

DISSERTATION

EFFECTS OF AGRICULTURAL MANAGEMENT ON GREENHOUSE GAS EMISSIONS,
CARBON AND NITROGEN SEQUESTRATION, AND DAYCENT SIMULATION
ACCURACY

Submitted by

Phasita Toonsiri

Department of Soil and Crop Sciences

In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Summer 2017

Doctoral Committee:

Advisor: Jessica G. Davis

M. Francesca Cotrufo
Richard T. Conant
Stephen J. Del Grosso

Copyright by Phasita Toonsiri 2017

All Rights Reserved

ABSTRACT

EFFECTS OF AGRICULTURAL MANAGEMENT ON GREENHOUSE GAS EMISSIONS, CARBON AND NITROGEN SEQUESTRATION, AND DAYCENT SIMULATION ACCURACY

Agricultural activities affect greenhouse gases (GHGs) sources and sinks in terrestrial ecosystems. Organic fertilizer provides nitrogen (N) and organic carbon (C) to soil, resulting in enhanced N and C substrates for nitrification and denitrification which produce nitrous oxide (N_2O) and for heterotrophic activity which generates carbon dioxide (CO_2). Therefore, reduction of N and C substrates for N_2O and CO_2 production can reduce these emissions. Proper organic fertilizer application can regulate or reduce the loss of N_2O from soil. In addition to reducing GHG production, increasing the potential of soil to sequester soil organic matter (SOM) is a key strategy for mitigating GHG emissions. Increasing organic inputs and reducing SOM turnover rate are keys for this mitigation. The persistence of SOM in agricultural soils is largely associated with the level of protection of C in stable aggregates. Therefore, applying proper practices to increase the stable aggregates can decrease the SOM decay rate, resulting in reduced loss of GHGs such as N_2O and CO_2 from soil.

The focus of my dissertation is the study of (i) N_2O and CO_2 emissions from a lettuce field which received different organic fertilizer applications, (ii) SOM persistence and stable aggregates in organic and conventional farming systems, and (iii) simulation of N_2O and CO_2 emissions in organic lettuce using the DAYCENT model.

The first study was performed in the summers of 2013 and 2014 at the Colorado State University Horticulture Research Center in Fort Collins, CO to determine the effects of environmental factors and four organic fertilizers (feather meal, blood meal, fish emulsion, and cyano-fertilizer) applied at different rates (0, 28, 56, and 112 kg N ha⁻¹) on N₂O and CO₂ emissions from a lettuce field (*Lactuca sativa* L.). Feather meal and blood meal were applied at the full rate (single application) prior to transplanting lettuce, and fish emulsion and cyano-fertilizer were applied five times (multiple applications) after transplanting. The results showed that single application treatments significantly increased cumulative N₂O emissions as compared with control, but multiple application treatments did not. However, single application treatments could be overestimated due to chamber placement over fertilizer bands. Cumulative CO₂ emissions from single application and multiple application treatments in 2013 were not different, while in 2014, single application treatments presented higher CO₂ emissions than multiple application treatments.

The second study evaluated the effect of management on aggregate stability and SOM protection and persistence. The study was conducted by collecting soil samples from conventional and organic vegetable fields in different locations (California, Colorado, and New York) and at different soil depths (0-10, 10-20, and 20-30 cm) and analyzing their properties, microbial biomass, and aggregate size distribution. The results showed that organic farming systems have more microbial biomass, thus resulting in enhanced aggregate stability and the formation of organo-mineral bonding of microbial products, thereby storing higher C and N stocks than conventional farming systems.

The last study compared N₂O and CO₂ emissions from field measurements with DAYCENT simulation. The data from the first study in 2014 was used to test the DAYCENT

model. The result showed that DAYCENT simulated N₂O and CO₂ emissions from feather meal and blood meal (single application) better than for fish emulsion and cyano-fertilizer (multiple applications). In addition, the DAYCENT model had low potential to simulate soil water content and soil temperature in irrigated organic lettuce.

Overall, the results of these studies show (i) multiple applications of cyano-fertilizer reduced N₂O and CO₂ emissions while maintaining lettuce yields, (ii) organic farming practices resulted in higher C inputs, microbial biomass, aggregate stability, and protected SOM relative to conventional farming practices, and (iii) DAYCENT reasonably simulated N₂O and CO₂ emissions from an irrigated organic lettuce field receiving solid organic fertilizers in single applications. These results should be used to support agricultural management decisions.

ACKNOWLEDGMENTS

I would like to acknowledge those who have helped me throughout my Ph.D. study. Without them, I cannot imagine how I could have accomplished my Ph.D. study and research. First, I would like to express my deepest gratitude to my advisor, Dr. Jessica Davis who has been a great advisor, mentor and role model. Dr. Davis has patiently and wisely taught me many things about scientific research and always given me support and motivation. Truly thanks for your trust and confidence in me. Next, I would like to thank my committee members, Dr. Stephen J. Del Grosso, who supported me in gas chromatography, guided me in measuring greenhouse gas emissions and using the DAYCENT model; Dr. M. Francesca Cotrufo, who advised and guided me about carbon sequestration and aggregation; and Dr. Richard Conant, who taught me about greenhouse gases and gave me suggestions for these projects. To Dr. Ann Hess, thank you for all of your statistical consulting. Thanks to the Royal Thai Government for funding my Ph.D. study, to the Colorado Agricultural Experiment Station, the USDA Western Sustainable Agriculture Research and Education program for supporting the first study, and to Russell Ranch Sustainable Agriculture Facility and Homer C. Thompson Vegetable Research Farm for their collaboration in the second study.

My dissertation would not have been possible without help in the field work, laboratory, and modelling from A. Sukor, D.P. Widiastuti, M. Smith, M. Reyes-Fox, J. Soong, H. Storteboom, R. Barminski, N. Yoder, S. Barstad, B. Fischer, A. Wickham, M. Abulobaida, H. Wang, J. Wenz, W. Suksavate, K. Kradangnga, K. Warrit, T. Deeney, W. Folsom, G. Gross, S. Fulton-Smith, D. Reuss, M. J. Wolff, C. Pinney, M. L. Haddix, Y. Zhang, M. Easter and many others.

Finally, thanks to the ones who always advise, support, encourage, and stand beside me in every situation - my family PGPK.

TABLE OF CONTENTS

ABSTRACT	ii
ACKNOWLEDGMENTS	v
OVERVIEW	1
REFERENCES	3
CHAPTER 1. GREENHOUSE GAS EMISSIONS FROM SOLID AND LIQUID ORGANIC FERTILIZERS APPLIED TO LETTUCE	5
1.1 Summary	5
1.2 Introduction	6
1.3 Materials and methods	9
1.3.1 Site description	9
1.3.2 Field experiment	10
1.3.3 Soil and fertilizer analyses	11
1.3.4 Gas sampling and analysis	12
1.3.5 Data analysis	14
1.4 Results	15
1.4.1 Environmental conditions	15
1.4.2 N ₂ O and CO ₂ emission during the growing season	16
1.4.3 Effect of time of gas sampling and irrigation schedule on N ₂ O and CO ₂ fluxes	16
1.4.4 Effect of sources of fertilizer and treatments (combination of sources of fertilizer and N rates) on cumulative N ₂ O emission	17

1.4.5 Effect of sources of fertilizer and treatments (combination of sources of fertilizer and N rates) on cumulative CO ₂ emission.....	17
1.5 Discussion.....	18
1.5.1 Effect of organic fertilizers and application method on cumulative N ₂ O emissions.....	18
1.5.2 Effect of organic fertilizers and application method on cumulative CO ₂ emissions.....	21
1.5.3 Effect of environmental factors on N ₂ O and CO ₂ emissions.....	22
1.6 Conclusion.....	22
TABLES.....	24
FIGURES.....	30
REFERENCES.....	34
 CHAPTER 2. SOIL ORGANIC CARBON AND AGGREGATE STABILITY IN CONVENTIONAL AND ORGANIC VEGETABLE FARMING SYSTEMS.....	
2.1 Summary.....	43
2.2 Introduction.....	44
2.3 Materials and methods.....	46
2.3.1 Site description.....	46
2.3.2 Experimental design and soil sampling.....	47
2.3.3 Soil property analyses.....	47
2.3.4 Soil microbial biomass.....	48
2.3.5 Physical fractionation.....	48
2.3.6 Carbon and nitrogen analysis.....	49
2.3.7 Data analysis.....	49
2.4 Results.....	51

2.4.1 Whole soil characteristics	51
2.4.2 Soil microbial biomass	52
2.4.3 Aggregate size distribution	52
2.4.4 Fractions within macroaggregates	53
2.4.5 Soil organic carbon stock and dissolved organic carbon	53
2.4.6 Nitrogen stock and dissolved nitrogen	55
2.4.7 Physical and chemical protection	56
2.4.8 Correlations of soil microbial biomass, aggregate stability, carbon and nitrogen protections	57
2.5 Discussion	57
2.5.1 Farming system effect on microbial biomass	58
2.5.2 Aggregate stability	58
2.5.3 Soil organic carbon and nitrogen stocks	59
2.5.4 Soil organic matter stabilization	60
2.6 Conclusions	61
TABLES	63
FIGURES	79
REFERENCES	92
 CHAPTER3. SIMULATED GREENHOUSE GAS EMISSIONS FROM SOIL-APPLIED SOLID AND LIQUID ORGANIC FERTILIZERS USING THE DAYCENT MODEL	
3.1 Summary	99
3.2 Introduction	100
3.3 Materials and Methods	101

3.3.1 Field and experimental data.....	101
3.3.2 DAYCENT model description.....	102
3.3.3 DAYCENT application.....	103
3.4 Results.....	106
3.4.1 N ₂ O emission.....	107
3.4.2 CO ₂ emission.....	108
3.4.3 Soil water content and soil temperature.....	108
3.4.4 Yield.....	110
3.5 Discussion.....	110
3.5.1 N ₂ O emission.....	110
3.5.2 CO ₂ emission.....	112
3.5.3 Soil water content and soil temperature.....	114
3.5.4 Yield.....	115
3.6 Conclusion.....	117
TABLES.....	118
FIGURES.....	120
REFERENCES.....	124
OVERALL CONCLUSIONS.....	130
RECOMMENDATIONS.....	132

OVERVIEW

Agriculture can play a critical role in greenhouse gas (GHG) mitigation. Agricultural lands occupy about 45% of the Earth's land surface, and agriculture accounts for 10 to 12 % of total global anthropogenic emissions of GHGs in 2010 (Corsi *et al.*, 2012; Smith *et al.*, 2014; Smith *et al.*, 2007). Nitrous oxide (N₂O) can be released from the soil through microbial activities which transform N in soil (Smith and Conen, 2004); however, N₂O emissions can be reduced through proper agricultural management practices such as avoiding over application of N fertilizer (Venterea *et al.*, 2012). In addition, CO₂ emissions are associated with the microbial decay of soil organic matter (SOM) (Al-Kaisi *et al.*, 2008). Therefore, protecting SOM from microbial decomposition can slow the decay process, while reducing CO₂ emissions. Although decomposition of OM generates CO₂, it is essential for crop production because OM decomposition provides nutrients to support plant growth (Schlesinger and Bernhardt, 2013). Therefore, stimulating decomposition of OM for optimization of soil fertility and regulating CO₂ emissions from decomposition simultaneously is challenging. The addition of organic amendments in organic farming systems can increase GHG emissions such as CO₂ and CH₄ (Halvorson *et al.*, 2016; Shrestha *et al.*, 2013). However, amending soil with proper practices can achieve a net gain of C due to the positive balance between C-input and C-output (Shrestha *et al.*, 2013).

In this context, the biogeochemical model, DAYCENT, plays an important role in simulating the response of GHG emissions to agricultural management, and the model also helps researcher to estimate the magnitude of the response in situations that are difficult to evaluate through experimentation. Additionally, the DAYCENT model is used by the EPA in its GHG

agricultural inventory and in COMET farm (Eco&Sols, 2016; USEPA, 2015). However, the model has not yet been tested for all crops and management systems; therefore, DAYCENT must be verified in these simulations prior to its use in simulation.

