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Differences of Soil Enzyme Activity after Incorporation with Chinese Milk Vetch Litter Cut at Different Growth Stages

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ABSTRACT Chinese milk vetch (CMV) is a winter legume that is commonly used as cover crop in Korea. Kill date of cover crop for addition into soil affects N content in cover crop and N availability in soil. This study was conducted to evaluate the effect of CMV as green manure cover crop according to kill dates before growing corn without artificial fertilizer. Top of CMV cut three times on 13 April, 27 April, and 11 May were added into soil at a rate of 600 kg per 10 a. Sugar content in CMV litter was persistently decreased from mid-April to late-May. The decrease of sugar content might be due to the transformation into starch and/or other storage or structural constituents. The decreased amount of sugars was greater than 12% and the increased amount of starch was less than 0.2%. Concentration of NH_4^+ in soil treated by CMV litter cut on May 11 was slightly higher than that in the treatment with early-cut (April 13) CMV, the concentration at 28 and 49 DAT (days after treatment) was higher in the treatment with late-cut CMV litter. Regardless of cut (kill) date of CMV, the phosphatase activity in the treatment of CMV litter was higher compared to the untreated control. Soil dehydrogenase activity was increased steadily by addition of CMV litter implying total microbial activities in the soil were increased. Our results demonstrate that the status of cover crop species at kill date is an important factor influencing soil enzyme activities derived from microorganisms. Therefore, the optimal kill date of cover crop should be examined to improve the efficiency of cover crop as green manure crop regarding the practical sequence in cropping system.

Keywords : *Astragalus sinicus*, cover crop, urease, phosphatase, dehydrogenase, soil enzyme

Chinese milk vetch (*Astragalus sinicus*; CMV) is a species belonging to Fabaceae. This species has been currently used for improving soil properties and reducing the amount of chemical fertilizers in concern with environmental-friendly agriculture. Chinese milk vetch (CMV) is a legume which exhibits relatively higher biological N-fixing activity (150 to 200 $kg \cdot ha^{-1} \cdot yr^{-1}$). CMV has been commonly used as green manure and cover crop, contributing to organic farming in Korea. Although the requirement of farmers for developing cropping system with CMV is increasing, the information of cultural practices that required for CMV-based cropping system is not much. Among several beneficial effects of green manure on agricultural soil (Roberson *et al.*, 1991), improvement of soil quality (Bandick & Dick, 1999), reduction of soil erosion (Delgado *et al.*, 1999), and weed suppression (Gallandt *et al.*, 1999; den Hollander *et al.*, 2007; Hartwig & Ammon, 2002), the relevance to soil quality is closely related to soil microbial activity (Ladd, 1978)

Chemical changes of CMV in soil are controlled by soil microorganisms via biotransformation of CMV constituents. Therefore, the chemical properties of CMV constituents are determining factor in field performance of CMV as a green manure crop. Because the growth stages of cover crop show different chemical composition of biomass, the effect of CMV litter on soil properties is various according to cutting and incorporated time. Although the cutting timing of the aerial part of CMV before incorporation into soil is dependent on the climatic and labor conditions, optimal timing for cutting should be elucidated for efficient performance of CMV litter in soil. As a green manure crop, the contribution of soil-incorporated CMV litter to successive crop has not been clarified clearly. However, many results showed the beneficial effects in case of other legume cover crops. The cover crops increase soil N availability (Clark *et al.*,

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1997a; Clark *et al.*, 1997b; Kuo *et al.*, 1996; Touchton *et al.*, 1994; Decker *et al.*, 1994) and the level of organic carbon and nitrogen and subsequently promote soil microbial activities (Janzen *et al.*, 1992; Van Gestel *et al.*, 1992). In temperature regions, winter cover crop contributes environmentally to agroecosystem in many aspects. The immobilization of inorganic nitrogen by cover crop results in a decrease of NO_3 leaching (Schertz and Miller, 1972) and slow release of the nutrient. Soil microorganisms participate into the process including degradation of organic matter and immobilization of inorganic nitrogen. In general, incorporation of cover crop into soil increases the microbial activity and improves soil health.

