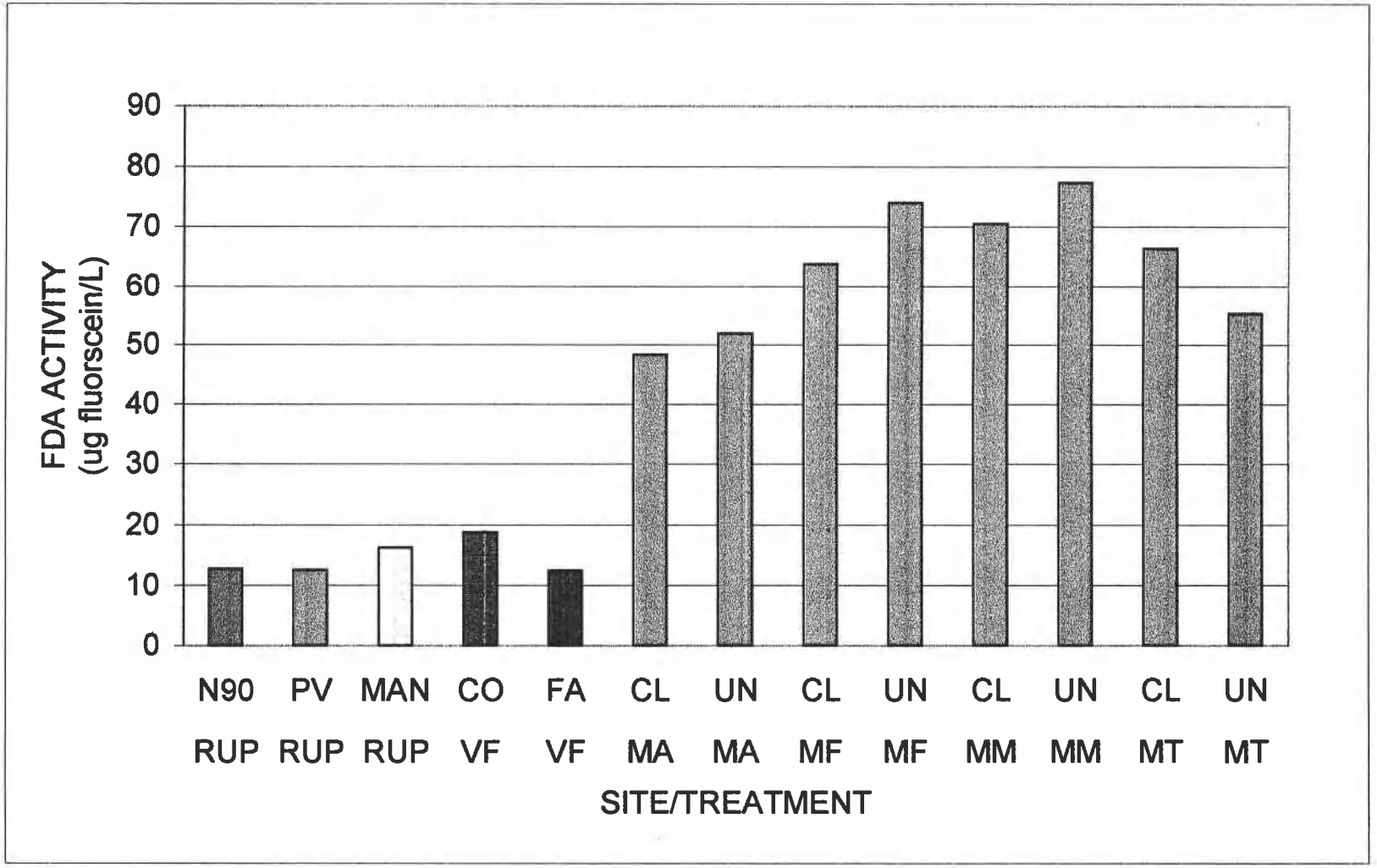

Figure 11. MWES ratios for FDA activity at agricultural sites samples in June.
RUP = Residue Utilization Plots, VF = Vegetable Farm, N90 = added N, PV = peavine ammendment, MAN = manure ammendment, CO = winter cover crop, and FA = winter fallow.