This dissertation consists of three sections which relate to the influence of agricultural management on GHG emissions and mitigation. In chapter 1, the objective was to investigate how organic fertilizers, fertilizer application methods, and environmental factors affect GHG emissions. In chapter 2, the objective was to investigate the effects of organic farming management as compared to conventional management on SOM stocks and persistence, through aggregate protection and mineral association, within the soil profile. Ultimately, the objectives of the last study (chapter 3) were to simulate the effect of organic fertilizers with differing application methods on N₂O and CO₂ emissions and to evaluate the performance of the DAYCENT simulation by comparing simulated data with measured data.

REFERENCES

- Al-Kaisi, M.M., M.L. Kruse, and J.E. Sawyer. 2008. Effect of nitrogen fertilizer application on growing season soil carbon dioxide emission in a corn–soybean rotation. *Journal of environmental quality* 37: 325-332. doi:10.2134/jeq2007.0240
- Corsi, S., T. Friedrich, A. Kassam, M. Pisante, and J.d.M. Sà. 2012. Soil organic carbon accumulation and greenhouse gas emission reductions from conservation agriculture: a literature review. Food and Agriculture Organization of the United Nations (FAO), Rome.
- Eco&Sols. 2016. Comet-global. <http://www.umr-ecosols.fr/index.php/en/recherche/projets/50-comet> (accessed 9 May 2017).
- Halvorson, A.D., S.J. Del Grosso, and C.E. Stewart. 2016. Manure and inorganic nitrogen affect trace gas emissions under semi-arid irrigated corn. *Journal of Environmental Quality* 45: 906-914.
- Schlesinger, W.H., and E.S. Bernhardt. 2013. *Biogeochemistry an analysis of global change*. Elsevier/Academic Press, New York.
- Shrestha, R.K., R. Lal, and B. Rimal. 2013. Soil carbon fluxes and balances and soil properties of organically amended no-till corn production systems. *Geoderma* 197: 177-185.
- Smith, K.A., and F. Conen. 2004. Impacts of land management on fluxes of trace greenhouse gases. *Soil use and management* 20: 255-263.
- Smith, P., M. Bustamante, H. Ahammad, H. Clark, H. Dong, E.A. Elsidig, H. Haberl, R. Harper, J. House, M. Jafari, O. Masera, C. Mbow, R.N. H., C.W. Rice, C. Robledo Abad, A. Romanovskaya, F. Sperling, and F. Tubiello. 2014. *Agriculture, Forestry and Other*

- Land Use (AFOLU). In: O. Edenhofer et al., editors, Climate change 2014: mitigation of climate change and contribution of working group III to the fifth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- Smith, P., D. Martino, Z. Cai, D. Gwary, H. Janzen, P. Kumar, B. McCarl, S. Ogle, F. O'Mara, C. Rice, B. Scholes, and O. Sirotenko. 2007. Agriculture. In: B. Metz et al., editors, Climate Change 2007: mitigation and contribution of working group III to the fourth assessment report of the intergovernmental panel on climate change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
- USEPA. 2015. Inventory of U.S. greenhouse gas emissions and sinks: 1990-2013. <https://www3.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2015-Main-Text.pdf> (accessed 6 May 2015).
- Venterea, R.T., A.D. Halvorson, N. Kitchen, M.A. Liebig, M.A. Cavigelli, S.J. Del Grosso, P.P. Motavalli, K.A. Nelson, K.A. Spokas, and B.P. Singh. 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. *Frontiers in Ecology and the Environment* 10: 562-570. doi:10.1890/120062

CHAPTER 1. GREENHOUSE GAS EMISSIONS FROM SOLID AND LIQUID ORGANIC FERTILIZERS APPLIED TO LETTUCE¹

1.1 Summary

Improper application of nitrogen (N) fertilizer and environmental factors can cause the loss of nitrous oxide (N₂O) to the environment. Different types of fertilizers with different C/N ratios may have different effects on the environment. The focus of this study was to evaluate the effects of environmental factors and four organic fertilizers (feather meal, blood meal, fish emulsion, and cyano-fertilizer) applied at different rates (0, 28, 56, and 112 kg N ha⁻¹) on N₂O emissions and to track CO₂ emissions from a lettuce field (*Lactuca sativa* L.). The study was conducted in 2013 and 2014 and compared preplant-applied solid fertilizers (feather meal and blood meal) and multiple applications of liquid fertilizers (fish emulsion and cyano-fertilizer). Three days a week, N₂O and CO₂ emissions were measured twice per day in 2013 and once per day in 2014 using a closed-static chamber, and gas samples were analyzed by gas chromatography. Preplant-applied solid fertilizers significantly increased cumulative N₂O emissions as compared with control, but multiple applications of liquid fertilizers did not. Emission factors for N₂O ranged from 0 to 0.1% for multiple applications of liquid fertilizers and 0.6 to 11% for preplant-applied solid fertilizers, which could be overestimated due to chamber placement over fertilizer bands. In 2014, solid fertilizers with higher C/N ratios (3.3–3.5) resulted in higher CO₂ emissions than liquid fertilizers (C/N ratio, 0.9–1.5). Therefore,

¹ Toonsiri, P., S. Del Grosso, A. Sukor, and J. Davis. 2016. Greenhouse gas emissions from solid and liquid organic fertilizers applied to lettuce. *Journal of Environmental Quality*. 45: 1812. doi:10.2134/jeq2015.12.0623

organic farmers should consider the use of multiple applications of liquid fertilizers as a means to reduce soil greenhouse gas emissions while maintaining high yields.

1.2 Introduction

Agriculture plays a significant role in the emissions of greenhouse gases (GHGs) (Paustian *et al.*, 2004). Total US GHG emissions were approximately 6673 million t CO₂ equivalent in 2013, and about 8% of this total was derived from agriculture, with soil management activities such as fertilization being a major contributor (USEPA, 2015). Fertilizer use in the United States trended steadily upward until about 1981 to support increasing crop yields to feed a growing population and exhibits some inter-annual variability (e.g., fertilizer consumption increased by 5% from 2010 to 2011 due to reduced fertilizer prices) (Cavigelli *et al.*, 2012; USDA–ERS, 2013).

Nitrogen (N) fertilizer can affect nitrous oxide (N₂O) emissions from soil by influencing the processes of nitrification and denitrification (Aguilera *et al.*, 2013; Amos *et al.*, 2005). Also, fertilizer can affect carbon dioxide (CO₂) emissions from soil by influencing the processes of microbial decomposition and root respiration (Al-Kaisi *et al.*, 2008; Sainju *et al.*, 2008).

Nitrous oxide is produced from nitrification and denitrification in soil, and the rates of these processes depend on plant available N, carbon (C) availability, and microbial activity (Amos *et al.*, 2005; Guzman *et al.*, 2015; Millar *et al.*, 2010). Nitrogen fertilizers are important for crop production, but excessive N fertilizer application, whether organic or synthetic, can increase N₂O emissions (Aguilera *et al.*, 2013; Liu *et al.*, 2012; Millar *et al.*, 2010; Mosier *et al.*, 2003; Tan *et al.*, 2009; Venterea *et al.*, 2012; Zebarth *et al.*, 2008). When N from organic or synthetic fertilizers is applied to soil, ammonium (NH₄⁺) and nitrate (NO₃⁻) concentrations are

increased, resulting in enhanced potential for N₂O production (Asgedom *et al.*, 2014; Vallejo *et al.*, 2006). Therefore, reducing the available N supply through multiple smaller N applications can limit N₂O production. Dalal *et al.* (2003) showed that applying N fertilizer at the appropriate rate and time to meet crop demand through split application was an effective strategy to minimize N₂O emission. Burton *et al.* (2008) and Allen *et al.* (2010) reported that split N fertilizer application was associated with a reduction of N₂O emissions. However, the effect of split N fertilizer application on N₂O emission has not been consistent in previous studies (Burton *et al.*, 2008; Venterea *et al.*, 2012) because N₂O emissions are also influenced by other factors, such as soil moisture and soil temperature (Abbasi and Adams, 2000; Luo *et al.*, 2013; Outlaw and Ernstes, 2008; Signor and Cerri, 2013; Smith *et al.*, 1998). Several recent studies have investigated the impacts of N fertilizer management on N₂O emissions and found that soil N₂O emissions vary with N source (Asgedom *et al.*, 2014; Guzman *et al.*, 2015; Halvorson *et al.*, 2008) due to the chemical form of N in the fertilizer and N release rates (Cayuela *et al.*, 2010). Nitrous oxide emission depends on N availability in the soil, and altering the rate and timing of N fertilizer application is an important strategy to mitigate N₂O emission.

Carbon dioxide emission from soil can be attributed to soil respiration by plant roots and soil microorganisms (Al-Kaisi *et al.*, 2008; Sainju *et al.*, 2008). The rate of CO₂ emission depends on plant growth, availability of C substrate, and environmental factors (e.g., H₂O, temperature, pH) that control microbial activity and N availability (Al-Kaisi *et al.*, 2008; Guzman *et al.*, 2015). Previous studies observing the effect of N fertilizer application on CO₂ emission have presented varying results, including no effects (Rochette and Gregorich, 1998), reduced CO₂ emission (Al-Kaisi *et al.*, 2008), or increased CO₂ emission (Sainju *et al.*, 2008), but these studies evaluated only synthetic N fertilizers. Rochette and Gregorich (1998) also

showed that manure application increased CO₂ flux due to the addition of available C substrates to soil, but synthetic fertilizer application did not. Organic fertilizers supply not only N but also organic C to soil, thereby enhancing C substrate for heterotrophic activity and CO₂ production (Guzman *et al.*, 2015; Sainju *et al.*, 2008). Because organic N fertilizers have varying amounts of C, it is important to quantify and investigate CO₂ emission as a measure of soil respiration due to variable C/N ratios.

In organic vegetable production, macronutrient availability, especially N, is often the yield-limiting factor (Lammerts van Bueren *et al.*, 2011), especially for leafy vegetables such as lettuce (*Lactuca sativa* L.). Lettuce is an internationally grown cash crop that is ranked second among all vegetables produced for consumption in the United States (Coelho *et al.*, 2005). Lettuce requires a fertilizer N input ranging from 100 to 150 kg ha⁻¹ or more (Bottoms *et al.*, 2012), depending on soil properties and cropping history. These fertilizer N rates have raised some concerns about potential environmental impacts, such as GHG emissions (Al-Kaisi *et al.*, 2008; Sainju *et al.*, 2008; Venterea *et al.*, 2012) and water quality impacts from NO₃⁻ leaching (Sebilo *et al.*, 2013; Zhou and Butterbach-Bahl, 2014).

There are many organic fertilizers available, including commercial organic fertilizers such as fish emulsion, blood meal, and feather meal, or alternative organic fertilizers that can be produced on-farm, such as cyano-fertilizer (Barminski *et al.*, 2016). However, optimal management of these fertilizers to provide timely N supply for crops while protecting the environment is not well established.

With concern growing for sustainable agricultural systems that minimize environmental impacts, organic wastes such as blood meal and feather meal have been promoted as alternative sources of N (Aguilera *et al.*, 2013; Cayuela *et al.*, 2010; Sanchez-Martin *et al.*, 2008; Vallejo *et*

al., 2006). However, little is known regarding the effects of commercial and alternative organic fertilizers on GHG emissions, particularly from horticultural crops. To investigate how organic fertilizers, fertilizer application methods, and environmental factors affect GHG emissions, the effects of four organic fertilizers (feather meal, blood meal, fish emulsion, and cyano-fertilizer) with differing forms (solid or liquid), applications (single or multiple application), N rates (0, 28, 56, and 112 kg N ha⁻¹), and C/N ratios on N₂O and CO₂ emissions from a lettuce field were evaluated.