Soil quality can be determined by the level of soil organic matter that is influenced by not only chemical and physical properties but also biological properties. Because soil microbial activity is subjected to soil properties, soil microbial activity is controlled by soil quality (Cole *et al.*, 1987). Therefore, soil enzyme activities have been shown to be sensitive indicators of soil health under different cropping systems (Kennedy & Papendick, 1995). Biological activity of soil and soil healthy can be estimated based on the soil enzyme activities that reflect activities of microorganisms. Of many soil enzymes assayed to estimate soil quality, activities of soil urease, dehydrogenase, and phosphatase have been used properly as an indicator for soil health.

Organic matter addition increases various soil enzyme activities (Crecchio *et al.*, 2001; Madejon *et al.*, 2001; Kandeler *et al.*, 1999). An increased amount of organic matter in soil implies the increase of substrates for soil microorganisms. Different C/N ratio and degradability of organic litter added into soil affect the decomposition rate and can change the microbial community structure (Bengtsson *et al.*, 2003). Therefore, the timing of cutting for addition of organic matter into soil can affect the degradation event of organic matter derived from CMV.

The objectives of this experiment are to verify the effect of CMV on soil healthy based on the enzyme activity and to know the proper timing of cutting and addition of CMV during growth stages for improving soil quality with respect to soil microbial activities. The results will contribute to the development of cover crop-based cropping system for environmentally friendly agriculture.

MATERIALS AND METHODS

Plant material and growth condition

CMV seeds were sown at Sep. 5 on experimental farm of Gyeongsang National University in 2004 at a sowing rate of 5 kg per 10 a. After sowing, the seeds were covered with soil to a depth of about 3 cm using rotary cultivator. Any fertilizer or pesticide has not been applied during the experiment. The CMV litters cut three times on Apr. 13, Apr. 27, and May 11. CMV litter were added into pot packed with sandy loam soil at a rate of 600 kg per 10 a. Two corn plants (cv. Chalok 2) were seeded in a pot and grown in a greenhouse.

Plant and soil analysis

Total nitrogen contents of CMV litter were determined by kjeldahl method (AACC, 1969). Sugar content was determined by the method of Bernfeld (1955) after extraction with 80% ethanol. Starch content of CMV was determined by enzymatic method using amyloglucosidase (Rose *et al.*, 1991). For determining NH_4^+ and NO_3^- in soil, 25 g soil was extracted with 25 ml of 2 M KCl for 1 h using orbital shaker. The soil solution was filtered using filter paper (Whatman #1). NH_4^+ content was determined spectrophotometrically. In brief, an aliquot of filtrate (5 ml) was mixed with phenol solution (0.2 ml), nitroferricyanide solution (0.2 ml) and oxidizing reagent (0.5 ml). The mixture was mixed vigorously and placed at room temperature for 1 h. The absorbance of solution was measured at 630 nm and the concentration was determined based on the standard curve of $NH_4^+ \cdot NO_3^-$ concentration was determined using NO_3^- ion selective electrode.

Soil enzyme assay

Dehydrogenase activity was determined based on the reduction of 2,3,5-triphenyltretrazolium (TTC) to triphenyl formazan (TPF) by the method of Tabatabai (1982) with slight modification. Air-dried soil (2 g) samples were placed in 15 ml centrifuge tubes and add 2ml of TTC solution (1% in 0.5 M Tris buffer, pH 7.6). The tubes were stoppered, mixed and incubated for 24 h at 37 °C in dark condition. After incubation, added 5 ml of methanol and mixed vigorously. The tubes were centrifuged at 3000 rpm for 10 min and the supernatant was collected. Aliquots (1.5 ml) were removed and the absorbance of the supernatant was measured at 485 nm. The amount of TPF was determined based on the standard curve of TPF. Enzyme activity was expressed in OD g^{-1} h^{-1}.

Urease activity was assayed based on the method of Kandeler and Gerber (1988). Wet soil of 0.5 g was mixed with 0.25 ml urea (80 mM) and 2 ml 75 mM borate buffer (pH 10.0). The mixture was incubated with shaking for 2 h at 20 °C. After incubation, added 3ml of 2 M KCl. The mixtures were shaken on an orbital shaker for 30 min and centrifuged (3000 g, 15 min). An aliquot (0.2 ml) of supernatant was mixed with 1.8 ml distilled water, 1 ml sodium salicylate/NaOH solution and 0.4 ml dichloroisocyanuric acid. The mixture was placed at room temperature for 20 min. Absorbance was measured at 690 nm. NH_4^+ concentrations were determined using a calibration curve of NH_4Cl standard solutions from 0 to 2.5 μg ml^{-1}.