1.3 Materials and methods

1.3.1 Site description

The experiment was conducted 1 July to 9 Aug. 2013 (40 d) and repeated on 9 June to 4 Aug. 2014 (57 d) on certified organic land at the Colorado State University Horticulture Field Research Center in Fort Collins, CO (40°36'39.78" N, 104°59'48.25" W). Nunn clay loam (fine, smectitic, mesic Aridic Argiustoll) is the soil type of the experimental site (NRCS, 1980). The annual precipitation was 302 and 324 mm in 2013 and 2014, respectively. Soil pH was 7.5, organic matter concentration was 2.6%, and mineral N concentrations (NH₄⁺-N and NO₃⁻-N) in 2013 were higher than in 2014 due to previous compost application (Table 1.1).

The experimental site has been certified organic since 2003. In 2013, the experimental site was located in the southwestern part of the certified organic land. In 2014, the experimental site was moved to the east of the 2013 site to avoid residual N from the 2013 experiment.

1.3.2 Field experiment

Twenty-seven lettuce rows were used for this study. Eight fertilized treatments and the control (an unfertilized treatment) were placed randomly using a randomized complete block design with three replications.

Organic lettuce (*L. sativa*) ‘Summer Crisp’ seeds from Johnny’s Selected Seed were germinated in PRO-MIX perlite (PRO-MIX) in a greenhouse at Colorado State University for one month and were transplanted to the experimental site. Lettuce was irrigated using surface drip irrigation.

Four organic fertilizers—two solid fertilizers (feather meal and blood meal [Down To Earth Inc.]) and two liquid fertilizers (fish emulsion [Central Garden & Pet Co.] and cyanofertilizer [*Anabaena* sp., cultivated on the farm])—were evaluated. These fertilizers were classified according to form (solid vs. liquid) and application method. Solid fertilizers were applied by subsurface banding (0.06 m deep and 0.06 m from the row) at the full rate one day before lettuce was transplanted. Liquid fertilizers were applied five times after transplanting by using a watering can (to simulate fertigation). All fertilizers were applied at rates of 56 or 112 kg N ha⁻¹ in 2013. During liquid fertilizer application, other rows were watered to maintain equal amounts of water applied. The lettuce was damaged by a hailstorm on 22 June 2014 and needed time to recover before liquid fertilizer application. Therefore, the liquid fertilizer applications were delayed in 2014.

Field management was different in 2014 compared with 2013. First, the row length was longer than in 2013 (Table 1.2) because the space was needed for additional measurements. Second, the irrigation schedule was changed from twice a day at 9 AM and 3 PM in 2013 to once a day at 6 AM in 2014 to reduce evaporation due to high afternoon temperatures. Finally, the N

rates were reduced to half the rates used in 2013 because there was no rate effect on lettuce yield in 2013 (Sukor, 2016). Therefore, solid fertilizers were subsurface banded in 2014, similar to 2013, at the full rate 4 d before lettuce was transplanted, and liquid fertilizers were fertigated five times after transplanting through drip lines for a total of 28 or 56 kg N ha⁻¹.

1.3.3 Soil and fertilizer analyses

Soil in the experimental field was analyzed before the experiment was set up. Soil samples were randomly taken from the 0- to 30-cm soil depth on 19 June 2013 and 13 May 2014. Chemical and physical properties of soil samples were analyzed (Table 1.1). Soil pH and electrical conductivity were measured in a 1:1 soil to water suspension using a Mettler Toledo pH meter (Thermo Fischer Scientific). Cation exchange capacity was determined using displacement with sodium acetate (Soil Survey Staff, 2004). Organic matter content was analyzed by the loss on ignition method (Blume *et al.*, 1990). Inorganic N (NH₄⁺-N and NO₃⁻-N) concentrations were determined using 2 mol L⁻¹ KCl extraction (Keeney and Nelson, 1982), and the extract was analyzed for NH₄⁺ and NO₃⁻ using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical). Soil texture was determined using the hydrometer method based on Stoke's law (Gee and Bauder, 1979).

Total C and N concentrations were analyzed in the fertilizers (Table 1.3) using a LECO CNH analyzer (Leco Corp.), and C/N ratios were calculated (Keeney and Nelson, 1982). Concentrations of NH₄⁺-N and NO₃⁻-N were analyzed in the fertilizer using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical).

Because the total C concentrations and C/N ratios of the four different organic fertilizers varied considerably (Table 1.3) and N fertilizer supplied substrate to nitrification and

denitrification, we expected that there could be a difference in N₂O and CO₂ emissions. Therefore, N₂O and CO₂ were measured in this study.

1.3.4 Gas sampling and analysis

A closed static chamber method (Hutchinson and Mosier, 1981) was used for gas sampling. Chambers were installed between lettuce plants. Chambers covered the fertilizer band, and the drip irrigation line for fertigation crossed over the chambers with an emitter precisely in the middle of each chamber. Both the fertilizer band and the dripline were placed directly in the middle of each chamber. The gas was sampled according to USDA–ARS GRACEnet project protocols (Parkin and Venterea, 2010). Chamber lids were made with polyvinyl chloride cylinders (10 cm height, 20 cm inner diameter) that contained a built-in vent tube and a sampling port. Chamber anchors were made from polyvinyl chloride cylinders (20 cm inner diameter, 15 cm height). The anchors were inserted 10 cm into the soil at least 24 h before the first gas sampling.

Gas was sampled from 28 June to 23 Aug. 2013, 3 d days a week and twice per day at around 6 AM and 6 PM because irrigation occurred at 9 AM and 3 PM, and from 23 May to 11 Aug. 2014, 3 d a week and once per day at around 9 AM. In 2013, the lettuce was irrigated three times per day (9 AM, 12 PM, and 3 PM) for 15 min each in the first 2 wk of the growing season, and then the irrigation schedule was changed to two times per day (9 AM and 3 PM) after plant establishment. Considering the percolation and sampling times, it was not possible to finish the sampling between irrigations. Therefore, gas samples were collected at 6 AM (before irrigation) and 6 PM (after irrigation). Data from the two sampling times were averaged to calculate the daily mean flux. In 2014, the irrigation schedule was changed from twice per day to once per

day. Gas sampling in 2014 was also changed to once per day during mid-morning after irrigation because midmorning measurements using automated chambers have been shown to be representative of mean daily fluxes, and triweekly sampling is sufficiently frequent (Parkin, 2008; Reeves and Wang, 2015). Gas samples were drawn from the chambers using an airtight syringe. Three samples were taken from each chamber at 0, 15, and 30 min after covering the chamber, for a total sampling time of 30 min. The 25-mL samples were transferred to 12-mL vacuum vials that were sealed with butyl rubber septa. The samples were transported to the USDA–ARS laboratory in Fort Collins for analysis with a gas chromatograph (Varian 450GC, Varian, Inc.) equipped with electron capture and thermal conductivity detectors to quantify N₂O and CO₂, respectively (Halvorson *et al.*, 2008; Mosier *et al.*, 2005). The N₂O and CO₂ emission fluxes were calculated from the linear or nonlinear increase in concentration in the chamber headspace with time (Parkin and Venterea, 2010). The fluxes between sampling days were calculated from a linear interpolation between adjacent sampling dates (Halvorson *et al.*, 2008).

Soil water content and temperature were monitored by using 5TM and 5EC-TM probes (Decagon Devices, Inc.). Twelve probes were installed randomly into 12 rows to measure soil water content and temperature.

Air temperature and precipitation were recorded by CoAgMet at the Colorado State University Agricultural Research, Development and Education Center located within 6 km of the experimental site.

1.3.5 Data analysis

In 2013, three sets of data were analyzed. The data included N₂O and CO₂ fluxes in the morning before irrigation, N₂O and CO₂ fluxes in the evening after irrigation, and cumulative N₂O and CO₂ emissions over the growing season.

Morning and evening fluxes of N₂O and CO₂ were calculated from N₂O and CO₂ emissions from 5 July until 9 Aug. 2013 in the morning and the evening. The cumulative N₂O and CO₂ emissions were calculated from the average of the daily fluxes in the morning and evening from 28 June until 9 Aug. 2013 (from 2 d before initial N application until harvest). However, emissions from 28 June until 4 July 2013 were measured only once per day.

In 2014, only the cumulative N₂O and CO₂ emissions over the entire growing season were analyzed. The cumulative N₂O and CO₂ emissions were calculated from the daily N₂O and CO₂ emissions from 3 June until 4 Aug. 2014 (from two days before initial N application until harvest). During both years, cumulative emissions were calculated by using linear interpolation between successive measurement days.

All data were analyzed in SAS version 9.3 (SAS Institute Inc.). Nitrous oxide emission values were log transformed before ANOVA based on the results of the normality test. Treatments including source and N rate effects on cumulative N₂O and CO₂ emissions were analyzed using the Proc Mixed procedure in SAS.

The treatment effects on N₂O and CO₂ fluxes separated by the time of gas collection in 2013 were analyzed using the Proc Mixed procedure and collection time (AM and PM) as repeated measures. In the repeated measures analysis [type = AR(1)], time of gas collection and treatments were fixed effects, and blocks were random effects. The differences in emissions

during the time of gas collection within each treatment were analyzed by the SLICE option and identified by least square means with a Tukey–Kramer test ($p < 0.05$).

The treatment effects on cumulative N₂O and CO₂ emissions were analyzed using Proc Mixed. Treatments (nine levels) were fixed effects, and blocks (three levels) were random effects. Because the control had a N rate of only 0 kg ha⁻¹, a factorial model would have been incomplete; therefore, a linear mixed model was used. The differences in emissions among nine treatments (an unfertilized control and eight combinations of fertilizer source and N rate) were analyzed by the PDIFF option and identified by least square means with a Tukey–Kramer test ($p < 0.05$). The differences in emissions among sources were analyzed by the LSMESTIMATE option and identified by least square means ($p < 0.05$).

1.4 Results

1.4.1 Environmental conditions

Mean air temperature ranged from 17 to 25°C in 2013 and from 13 to 25°C in 2014 during the growing season (Fig. 1.1). In 2013, mean soil temperature in the evening (26°C) was higher than in the morning (20°C). Across years, the average soil temperatures were 23°C in 2013 and 19°C in 2014 (Fig. 1.2); this difference is probably due to higher precipitation and soil moisture in 2014, leading to cooler soil temperatures.

Total precipitation during the growing season in 2014 (108 mm) was higher than in 2013 (60 mm), and there were more frequent precipitation events in 2014 than in 2013. The highest precipitation events were 23 mm in 2013 and 18 mm in 2014 (Fig. 1.1). Additionally, mean soil water content in the evening of 2013 (26%) was higher than in the morning (23%), and mean soil water content during measurement in 2014 (31%) was higher than in 2013 (24%). In addition to

the higher moisture in 2014, the lettuce crop was damaged by a hailstorm on 22 June 2014, resulting in a setback, a subsequent longer growing period, and delayed liquid fertilizer application.

1.4.2 N₂O and CO₂ emission during the growing season

The effect of organic fertilizer application on N₂O and CO₂ emissions from soil was evaluated in 2013 and 2014. Several peaks of N₂O and CO₂ emissions were found during the growing seasons (Fig. 1.3 and 1.4). Emissions of N₂O and CO₂ were relatively high in the early and mid-growing season and began to decline late in the growing season. However, daily CO₂ flux recorded more fluctuation during the growing season in 2014 than in 2013. Generally, the peak N₂O and CO₂ emissions were observed after rain events, although water inputs from irrigation contributed to some of the emission pulses (Fig. 1.3 and 1.4).

1.4.3 Effect of time of gas sampling and irrigation schedule on N₂O and CO₂ fluxes

Significant differences in N₂O and CO₂ fluxes were observed at different sampling times. Fluxes of N₂O and CO₂ in the evening after irrigation tended to be higher than in the morning before irrigation (Table 1.4).

Due to the effect of irrigation schedule and time of gaseous sampling, N₂O fluxes measured in the evening after irrigation from both rates (56 and 112 kg N ha⁻¹) of blood meal, feather meal, and fish emulsion treatments were significantly higher than the N₂O flux measured in the morning before irrigation (Table 1.4). However, N₂O fluxes from the unfertilized control and both rates of cyano-fertilizer treatments showed no significant difference due to sampling

time (Table 1.4). Similarly, CO₂ fluxes from the unfertilized control and feather meal treatment at 112 kg N ha⁻¹ showed no significant differences due to sampling time.

1.4.4 Effect of sources of fertilizer and treatments (combination of sources of fertilizer and N rates) on cumulative N₂O emission

Comparing the cumulative N₂O emission by fertilizer source (Table 1.5), blood meal and feather meal had higher cumulative N₂O emissions compared with the unfertilized control in 2013 and 2014. The cumulative N₂O emissions in 2013 and 2014 from the solid fertilizer (blood meal and feather meal) treatments were significantly higher than emissions from the liquid fertilizer (fish emulsion and cyano-fertilizer) treatments except blood meal treatment at 56 kg N ha⁻¹ in 2013 (Table 1.6). However, cumulative N₂O emissions in both years from the liquid fertilizer treatments were not different from the control. Considering the cumulative N₂O emission between N rates within each fertilizer (Table 1.6), the cumulative N₂O emissions in 2014 from solid fertilizers applied at 56 kg N ha⁻¹ were significantly higher than the 28 kg N ha⁻¹ rate. Comparing within treatments (combination of sources of fertilizer and N rates), there were no significant differences in the cumulative N₂O emissions in 2013 or 2014 from either N rate of fish emulsion or cyano-fertilizer treatments compared with the control (Table 1.6).

1.4.5 Effect of sources of fertilizer and treatments (combination of sources of fertilizer and N rates) on cumulative CO₂ emission

Comparing the cumulative CO₂ emission by fertilizer source (Table 1.5), there were significant increases in CO₂ emission from all fertilized treatments compared with the unfertilized control (768.8 kg C ha⁻¹ season⁻¹) in 2013 except for the cyano-fertilizer treatment

(997.4 kg C ha⁻¹ season⁻¹). However, only the cumulative CO₂ emission from the blood meal treatments (1292.5 kg C ha⁻¹ season⁻¹) was higher than the control (936.0 kg C ha⁻¹ season⁻¹) in 2014. Comparing within fertilizer form or treatments (combination of sources of fertilizer and N rates), there were no significant differences in the cumulative CO₂ emissions in 2013 and 2014 compared with the control (Table 1.6). Considering the differences in the cumulative CO₂ emission between N rates within each fertilizer (Table 1.6), the cumulative CO₂ emission was not affected by N rate in either 2013 or 2014.

1.5 Discussion

1.5.1 Effect of organic fertilizers and application method on cumulative N₂O emissions

In this study, the solid fertilizers (feather meal and blood meal) had cumulative N₂O emissions higher than the liquid fertilizers (fish emulsion and cyano-fertilizer) in both years (Tables 1.5 and 1.6). However, the cumulative N₂O emissions from the liquid fertilizers were not higher than the unfertilized control. The solid and liquid fertilizers were applied at the same N rate, but the fertilizer application method was different. The solid fertilizers were applied in full before transplanting, but the liquid fertilizers were applied in multiple doses to simulate fertigation in 2013 and were actually fertigated in 2014. Therefore, early in the season, solid fertilizers supplied more available N to plot rows than liquid fertilizers, which contributed to enhanced N₂O production. Also, the higher C/N ratio of the solid fertilizers may have stimulated microbial activity to use excess N from preplant solid fertilizer application in the early season, resulting in increased N₂O emissions.

We have presented strong evidence that fertilizer type and application method influence N₂O emissions; however, due to solid fertilizers being applied preplant and liquid fertilizers

being applied through multiple applications, we cannot separate the effects of fertilizer type from the application method. In relation to this finding, Cole *et al.* (1997) reported that adding N when it is needed by a crop results in decreased available N for conversion to N₂O, and Burton *et al.* (2008) observed that multiple applications of conventional fertilizer (NH₄NO₃) can reduce N₂O emissions in some years due to minimizing the supply of NO₃⁻ for the denitrification process. However, not all studies reported reduced N₂O emissions with multiple applications of N fertilizer (Burton *et al.*, 2008; Venterea and Coulter, 2015). The number of applications or other factors (e.g., management, cropping system, and environment) may contribute to this inconsistency.

There was no significant difference in N₂O emissions between high (112 kg N ha⁻¹) and low (56 kg N ha⁻¹) application rates in 2013 (Table 1.6). This may have been due to high residual soil inorganic N from previous compost application (Table 1.1). However, there was a significant difference between high (56 kg N ha⁻¹) and low (28 kg N ha⁻¹) rates of blood meal and feather meal treatments in 2014, with the lower N rate having lower emissions (Table 1.6). These results partially support methods [e.g., IPCC (2006) Tier 1 methodology; Millar *et al.* (2010)] that assume that N₂O emissions increase linearly or exponentially with fertilizer N inputs because adding more N to soils results in increased available N for nitrification and denitrification, thereby increasing the amount of N₂O emitted.

The percentage of N lost as N₂O (N₂O emission factor) from liquid fertilizer treatments ranged from 0 to 0.06% in 2013 and from 0 to 0.10% in 2014, and from solid fertilizer treatments ranged from 0.6 to 1.9% in 2013 and from 2.6 to 11% in 2014. For comparison, the default IPCC Tier 1 emission factor is 1% (Millar *et al.*, 2010). The lower N₂O loss from liquid fertilizer treatments was probably due to the application method (fertigation with multiple

applications), which reduced available N for N₂O production early in the growing season when plant N demand was relatively low. One limitation of our results is that fertilizer-induced emissions may be overestimated because fertilizers were banded, but the circular sampling chambers did not cover the unfertilized area between rows. Pereira *et al.* (2015) reported a 0 to 0.05% N₂O emission factor from lettuce that received biochar and a compost mix of animal and plant manure. With other organic N fertilizers, Sanchez-Martin *et al.* (2010) found that N₂O emission factors from anaerobically digested pig slurry and a mixture of goat and hen residues applied to onions were 0.71 and 0.54%, respectively. Based on our results, the emission factor of the liquid fertilizers with multiple applications was $\leq 0.1\%$; therefore, using liquid fertilizer applied in multiple applications resulted in N₂O emissions an order of magnitude less than the IPCC default value, in spite of the likely overestimation of our sampling method. Therefore, this approach to N application is a potential mitigation strategy for reducing N₂O emissions. Similar to Kennedy *et al.* (2013), our results suggest that applying fertilizer in multiple doses with irrigation water is an effective strategy to mitigate soil N₂O emissions while maintaining high crop yields.

The lettuce yield ranged from 900 to 1050 kg fresh weight ha⁻¹ in 2013 and from 700 to 1500 kg fresh weight ha⁻¹ in 2014, and there were no lettuce yield differences among fertilizer treatments in 2013. However, in 2014 the marketable weight from the fish emulsion treatment (920 kg fresh weight ha⁻¹) was significantly lower compared with other treatments (average weight of 1290 kg fresh weight ha⁻¹) at the 56 kg N ha⁻¹ application rate (Sukor, 2016). Also, there was no significant difference in N use efficiency (NUE) among treatments, but increasing the N rate reduced NUE. Specifically, the 28, 56, and 112 kg N ha⁻¹ rates had average NUE values of 53, 20, and 9 kg yield kg N applied⁻¹, respectively (Sukor, 2016). Therefore, the use of

multiple applications of cyano-fertilizer was the best option of those we evaluated in terms of reducing GHG emissions while maintaining crop productivity.

1.5.2 Effect of organic fertilizers and application method on cumulative CO₂ emissions

Cumulative CO₂ emissions from the blood meal treatment were significantly higher than the control in both years (Table 1.5). The feather meal and fish emulsion treatments also had CO₂ emissions greater than the control in 2013. These differences in 2013 that were not repeated in 2014 may be attributed to the higher rates of fertilizer application in 2013 (Table 1.2). The cumulative CO₂ emissions from solid fertilizer (blood meal and feather meal) treatments were significantly higher than from liquid fertilizer (fish emulsion and cyano-fertilizer) treatments in 2014 (Table 1.5). This may be due to the higher C/N ratios of the solid fertilizers compared with the liquid fertilizers (Table 1.3). To balance nutrient and energy needs, soil microorganisms require a foodstuff with a C/N ratio of about 24:1 (Mesic *et al.*, 2014; USDA-NRCS, 2011). Fertilizers in this study contained C/N ratios ranging from 0.9 to 3.5 (Table 1.3), considerably lower than 24:1 in all cases. With the lower C/N ratios, the microorganisms are likely to leave excess N in the soil for plants or other microorganisms to use. At the same N rate, more C was applied with the solid fertilizer treatments than with the liquid fertilizer treatments due to their higher C/N ratios. Therefore, more C in the N sources might enhance decomposition, resulting in higher CO₂ emissions (Spohn, 2015). Leu (2006) showed that high nutrient availability stimulates microbial growth and activity, thus resulting in increased decomposition rates and thereby increasing CO₂ emissions. In addition to enhancing microbial activity, N fertilization may increase soil respiration (Gallardo and Schlesinger, 1994).

1.5.3 Effect of environmental factors on N₂O and CO₂ emissions

The time of gas sampling influenced N₂O and CO₂ fluxes. The fluxes measured in the late afternoon 2.5 h after irrigation were higher than in the morning before irrigation (Table 1.4). As expected, mean soil temperature (25.3°C) and soil water content (26.2%) in the evening were higher than in the morning before irrigation (Fig. 1.2). The higher soil temperature and soil water content may explain the increased N₂O and CO₂ emissions. In relation to our finding, Parkin and Kaspar (2003) and Smith *et al.* (1998) have observed diurnal fluctuations in N₂O and CO₂ emissions and reported maximum emissions occurring in the afternoon due to higher soil temperatures. Higher soil moisture can increase N₂O and CO₂ emissions after a rainfall event (Burton *et al.*, 2008; Liu *et al.*, 2006; Parkin and Kaspar, 2003; Perdomo *et al.*, 2009; Venterea and Coulter, 2015). Additionally, Al-Kaisi *et al.* (2008) found that increased soil moisture and temperature resulted in higher rates of CO₂ emission, and Signor and Cerri (2013) reported that soil moisture and temperature influenced N₂O emissions because of their effect on microbial activity and nitrification and denitrification processes. Therefore, the higher N₂O emissions from solid fertilizers in 2014 compared with 2013 may be due to the higher precipitation and subsequent higher soil moisture contents (Signor and Cerri, 2013; Venterea and Coulter, 2015) and the longer growing season in 2014.

1.6 Conclusion

Results from this study showed that (i) solid fertilizers applied preplant significantly increased cumulative N₂O emissions compared with the control, but the liquid fertilizers with multiple N applications were not different in N₂O emissions from the control; (ii) N application rates significantly affected cumulative N₂O emissions in the solid fertilizer treatments but not in

the liquid fertilizer treatments; (iii) fertilizer application schedule and application method can influence cumulative N₂O emissions, but these effects were not separated from fertilizer source in this study; and (iv) high soil temperature and high soil water content in the afternoon after irrigation increased N₂O and CO₂ fluxes from most fertilizer treatments but not from the control. Multiple applications of liquid fertilizer could be a reliable strategy for reducing N₂O emissions from organic lettuce cropping systems. In addition to application method, N₂O emissions are influenced by fertilizer source, N rate, and soil temperature and water content.

TABLES

Table 1.1. Initial chemical and physical properties of the Nunn sandy clay loam at the 0-30 cm depth in 2013 and 2014.