Phosphatase activity was determined using *p*-nitrophenyl phosphate disodium (PNPP, 0.115 M) as substrate. Soil samples of 2 g were placed in a test tube. Add 0.5 M acetate buffer (pH 5.5) of 0.2 ml and 0.115 M *p*-nitrophenyl phosphate disodium of 0.2 ml. The mixtures were incubated at 37°C for 90 min. The reaction was stopped by cooling at 2°C for 15 min. Then, 0.5 ml of 0.5 M $CaCl_2$ and 2 ml of 0.5 M NaOH were added, and the mixture was centrifuged at 3000 rpm for 10 min. The *p*-nitrophenol formed was determined by a spectrophotometer at 398 nm (Tabatabai & Bremner, 1969).

RESULTS AND DISCUSSION

Nitrogen and carbohydrates contents

The content of N in CMV was presented in Fig. 1. The content was changed according to the growth stage after overwintering. Because most nitrogen in CMV is organic form, the nitrogen would be mineralized into inorganic N by soil microorganisms. N content is an important factor in CMV as green manure crop. To maximize the effect of CMV in a sequence of cropping, CMV litter should be added into soil when the N content reaches to the highest level. In this study, the content in top of CMV steadily decreased until mid-May. It has been known that N content in leaf is highly correlated with leaf protein content and photosynthetic rate. However, there is a little discrepancy between the two dates showing the highest nitrogen content and the highest biomass of CMV. Because the nitrogen content and biomass in aerial part were highest around flowering stage in CMV, the addition of CMV litter into soil at the flowering stage would be more beneficial to increase N pool in soil.

Sugar content (Fig 2.) was persistently decreased from mid-April until late-May. The decrease of sugar content might be due to the transformation into starch and/or other

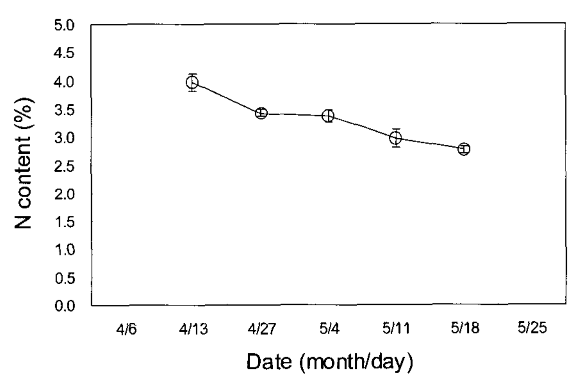

Fig. 1. Changes of nitrogen content in Chinese milk vetch shoot during reproductive growth from April to May.

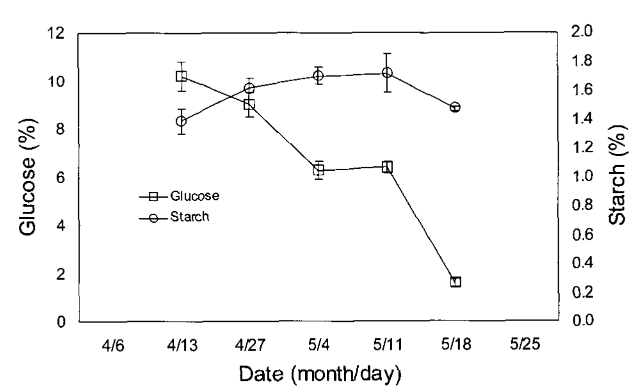

Fig. 2. Changes of glucose and starch content in Chinese milk vetch shoot during reproductive growth from April to May.

storage or structural constituents. The decreased amount of sugars was greater than 12% and the increased amount of starch was less than 0.2%. Therefore, the carbohydrates of CMV might be changed into less degradable forms like cellulose that degrades slowly in soil. Carbohydrate contents in cover crop influence the mineralization rate of cover crop residue in soil (Vigil *et al.*, 1991; Schomberg & Cabrera, 2001). Our result suggests that the degradation of CMV litter added into soil would be degraded with different degradation rate according to the growth stage of CMV in spring. After late-April, the sugar content decreased dramatically reflecting the internal metabolic activities related to carbon assimilation was lowering.