Soil properties	2013 [#]	2014 [#]
pH [‡]	7.5	7.5
Electrical conductivity [‡] (dS m ⁻¹)	0.6	0.6
Cation exchange capacity [†] (cmol kg ⁻¹)	29	29
Organic matter loss on ignition (%)	2.5	2.7
NH ₄ ⁺ -N [§] (mg kg ⁻¹)	42.5	2.4
NO ₃ ⁻ -N [§] (mg kg ⁻¹)	11.4	4.0
Soil texture	Sandy clay loam	Sandy clay loam

[#] Dates of soil sampling were June 19, 2013 and May 13, 2014.

[‡] Determined with 1:1 soil/water extraction.

[†] Displacement with sodium acetate (pH 8.2).

[§] Determined with 2 mol L⁻¹ KCl extraction.

Table 1.2. Experimental design in 2013 and 2014.

Experimental design	2013	2014
<u>Crop</u>	Organic lettuce variety ‘Summer Crisp’ (<i>Lactuca sativa</i>)	Organic lettuce variety ‘Summer Crisp’ (<i>Lactuca sativa</i>)
<u>Plot</u>		
Plot size	0.75 m X 3 m	0.75 m X 4.5 m
Distance between rows	0.75 m	0.75 m
Within row distance between plants	0.2 m	0.2 m
<u>Irrigation</u>		
Irrigation method	Drip	Drip
Irrigation schedule	Every day at 9 A.M. and 3 P.M. for 15 minutes	Every day at 6 A.M. for 60 minutes
<u>Planting</u>		
Planting date in greenhouse	June 1	May 9
Transplanting date	July 1	June 9
Harvest date	August 9	August 4
Length of growing season	40 days	57 days
<u>Fertilizer</u>		
Fertilizer type	Feather meal Blood meal Fish emulsion Cyano-fertilizer	Feather meal Blood meal Fish emulsion Cyano-fertilizer
Fertilizer application rate	56 kg N ha ⁻¹ and 112 kg N ha ⁻¹	28 kg N ha ⁻¹ and 56 kg N ha ⁻¹
Fertilizer application		
Feather meal	Banding	Banding
Blood meal	Banding	Banding
Fish emulsion	Watering can	Fertigation
Cyano-fertilizer	Watering can	Fertigation
Fertilizer application schedule		
Feather meal	Full amount prior to transplanting	Full amount prior to transplanting
Blood meal	Full amount prior to transplanting	Full amount prior to transplanting
Fish emulsion	5 times after transplanting	5 times after transplanting
Cyano-fertilizer	5 times after transplanting	5 times after transplanting

Table 1.3. Fertilizer C and N analyses.

Fertilizer	NH ₄ -N [§]	NO ₃ -N [§]	Total C [‡]	Total N [‡]	C/N
	-----mg kg ⁻¹ -----		-----%-----		
Feather meal	1230	2.30	50.5	14.4	3.5
Blood meal	27.7	8.40	51.4	15.5	3.3
Fish emulsion	23.7	0.12	0.066	0.045	1.5
Cyano-fertilizer	4.70	0.01	0.038	0.041	0.9

[§] Alpkem Flow Solution IV Auto Analyzer.

[‡] LECO CHN analyzer.

Table 1.4. Means of N₂O and CO₂ fluxes in 2013 separated by time of gas sampling and irrigation schedule.

Source of fertilizer	Nitrogen rate	Time [§]	N ₂ O flux	CO ₂ flux
	kg N ha ⁻¹		g N ha ⁻¹ hour ⁻¹	kg C ha ⁻¹ hour ⁻¹
Control	0	AM	0.10a [‡]	0.652a
		PM	0.14a	0.794a
Blood meal	56	AM	0.26b	0.844b
		PM	0.68a	1.112a
	112	AM	0.79b	0.886b
		PM	1.70a	1.100a
Feather meal	56	AM	0.71b	0.923b
		PM	1.43a	1.198a
	112	AM	0.62b	0.940a
		PM	1.44a	1.101a
Fish emulsion	56	AM	0.15b	0.789b
		PM	0.23a	0.997a
	112	AM	0.10b	0.888b
		PM	0.27a	1.104a
Cyano-fertilizer	56	AM	0.11a	0.682b
		PM	0.14a	0.943a
	112	AM	0.12a	0.769b
		PM	0.17a	1.210a
Treatment (Combination of source of fertilizer and nitrogen rate)			<0.0001*	0.0941
Time			<0.0001*	<0.0001*
Treatment x time			0.2520	0.3203

* Significant at the 0.05 probability level.

[§] Time was separated to AM and PM; AM refers to the time of gaseous sampling in the morning prior to irrigation, and PM refers to the time of gaseous sampling in the late afternoon after irrigation (2.5 hrs after irrigation).

[‡] In the same fertilizer and rate, sampling time means followed by the same letter are not significantly different according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 1.5. Cumulative N₂O and CO₂ emissions throughout the 2013 and 2014 growing seasons as affected by sources of fertilizer.

Source of fertilizer	2013		2014	
	Cumulative N ₂ O emission Mean ± SE	Cumulative CO ₂ emission Mean ± SE	Cumulative N ₂ O emission Mean ± SE	Cumulative CO ₂ emission Mean ± SE
	kg N ha ⁻¹ season ⁻¹	kg C ha ⁻¹ season ⁻¹	kg N ha ⁻¹ season ⁻¹	kg C ha ⁻¹ season ⁻¹
Control	0.247±0.039b‡	768.8±59.738b	0.308±0.046c	936.0±311.191bc
Blood meal	0.969±0.214a	1117.3±58.324a	4.489±1.011a	1292.5±28.230a
Feather meal	1.265±0.184a	1167.4±17.304a	2.802±0.804b	1138.4±182.794ab
Fish emulsion	0.257±0.016b	1042.2±84.046a	0.285±0.053c	849.3±85.272c
Cyano-fertilizer	0.262±0.030b	997.4±97.074ab	0.226±0.016c	591.9±72.312c

‡ Values are means ± SE. Within each column, means followed by the same lowercase letter are not significantly different according to the least square means ($p < 0.05$).

Table 1.6. Cumulative N₂O and CO₂ emissions throughout the 2013 and 2014 growing seasons as affected by treatments (combination of sources of fertilizer and nitrogen rates).

Source of fertilizer	Nitrogen rate	2013		2014	
		Cumulative N ₂ O emission Mean ± SE	Cumulative CO ₂ emission Mean ± SE	Cumulative N ₂ O emission Mean ± SE	Cumulative CO ₂ emission Mean ± SE
	kg N ha ⁻¹	kg N ha ⁻¹ season ⁻¹	kg C ha ⁻¹ season ⁻¹	kg N ha ⁻¹ season ⁻¹	kg C ha ⁻¹ season ⁻¹
Control	0	0.247±0.039b [‡]	768.8±59.7a	0.308±0.046d	936.0±311.2abc
Blood meal	28	N/A [§]	N/A	2.447±0.343bc	1285.4±18.8ab
	56	0.578±0.259ab	1115.6±74.4a	6.530±0.911a	1299.5±59.8ab
	112	1.360±0.101a	1118.9±107.1a	N/A	N/A
Feather meal	28	N/A	N/A	1.034±0.306c	802.9±137.4abc
	56	1.291±0.145a	1167.5±36.9a	4.571±0.102ab	1473.8±188.8a
	112	1.240±0.384a	1167.2±11.5a	N/A	N/A
Fish emulsion	28	N/A	N/A	0.206±0.075d	861.8±44.8abc
	56	0.269±0.030b	1014.7±128.0a	0.365±0.048d	836.8±184.9abc
	112	0.245±0.014b	1069.8±134.8a	N/A	N/A
Cyano-fertilizer	28	N/A	N/A	0.258±0.009d	658.1±105.7bc
	56	0.213±0.027b	861.6±95.1a	0.194±0.013d	525.6±102.9c
	112	0.310±0.036b	1133.1±140.2a	N/A	N/A
		<i>P > F</i>			
Treatment (Combination of source of fertilizer and nitrogen rate)		<0.0001*	0.0939	<0.0001*	0.0019*

* Significant at the 0.05 probability level.

§ N/A refers to no data because the study did not apply this N rate in both years.

‡ Values are means ± SE. Within each column, means followed by the same letter are not significantly different according to the least square means with a Tukey-Kramer test ($p < 0.05$).

FIGURES

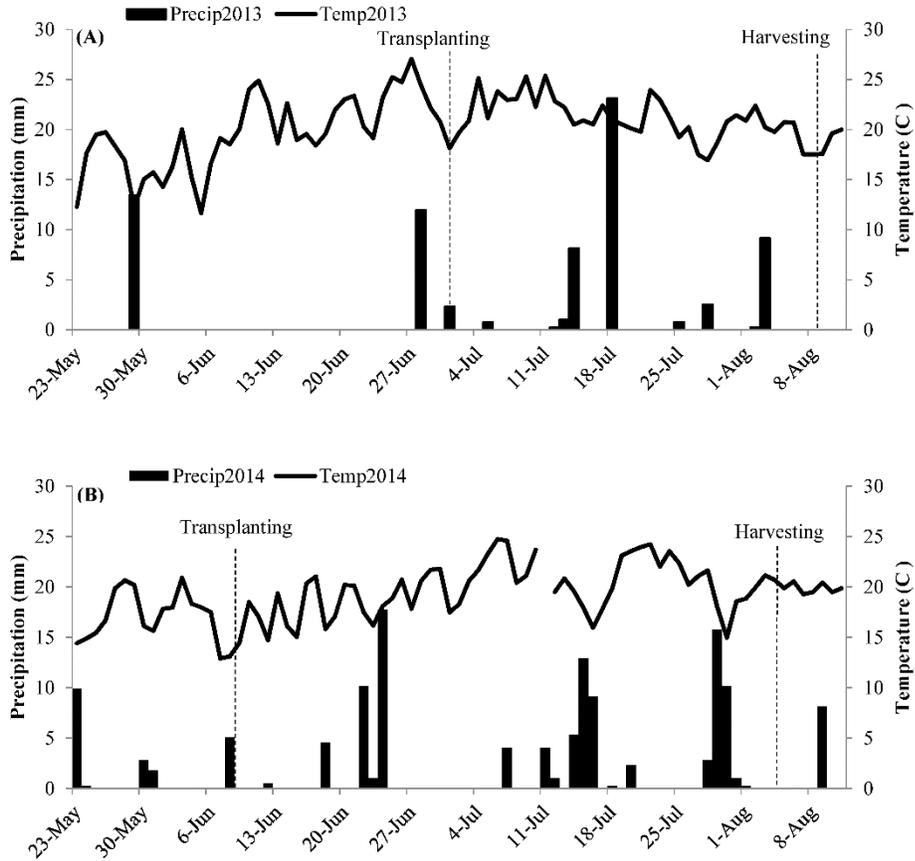


Fig. 1.1. Average daily air temperature (Temp) and precipitation (Precip) from CoAgMet at the Colorado State University Agricultural Research, Development and Education Center (ARDEC) during the 2013 (A) and 2014 (B) growing seasons.

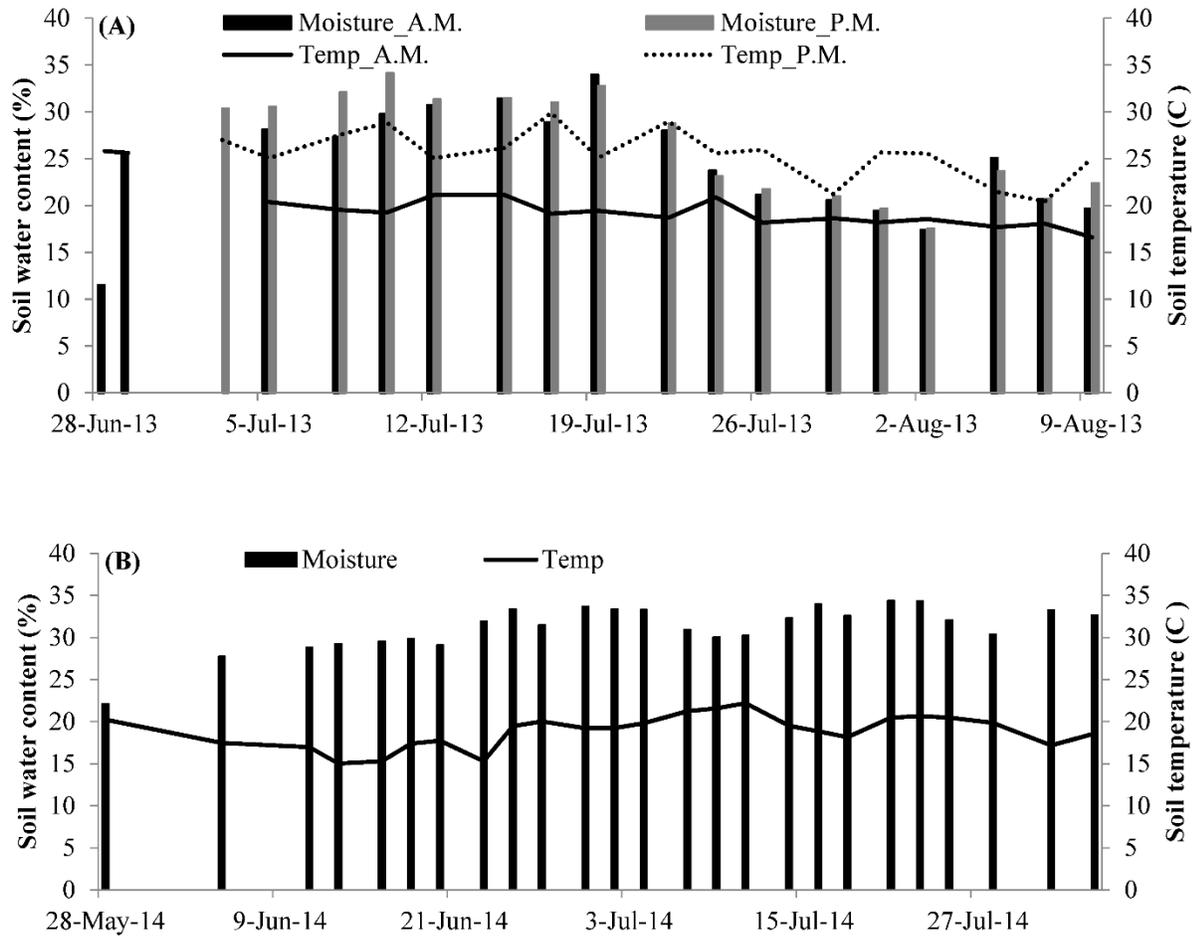


Fig. 1.2. Soil temperature (Temp) and soil water content (Moisture) measured during the 2013 and 2014 growing seasons. (A) Soil temperature and moisture in the 2013 growing season. In 2013, measurements were made twice per day. Temp_A.M. and Moisture_A.M. refer to soil temperature and soil water content measured in the morning before irrigation. Temp_P.M. and Moisture_P.M. refer to soil temperature and soil water content measured in the evening following irrigation. (B) Soil temperature and soil water content in the 2014 growing season. Note: No measurements were taken on 28 and 29 June 2013 in the evening or on 3 July 2013 in the morning.

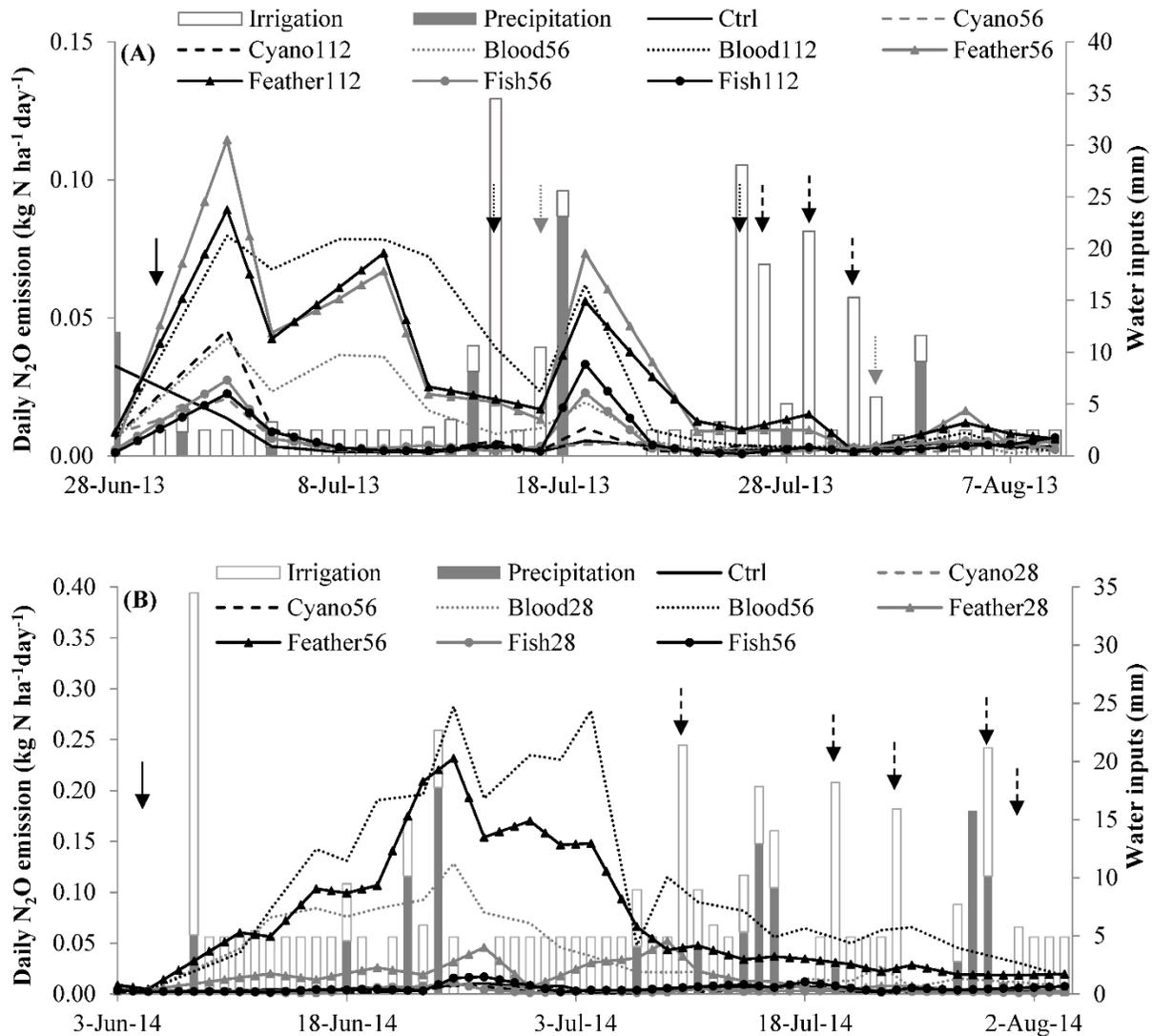


Fig. 1.3. Effect of organic fertilizers on (A) daily N_2O emissions in 2013 and (B) daily N_2O emissions in 2014 (Blood, blood meal; Ctrl, control; Cyano, cyano-fertilizer; Feather, feather meal; Fish, fish emulsion). Number after name of fertilizer refers to N application rate ($kg\ N\ ha^{-1}$) (e.g., Fish56 refers to applying fish emulsion at $56\ kg\ N\ ha^{-1}$). Solid arrows indicate timing of solid fertilizer application (blood meal and feather meal), dashed arrows indicate timing of liquid fertilizer application (fish emulsion and cyano-fertilizer), black dotted arrows indicate timing of cyano-fertilizer application alone, and gray dotted arrows indicate timing of fish emulsion application alone.

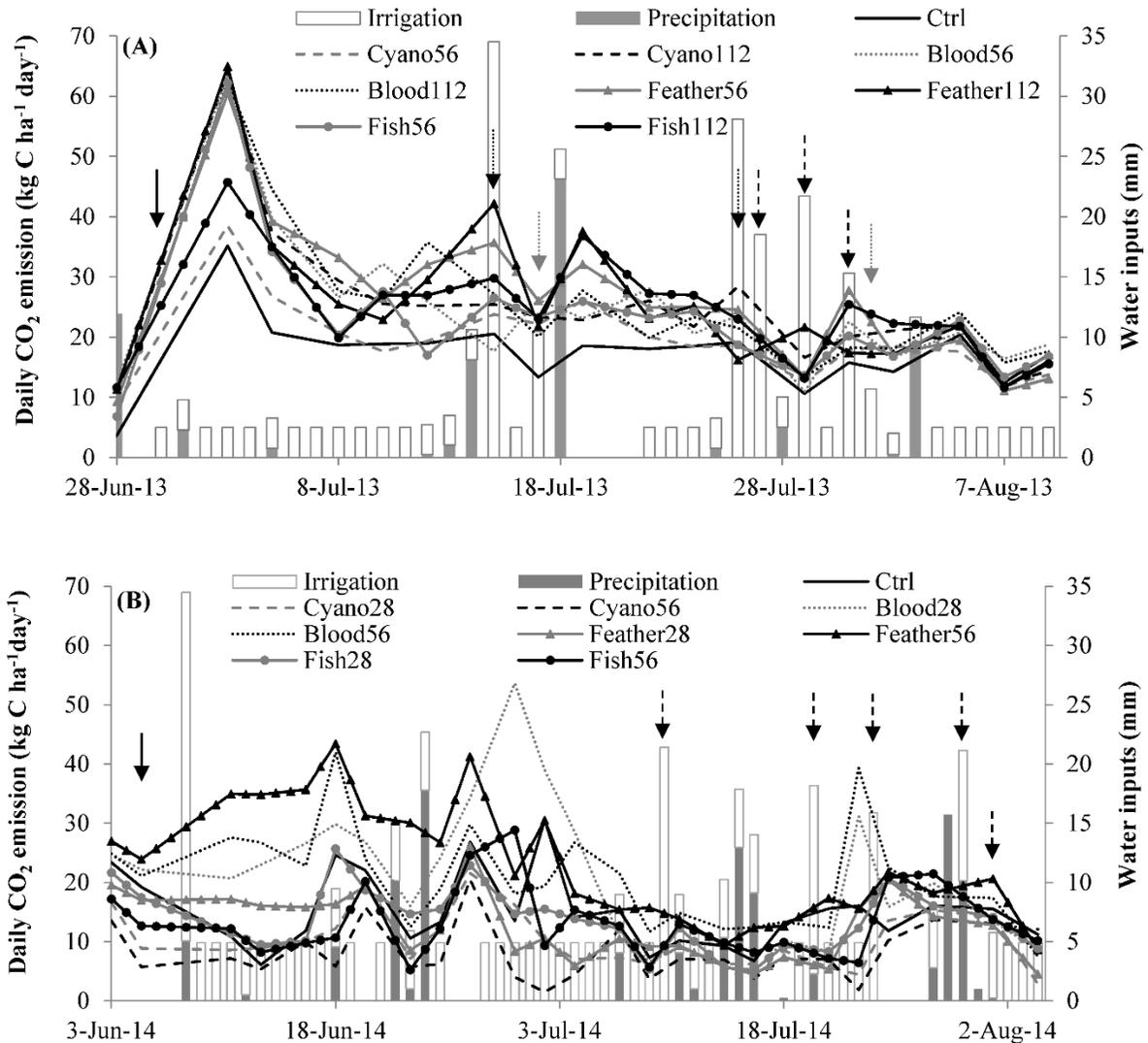


Fig. 1.4. Effect of organic fertilizers on (A) daily CO₂ emissions in 2013 and (B) daily CO₂ emissions in 2014 (Blood, blood meal; Ctrl, control; Cyano, cyano-fertilizer; Feather, feather meal; Fish, fish emulsion). Number after name of fertilizer refers to N application rate (kg N ha⁻¹) (e.g., Fish56 refers to applying fish emulsion at 56 kg N ha⁻¹). Solid arrows indicate timing of solid fertilizer application (blood meal and feather meal), dashed arrows indicate timing of liquid fertilizer application (fish emulsion and cyano-fertilizer), black dotted arrows indicate timing of cyano-fertilizer application alone, and gray dotted arrows indicate timing of fish emulsion application alone.