Changes in NO_3^- and NH_4^+–N concentration in soil after addition of CMV

NO_3^- contents in the treated soil were presented in Fig. 3. Spring kill date of cover crop affects the level of N in cover crop and subsequently, the N availability also can be different after addition into soil (Clark *et al.*, 1997a; Clark *et al.*, 1997b). Addition of CMV increased greatly the soil NO_3^- content. Previous report about corn plant showed that corn growth was highly dependent on the amount of CMV litter added into pot (Park & Shim, 2005). The positive effect of CMV litter on the corn growth might be due to the increased level of NO_3^-. Soil NO_3^- content showed the highest level by addition of early-cut CMV (April 13) litter as compared to the CMV litter cut later. Because a part of NO_3^- in soil was absorbed and utilized by corn plants, the content was decreased as corn plants grown in all treatment. However, the soil added by the litter cut on April 13 showed slow decreasing tendency. This result might be due to the abundance of NO_3^- in the soil in which the CMV litter cut on April 13 was added. The soil added by the litter cut on April 27 and May 11 might be not abundant in NO_3^- to support corn growth. Therefore, the NO_3^- level was decreased more rapidly according to corn growth.

NH_4^+ content was presented in Fig. 4. NH_4^+ concentration in soil was different with that of NO_3^- in tendency. Although the concentration of NH_4^+ in soil treated by CMV litter cut on May 11 was slightly higher than that in the treatment with early-cut (April 13) CMV, the concentration at 28 and 49 DAT was higher in the treatment with late-cut CMV litter. It might be due to that corn plants grown under high NO_3^- content were grow well and uptake more amount NH_4^+ resulting in the lowered NH_4^+ content.

Soil enzyme activity

Soil phosphatase activities were measured three times, 14, 28, 47 days after treatment (DAT). Regardless of the cut time of CMV, the enzyme activity treated by CMV litter was higher than that of untreated control (Fig. 5). Overall tendency of enzyme activity was decreased over time. Soil phosphates have an important role for soil organic P mineralization and plant nutrition (Klose & Tabatabai, 2002). The increased activity of phosphatase might contribute to

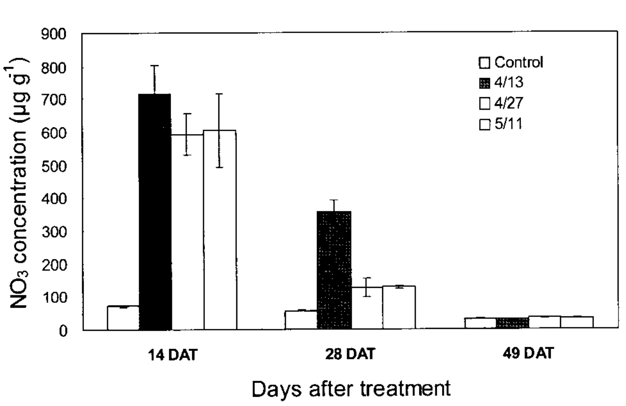

Fig. 3. Effect of the addition of Chinese milk vetch litter cut at different dates on soil NO_3 content.

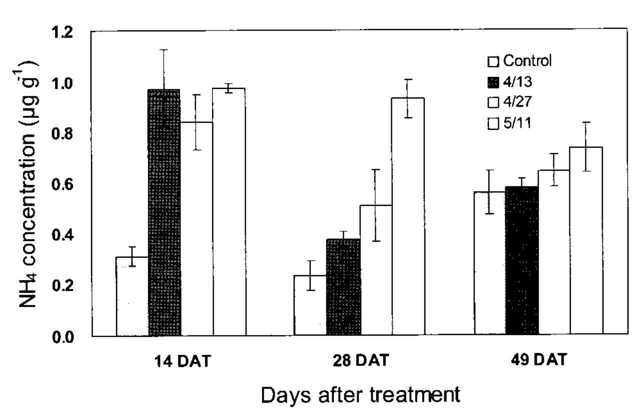

Fig. 4. Effect of the addition of Chinese milk vetch litter cut at different dates on soil NH_4 content.

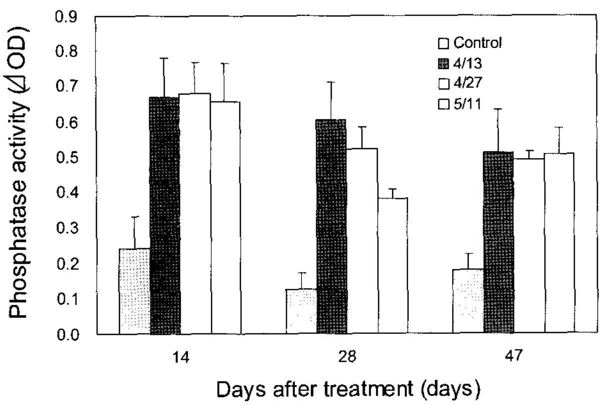

Fig. 5. Effect of kill date of CMV on soil phosphatase activity at each incubation date. The activities were expressed as the change of optical density at 485 nm.

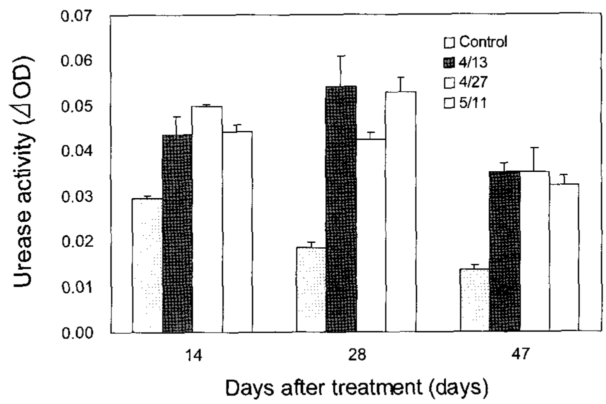

Fig. 6. Effect of kill date of CMV on soil urease activity at each incubation date. The activities were expressed as the change of optical density at 690 nm.

increase availability of P in soil. Therefore, increased activities of phosphatase by addition of CMV into soil suggest that CMV addition into soil can reduce the rate of P fertilizer that normally applied as chemical fertilizer in Korea. Soil urease catalyses the hydrolysis of urea and has also been widely used as an indicator of soil quality (Gil-Sotres *et al.*, 2005). The activity of this enzyme is increased by addition of organic matters (Pascual *et al.*, 1999). The activities of this enzyme showed less distinctive differences among cutting times of CMV.

Urease activities were increased by addition of CMV litter, however, the degree of effect was lower than that of phosphatase (Fig. 6). Although N pool was increased by addition of CMV litter, there was no significant difference in the effect of CMV addition on urease between kill dates of CMV. The promotive effect of the addition of CMV litter into soil was diminished at 47 DAT. Because urease activity in soil is highly related to the addition of organic matter, the lowered activity at 47 DAT means the decrease of organic nitrogen derived from CMV litter. Therefore, the enzymatic process for degrading organic N from CMV was no longer persistent at a higher level reflecting that N mineralization was reduced after 28 DAT in this experiment.

Dehydrogenase is a common and ubiquitous enzyme in biological system and has been used as an indicator of viability of organisms (Bentham *et al.*, 1992). Regardless of nutrient level in soil, the dehydrogenase activity was increased steadily during the experiment (Fig. 7). This result implies that total microbial activities in the soil were increased by addition of CMV litter. The effect was greater in the treatment of early-cut CMV than that of late-cut CMV. Unlike other enzyme, activity of dehydrogenase was not lowered significantly at 47 DAT. This result suggests that the addition of CMV litter improves the soil quality that was represented by the enzyme activity of soil microorganisms.

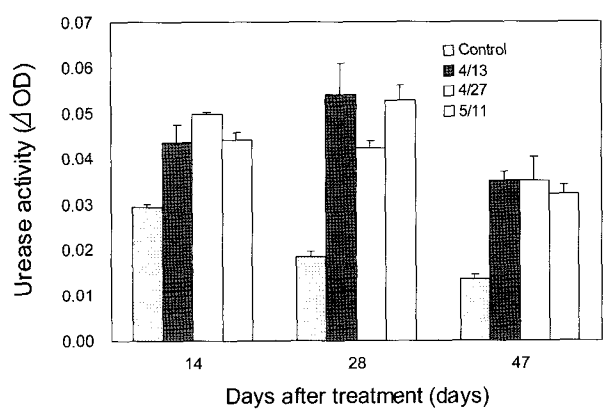

Fig. 7. Effect of kill date of CMV on soil dehydrogenase activity at each incubation date. The activities were expressed as the change of optical density at 398 nm.