REFERENCES

- Abbasi, M.K., and W.A. Adams. 2000. Gaseous N emission during simultaneous nitrification–denitrification associated with mineral N fertilization to a grassland soil under field conditions. *Soil biology & biochemistry*. 32: 1251-1259. doi:doi:10.1016/S0038-0717(00)00042-0
- Aguilera, E., L. Lassaletta, A. Sanz-Cobena, J. Garnier, and A. Vallejo. 2013. The potential of organic fertilizers and water management to reduce N₂O emissions in Mediterranean climate cropping systems. A review. *Agriculture, ecosystems & environment*. 164: 32-52. doi:10.1016/j.agee.2012.09.006
- Al-Kaisi, M.M., M.L. Kruse, and J.E. Sawyer. 2008. Effect of nitrogen fertilizer application on growing season soil carbon dioxide emission in a corn–soybean rotation. *Journal of environmental quality*. 37: 325-332. doi:10.2134/jeq2007.0240
- Allen, D., G. Kingston, H. Rennenberg, R. Dalal, and S. Schmidt. 2010. Effect of nitrogen fertilizer management and waterlogging on nitrous oxide emission from subtropical sugarcane soils. *Agriculture, ecosystems & environment*. 136: 209-217. doi:10.1016/j.agee.2009.11.002
- Amos, B., T.J. Arkebauer, and J.W. Doran. 2005. Soil surface fluxes of greenhouse gases in an irrigated maize-based agroecosystem. *Soil Science Society of America Journal*. 69: 387-395. doi:10.2136/sssaj2005.0387
- Asgedom, H., M. Tenuta, D.N. Flaten, X. Gao, and E. Kebreab. 2014. Nitrous oxide emissions from a clay soil receiving granular urea formulations and dairy manure. *Agronomy Journal*. 106: 732-744. doi:10.2134/agronj2013.0096

- Barminski, R., H. Storteboom, and J.G. Davis. 2016. Development and evaluation of an organically certifiable growth medium for cultivation of cyanobacteria. *Journal of Applied Phycology*. 1-8. doi:10.1007/s10811-016-0819-2
- Blume, L.J., P.W. Schumacher, K.A. Schaffer, K.A. Cappo, M.L. Papp, R.D. Remortel, D.S. Coffey, M.G. Johnson, and D.J. Chaloud. 1990. Handbook of methods for acid-deposition studies. Laboratory analyses for soil chemistry. USEPA, Environmental Monitoring Systems Laboratory, Las Vegas, NV.
- Bottoms, T.G., R.F. Smith, M.D. Cahn, and T.K. Hartz. 2012. Nitrogen requirements and N status determination of lettuce. *HortScience*. 47: 1768-1774.
- Burton, D., B. Zebarth, K. Gillam, and J. MacLeod. 2008. Effect of split application of fertilizer nitrogen on N₂O emissions from potatoes. *Canadian Journal of Soil Science*. 88: 229. doi:10.4141/CJSS06007
- Cavigelli, M.A., S.J. Del Grosso, M.A. Liebig, C.S. Snyder, P.E. Fixen, R.T. Venterea, A.B. Leytem, J.E. McLain, and D.B. Watts. 2012. US agricultural nitrous oxide emissions: context, status, and trends. *Frontiers in Ecology and the Environment*. 10: 537-546. doi:10.1890/120054
- Cayuela, M., G. Velthof, C. Mondini, T. Sinicco, and J. Van Groenigen. 2010. Nitrous oxide and carbon dioxide emissions during initial decomposition of animal by-products applied as fertilisers to soils. *Geoderma*. 157: 235-242. doi:10.1016/j.geoderma.2010.04.026
- Coelho, C., A. Laouina, K. Regaya, A. Ferreira, T. Carvalho, M. Chaker, R. Naafa, R. Naciri, A. Boulet, and J. Keizer. 2005. The impact of soil water repellency on soil hydrological and erosional processes under Eucalyptus and evergreen Quercus forests in the Western Mediterranean. *Soil Research*. 43: 309-318. doi:10.1071/SR04083

- Cole, C., J. Duxbury, J. Freney, O. Heinemeyer, K. Minami, A. Mosier, K. Paustian, N. Rosenberg, N. Sampson, and D. Sauerbeck. 1997. Global estimates of potential mitigation of greenhouse gas emissions by agriculture. *Nutrient cycling in Agroecosystems*. 49: 221-228. doi:10.1023/A:1009731711346
- Dalal, R.C., W. Wang, G.P. Robertson, and W.J. Parton. 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. *Soil Research*. 41: 165-195. doi:10.1071/SR02064
- Gallardo, A., and W.H. Schlesinger. 1994. Factors limiting microbial biomass in the mineral soil and forest floor of a warm-temperate forest. *Soil Biology and Biochemistry*. 26: 1409-1415. doi:10.1016/0038-0717(94)90225-9
- Gee, G., and J. Bauder. 1979. Particle size analysis by hydrometer: a simplified method for routine textural analysis and a sensitivity test of measurement parameters. *Soil Science Society of America Journal*. 43: 1004-1007.
- Guzman, J., M. Al-Kaisi, and T. Parkin. 2015. Greenhouse gas emissions dynamics as influenced by corn residue removal in continuous corn system. *Soil Science Society of America Journal*. 79: 612-625. doi:10.2136/sssaj2014.07.0298
- Halvorson, A.D., S.J. Del Grosso, and C.A. Reule. 2008. Nitrogen, tillage, and crop rotation effects on nitrous oxide emissions from irrigated cropping systems. *Journal of Environmental Quality*. 37: 1337-1344. doi:10.2134/jeq2007.0268
- Hutchinson, G., and A. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal*. 45: 311-316. doi:10.2136/sssaj1981.03615995004500020017x

- IPCC. 2006. Vol. 4: Agriculture, forestry and other land use. In: S. Eggleston et al., editors, 2006 IPCC guidelines for national greenhouse gas inventories. IPCC, Geneva, Switzerland. p. 11.11-11.54.
- Keeney, D.R.A., and D.W. Nelson. 1982. Nitrogen—inorganic forms. In: A.L. Page, editor Methods of soil analysis. Part 2. Chemical and microbiological properties. Agron Monographs No 9. ASA and SSSA, Madison, WI. p. 643-698.
- Kennedy, T.L., E.C. Suddick, and J. Six. 2013. Reduced nitrous oxide emissions and increased yields in California tomato cropping systems under drip irrigation and fertigation. *Agriculture, ecosystems & environment*. 170: 16-27. doi:10.1016/j.agee.2013.02.002
- Lammerts van Bueren, E.T., S.S. Jones, L. Tamm, K.M. Murphy, J.R. Myers, C. Leifert, and M.M. Messmer. 2011. The need to breed crop varieties suitable for organic farming, using wheat, tomato and broccoli as examples: A review. *NJAS Wageningen journal of life sciences*. 58: 193-205. doi:10.1016/j.njas.2010.04.001
- Leu, A. Year. Organics and soil carbon: Increasing soil carbon, crop productivity and farm profitability. In: P. Kristiansen et al., editors, *Organics: Solutions to climate change*. Proceedings title. Place Citeseer. Conference name. p. 4-12.
- Liu, C., K. Wang, and X. Zheng. 2012. Responses of N₂O and CH₄ fluxes to fertilizer nitrogen addition rates in an irrigated wheat-maize cropping system in northern China. *Biogeosciences*. 9: 839-850. doi:10.5194/bg-9-839-2012
- Liu, X.J., A.R. Mosier, A.D. Halvorson, and F.S. Zhang. 2006. The impact of nitrogen placement and tillage on NO, N₂O, CH₄ and CO₂ fluxes from a clay loam soil. *Plant and soil*. 280: 177-188. doi:10.1007/s11104-005-2950-8

- Luo, G., R. Kiese, B. Wolf, and K. Butterbach-Bahl. 2013. Effects of soil temperature and moisture on methane uptake and nitrous oxide emissions across three different ecosystem types. *Biogeosciences*. 10: 3205-3219. doi:10.5194/bg-10-3205-2013
- Mesic, M., M. Birkás, Z. Zgorelec, I. Kistic, I. Sestak, A. Jurisic, and S. Husnjak. 2014. Soil Carbon Variability in Some Hungarian and Croatian Soils. In: A.E. Hartemink et al., editors, *Soil Carbon: Progress in soil science*. Springer International Publishing, Charm, Switzerland. p. 419-426. doi: 10.1007/978-3-319-04084-4_41.
- Millar, N., G.P. Robertson, P.R. Grace, R.J. Gehl, and J.P. Hoben. 2010. Nitrogen fertilizer management for nitrous oxide (N₂O) mitigation in intensive corn (Maize) production: an emissions reduction protocol for US Midwest agriculture. *Mitigation and Adaptation Strategies for Global Change*. 15: 185-204. doi:10.1007/s11027-010-9212-7
- Mosier, A., A. Halvorson, G. Peterson, G. Robertson, and L. Sherrod. 2005. Measurement of net global warming potential in three agroecosystems. *Nutrient Cycling in Agroecosystems*. 72: 67-76. doi:10.1007/s10705-004-7356-0
- Mosier, A., G. Peterson, and L. Sherrod. 2003. Mitigating net global warming potential (CO₂, CH₄ and N₂O) in upland crop productions. *Proceedings title. Place Conference name*. p. 273-280.
- NRCS. 1980. Soil survey of Larimer County area, Colorado.
http://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/colorado/larimerCO1980/Larimer.pdf (accessed 6 MAy 2015).
- Outlaw, J.L., and D.P. Ernstes. 2008. *The Lifecycle Carbon Footprint of Biofuels*. Farm Foundation, Oak brook, IL.

- Parkin, T.B. 2008. Effect of sampling frequency on estimates of cumulative nitrous oxide emissions. *Journal of Environmental Quality*. 37: 1390-1395. doi:10.2134/jeq2007.0333
- Parkin, T.B., and T.C. Kaspar. 2003. Temperature controls on diurnal carbon dioxide flux. *Soil Science Society of America Journal*. 67: 1763-1772. doi:10.2136/sssaj2003.1763
- Parkin, T.B., and R.T. Venterea. 2010. Chamber-based trace gas flux measurements. In: Editor, editor^editors, *Sampling protocols*. ISDA Washington, DC. p. 3-1-3-39.
<http://www.ars.usda.gov/research/GRACEnet> (accessed 6 May 2015).
- Paustian, K., B. Babcock, C. Kling, J. Hatfield, R. Lal, B. McCarl, S. McLaughlin, W.M. Post, A. Mosier, C. Rice, G. Robertson, N.J. Rosenberg, C. Rosenzweig, W.H. Schlesinger, and D. Zilberman. 2004. Agricultural mitigation of greenhouse gases: science and policy options. CAST report R141. Council on Agricultural Science and Technology, Ames, IA.
- Perdomo, C., P. Irisarri, and O. Ernst. 2009. Nitrous oxide emissions from an Uruguayan argiudoll under different tillage and rotation treatments. *Nutrient cycling in agroecosystems*. 84: 119-128. doi:10.1007/s10705-008-9231-x
- Pereira, E.I.P., E.C. Suddick, F.N. Mukome, S.J. Parikh, K. Scow, and J. Six. 2015. Biochar alters nitrogen transformations but has minimal effects on nitrous oxide emissions in an organically managed lettuce mesocosm. *Biology and Fertility of Soils*. 51: 573-582.
doi:10.1007/s00374-015-1004-5
- Reeves, S., and W. Wang. 2015. Optimum sampling time and frequency for measuring N₂O emissions from a rain-fed cereal cropping system. *Science of The Total Environment*. 530: 219-226. doi:10.1016/j.scitotenv.2015.05.117