In this study, we have not examined the effect of application rate of CMV litter. Therefore, the result of this study could be different under different application rate of CMV litter and environmental conditions like soil and meteorological condition. The treatment examined in this study, killing date, resulted in the changed enzyme activities derived from soil microorganisms and the level of nutrient pool required for crop growth. The results may be due to the differences in N status and chemical composition in cover crop (Clark *et al.*, 1997a; Clark *et al.*, 1997b) that affected by the killing date in spring. Because this experiment was conducted by simplified condition, further studies on the effect of CMV under various conditions should be conducted to know the more detailed effects of CMV addition in cropping system.

In conclusion, CMV addition into soil changed soil N pool and soil enzyme activity. The differences in the effects on soil were observed between the growth stages of CMV when the aerial part was cut. Although biomass production was different between the growth stages of CMV, early cutting has more beneficial effects on soil quality. Activities of urease and phosphates, soil enzymes that play roles in transformation of nutrients, were lowered as corn plants grew. However, dehydrogenase, a representative soil enzyme that reflects total soil microbial activity, did not show the decrease. Therefore, addition of CMV litter into soil improved the soil quality and nutritional condition for crop growth.

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REFERENCES

American Association of Cereal Chemists. 1969. Crude protein-improved Kjeldahl Method. Cereal Laboratory Method. 7th. Ed. Amer. Assoc. Cereal Inc., pp. 6-10.

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

Bengtsson, G., P. Bengtson, and K. F. Mansson. 2003. Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. Soil Biol. Biochem. 35 : 143-154.

Bentham, H., J. A. Harris, P. Birch, and K. C. Short. 1992. Habitat Classification and Soil Restoration Assessment Using Analysis of Soil Microbiological and Physico-chemical Characteristics. J. Appl. Ecol. 29 : 711-718.

Bernfeld, P. 1955. Amylase, α and β. Method. Enzymol. 1 : 149-158.

Clark, A. J., A. M. Decker, J. J. Meisinger, and M. S. McIntosh. 1997a. Kill date of vetch, rye, and a vetch-rye mixture. I. Cover crop and corn nitrogen. Agron. J. 89 : 427-434.

Clark, A. J., A. M. Decker, J. J. Meisinger, and M. S. McIntosh. 1997b. Kill date of vetch, rye, and a vetch-rye mixture. II. Soil moisture and corn yield. Agron. J. 89 : 434-441.

Cole, C. V., J. Williams, M. Schaffer, and J. Hanson. 1987. Nutrient and organic matter dynamics as components of agricultural production systems. In : Follet, R. F., J. W. B. Stewart, C. V. Cole (Eds.), Soil Fertility and Organic Matter as Critical Components of Production Systems. vol. 19. SSSA Special Publication. ASA, CSSA, and SSSA, Madison, WI, pp. 147-166.

Crecchio, C., M. Curci, R. Mininni, P. Ricciuti, and P. Ruggiero. 2001. Short-term effects of municipal solid waste compost amendments on soil carbon and nitrogen content, some enzyme activities and genetic diversity. Biol. Fert. Soil. 34 : 311-318.

Decker, A. A., J. Clark, J. J. Meisinger, F. R. Mulford, and M. S. McIntosh. 1994. Legume cover crop contribution to no-tillage corn production. Agron. J. 86 : 126-135.

Delgado, J. A., R. T. Sparks, R. F. Follett, J. L. Sharkoff, and R. R. Riggenbach. 1999. Use of Winter Cover Crops to Conserve Soil and Water Quality in the San Luis Valley of South Central Colorado, Soil Quality and Soil Erosion. Soil and Water Conservation Society, pp. 125-142.

den Hollander, N. G., L. Bastiaans, and M. J. Kropff. 2007. Clover as a cover crop for weed suppression in an intercropping design. I. Characteristics of several clover species. Eur. J. Agron. 26 : 92-103.

Gallandt, E. R., M. Liebman, and D. R. Huggins. 1999. Improving soil quality: implications for weed management, J. Crop Prod. 2 : 95-121.

Gil-Sotres, F., C. Trasar-Ceped, M. C. Leirós, and S. Seoane. 2005. Different approaches to evaluating soil quality using biochemical properties. Soil Biol. Biochem. 37 : 877-887.

Hartwig, N. L., and H. U. Ammon. 2002. Cover crops and living mulches. Weed Sci. 50 : 688-699.

Janzen, H. H., C. A. Campbell, S. A. Brandt, G. P. Lafond, and L. Townley-Smith. 1992. Light-fraction organic matter in soils from long-term crop rotations. Soil Sci. Soc. Am. J. 56 : 1799-1806.

Kandeler, E. and H. Gerber. 1988. Short-term assay of soil urease activity using colorimetric determination of ammonium. Biol. Fert. Soil. 6 : 68-72.

Kandeler, E., M. Stemmer, and E. M. Klimanek. 1999. Response of soil microbial biomass, urease and xylanase within particle size fractions to long-term soil management. Soil Biol. Biochem. 31 : 261-273.

Kennedy A. C. and R. I. Papendick. 1995. Microbial characteristics of soil quality. J. Soil Water Conserv. 50 : 243-248.

Klose, S. and M. A. Tabatabai. 2002. Response of phosphomonoesterases in soils to chloroform fumigation. J. Plant Nutr. Soil Sci. 165 : 429-434.

Kuo, S., U. M. Sainju, and E. J. Jellum. 1996. Winter cover cropping influence on nitrogen mineralization, presidedress soil nitrate test, and corn yields. Biol. Fertil. Soils. 22 : 310-317.

Ladd, J. N. 1978. Origin and range of enzymes in soil. In : Burns, R. G. (Eds.), Soil Enzymes. Academic Press, London, pp. 51-96.

Madejon, E., P. Burgos, R. Lopez, and F. Cabrera. 2001. Soil enzymatic response to addition of heavy metals with organic residues. Biol. Fert. Soil. 34 : 144-150.

Park, C. I. and S. I. Shim. 2006. Effect of soil-incorporated Chinese milk vetch on early growth of corn. Kor. J. Crop Sci. 51(S1) : 230-231.

Pascual, J. A., C. García, and T. Hernández. 1999. Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. Biol. Fert. Soil. 30 : 1-6.

Roberson, E. B., S. Sarig, and M. K. Firestone. 1991. Cover crop management of polysaccharide-mediated aggregation in an orchard soil, Soil Sci. Soc. Am. J. 55 : 734-739.

Rose, R., C. L. Rose, S. K. Omi, K. R. Forry, D. M. Durall, and W. L. Bigg. 1991. Starch determination by perchloric acid vs enzymes : evaluating the accuracy and precision of six colorimetric methods. J. Agri. Food Chem. 39 : 2-11.

Schertz, D. L. and D. A. Miller. 1972. Nitrate-N accumulation in the soil profile under alfalfa. Agron J. 64 : 660-664.

Schomberg, H. H. and M. L. Cabrera. 2001. Modeling *in situ* N mineralization in conservation tillage fields : comparison of two versions of the CERES nitrogen submodel. Ecol. Model. 145 : 1-15.

Tabatabai, M. A. and J. M. Bremner. 1969. Use of *p*-nitrophenyl phosphate in assay of soil phosphatase activity. Soil Biol. Biochem. 1 : 301-307.

Tabatabai, M. A. 1982. Soil enzymes. In : Page, A. L., E. M. Miller, D. R. Keeney. (Eds.), Part 2 : Methods of Soil Analysis, vol. 9, 2nd ed. Agron Monograph. ASA and SSSA, Madison, WI, pp. 501-538.

Touchton, J. D., D. H. Ricker, R. H. Walker, and C. E. Snipes. 1984. Winter legumes as a nitrogen source for no-tillage corn. Soil Tillage Res. 4 : 391-401.

Van Gestel, M., J. N., Ladd, and M. Amato. 1992. Microbial biomass responses to seasonal change and imposed drying regimes at increasing depths of undisturbed topsoil profiles. Soil Biol. Biochem. 24 : 103-111.

Vigil, M. F., D. E. Kissel, and S. J. Smith. 1991. Field crop recovery and modeling of nitrogen release from several winter annual cover crops. Soil Sci. Soc. Am. J. 55 : 1031-1037.