- Rochette, P., and E. Gregorich. 1998. Dynamics of soil microbial biomass C, soluble organic C and CO₂ evolution after three years of manure application. *Canadian Journal of Soil Science*. 78: 283-290. doi:10.4141/S97-066
- Sainju, U.M., J.D. Jabro, and W.B. Stevens. 2008. Soil carbon dioxide emission and carbon content as affected by irrigation, tillage, cropping system, and nitrogen fertilization. *Journal of Environmental Quality*. 37: 98-106. doi:10.2134/jeq2006.0392
- Sanchez-Martin, L., A. Vallejo, J. Dick, and U.M. Skiba. 2008. The influence of soluble carbon and fertilizer nitrogen on nitric oxide and nitrous oxide emissions from two contrasting agricultural soils. *Soil Biology and Biochemistry*. 40: 142-151.
doi:10.1016/j.soilbio.2007.07.016
- Sanchez-Martin, L., A. Sanz-Cobena, A. Mejjide, M. Quemada, and A. Vallejo. 2010. The importance of the fallow period for N₂O and CH₄ fluxes and nitrate leaching in a Mediterranean irrigated agroecosystem. *European journal of soil science*. 61: 710-720.
doi:10.1111/j.1365-2389.2010.01278.x
- Sebilo, M., B. Mayer, B. Nicolardot, G. Pinay, and A. Mariotti. 2013. Long-term fate of nitrate fertilizer in agricultural soils. *Proceedings of the National Academy of Sciences*. 110: 18185-18189. doi:10.1073/pnas.1305372110
- Signor, D., and C.E.P. Cerri. 2013. Nitrous oxide emissions in agricultural soils: a review. *Pesquisa Agropecuária Tropical*. 43: 322-338. doi:10.1590/S1983-40632013000300014
- Smith, K.A., P.E. Thomson, H. Clayton, I.P. McTaggart, and F. Conen. 1998. Effects of temperature, water content and nitrogen fertilisation on emissions of nitrous oxide by soils. *Atmospheric Environment*. 32: 3301-3309. doi:10.1016/S1352-2310(97)00492-5

- Soil Survey Staff. 2004. Method 4B4b. In: R. Burt, editor Soil survey laboratory methods manual. Soil Survey Investigations Report No. 42. Version 4.0. USDA-NRCS, Washington, DC. p. 202.
- Spohn, M. 2015. Microbial respiration per unit microbial biomass depends on litter layer carbon-to-nitrogen ratio. *Biogeosciences*. 12: 817-823. doi:10.5194/bg-12-817-2015
- Sukor, A. 2016. Organic nitrogen fertilizers influence nutritional value, water use efficiency, and nitrogen dynamics of drip irrigated lettuce and sweet corn. Ph.D. dissertation, Colorado State Univ., Fort Collins.
- Tan, I.Y., H.M. van Es, J.M. Duxbury, J.J. Melkonian, R.R. Schindelbeck, L.D. Geohring, W.D. Hively, and B.N. Moebius. 2009. Single-event nitrous oxide losses under maize production as affected by soil type, tillage, rotation, and fertilization. *Soil and Tillage Research*. 102: 19-26. doi:10.1016/j.still.2008.06.005
- Toonsiri, P., S. Del Grosso, A. Sukor, and J. Davis. 2016. Greenhouse gas emissions from solid and liquid organic fertilizers applied to lettuce. *Journal of Environmental Quality*. 45: 1812. doi:10.2134/jeq2015.12.0623
- USDA-NRCS. 2011. Carbon to nitrogen ratios in cropping systems. USDA, Washington, DC. <http://www.nrcs.usda.gov/wps/portal/nrcs/main/soils/health/mgmt/> (accessed 6 June 2016).
- USDA-ERS. 2013. Fertilizer use & markets. USDA, Washington, DC. <http://www.ers.usda.gov/topics/farm-practices-management/chemical-inputs/fertilizer-use-markets.aspx> (accessed 6 May 2015).

- USEPA. 2015. Inventory of U.S. greenhouse gas emissions and sinks: 1990-2013.
<https://www3.epa.gov/climatechange/Downloads/ghgemissions/US-GHG-Inventory-2015-Main-Text.pdf> (accessed 6 May 2015).
- Vallejo, A., U.M. Skiba, L. García-Torres, A. Arce, S. López-Fernández, and L. Sánchez-Martín. 2006. Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts. *Soil Biology and Biochemistry*. 38: 2782-2793. doi:10.1016/j.soilbio.2006.04.040
- Venterea, R.T., and J.A. Coulter. 2015. Split application of urea does not decrease and may increase nitrous oxide emissions in rainfed corn. *Agronomy Journal*. 107: 337-348.
doi:10.2134/agronj14.0411
- Venterea, R.T., A.D. Halvorson, N. Kitchen, M.A. Liebig, M.A. Cavigelli, S.J. Del Grosso, P.P. Motavalli, K.A. Nelson, K.A. Spokas, and B.P. Singh. 2012. Challenges and opportunities for mitigating nitrous oxide emissions from fertilized cropping systems. *Frontiers in Ecology and the Environment*. 10: 562-570. doi:10.1890/120062
- Zebarth, B., P. Rochette, and D. Burton. 2008. N₂O emissions from spring barley production as influenced by fertilizer nitrogen rate. *Canadian Journal of Soil Science*. 88: 197-205.
doi:10.4141/CJSS06006
- Zhou, M., and K. Butterbach-Bahl. 2014. Assessment of nitrate leaching loss on a yield-scaled basis from maize and wheat cropping systems. *Plant and soil*. 374: 977-991.
doi:10.1007/s11104-013-1876-9

CHAPTER 2. SOIL ORGANIC CARBON AND AGGREGATE STABILITY IN CONVENTIONAL AND ORGANIC VEGETABLE FARMING SYSTEMS²

2.1 Summary

Agricultural management can affect soil health and the ecosystem services provided by soil by altering soil biochemical properties and the soil's capacity to accrue and protect organic matter. The effect of management on soil biochemical properties, organic carbon (SOC), and total nitrogen (TN) stocks, and their protection in soil aggregates and organo-mineral complexes under conventional and organic vegetable farming systems were evaluated. Soil samples were collected from conventional and organic vegetable fields at different locations (California, Colorado, and New York) and at different soil depths (0-10, 10-20, and 20-30 cm). Soil samples were analyzed for their chemical properties, microbial biomass, and aggregate size distribution. SOC and TN were determined in bulk soils, as well as in aggregates and primary organo-mineral fractions within aggregates. The results showed that, as compared to conventional, organic farming: i) Sustained higher stocks of C and N, and a larger microbial biomass; ii) Increased SOM protection through enhancing aggregate stability, and the formation of organo-mineral complexes. We believe that the higher inputs in organic farming systems promoted a higher microbial biomass and activity, which stimulated aggregation while increasing the formation of organo-mineral bonding of microbial products. We conclude that organic farming practices should be considered as a means to promote soil organic matter accrual and protection, ultimately promoting soil health and mitigating climate change through soil C sequestration.

² In preparation for submission to Agriculture, Ecosystems & Environment with M. Francesca Cotrufo, Betsy A. Leonard, Emma Torbert, Kate M. Scow, and Jessica G. Davis

2.2 Introduction

Soil is a key factor in mitigating greenhouse gas (GHG) emissions, especially carbon dioxide (CO₂), since it can serve as a source and a sink of carbon (C) depending on soil management. The soil C pool is estimated at 2500 Pg comprising about 38% soil inorganic carbon (SIC) and 62% soil organic carbon (SOC) and has been a significant source of atmospheric CO₂ (Eswaran *et al.*, 2000; Lal, 2004). In contrast, the loss of CO₂ from soil can be offset by storing C in soil. Carbon sequestration is a strategy that increases SOC by transferring CO₂ from the atmosphere to plants, then storing C in soil as soil organic matter (SOM), and stabilizing and protecting SOC with a long turnover time (Lal, 2004).

Soil organic matter stocks depend on the balance between SOM inputs from plants or other organic amendments and SOM losses mostly through decomposition or erosion (Lehmann *et al.*, 2007; Smith *et al.*, 2015; Van Oost *et al.*, 2007). Thus, SOM sequestration management strategies ought to result in a net positive SOM change, by either increasing inputs or decreasing decay rates (Paustian *et al.*, 2016). The change in SOM is driven by many factors: e.g., soil environment (Smith *et al.*, 2015), soil management (Lal, 2004; Olson *et al.*, 2014), quality and quantity of SOM (Bulluck III *et al.*, 2002; Herencia *et al.*, 2007; Six *et al.*, 2002), and SOM protection mechanisms (Six *et al.*, 2002; Six *et al.*, 1998).

The persistence of SOM in soils is associated with the level of physical protection in stable aggregates or SOM mineral associations (Lehmann and Kleber, 2015). In fact, SOM is more stable and less bioavailable when it is occluded within soil aggregates (Banwart *et al.*, 2015; Six *et al.*, 2002). Microbial extracellular polymers from decomposition of SOM chemically bind soil particles, both organic and inorganic materials, to form larger aggregates (Amézketa, 1999; Banwart *et al.*, 2015). Besides serving as a binding material, SOM serves as a

nucleus for aggregate formation (Semenov *et al.*, 2010). Soil organic matter is also more stable when it is associated with minerals. This protection mechanism is regulated by the type of silt and clay particles (Lutzow *et al.*, 2006; Plante *et al.*, 2006) and their degree of C saturation (Castellano *et al.*, 2015), but also by the microbial activity and resource use efficiency (Cotrufo *et al.*, 2013), since most of the SOM associated with minerals is of microbial origin (Miltner *et al.*, 2012).

Agricultural management contributes to the loss or enhancement of SOM stocks and overall persistence (Green *et al.*, 2005; Mikha *et al.*, 2015; Mikha and Rice, 2004). Conventional farming practices such as intensive tillage and excessive fertilizer and pesticide applications generally lead to deterioration of soil structure and loss of SOM (Herencia *et al.*, 2007; Melero *et al.*, 2006; Tu *et al.*, 2006; Wells *et al.*, 2000). Conversely, application of organic soil amendments can enhance SOM and aggregate stability (Bulluck III *et al.*, 2002; Herencia *et al.*, 2007; Król *et al.*, 2013; Mikha *et al.*, 2015). Because of the concern regarding CO₂ emissions and the significance of SOM persistence on CO₂ mitigation, optimizing agricultural management to increase SOM stocks and protection is extremely urgent.

Agricultural management, particularly organic crop management has been suggested as a means to CO₂ mitigation (FAO, 2011). In relation to this suggestion, previous studies also support that organic management can increase and protect SOM stocks relative to conventional management (Bulluck III *et al.*, 2002; Herencia *et al.*, 2007; Król *et al.*, 2013). It is clear that organic management can increase SOM, but little is known about the effects of organic farming, particularly in vegetable crops, on SOM stocks and persistence.

The objectives of this study were to investigate the effects of organic farming management as compared to conventional management on SOM stocks and persistence, through

aggregate protection and mineral association, within the soil profile. In particular, we hypothesized that: i) soil aggregate stability will be higher under organic farming compared to conventional farming due to greater organic inputs and microbial biomass enhancing aggregate formation, ii) SOC and total N stocks will be higher under organic farming systems, compared to conventional farming systems, due to higher C inputs and higher physical and chemical protection resulting in more stable SOM, and iii) SOM enhancement in organic farming systems will be present throughout the soil profile to at least 30 cm depth. We compared conventional and organic vegetable farming systems in three locations (California, Colorado, and New York), and soil aggregation, SOM and microbial biomass C and N stocks were evaluated at three soil depths (0-10, 10-20, and 20-30 cm).

2.3 Materials and methods

2.3.1 Site description

This study was conducted on conventional and organic vegetable farms at three locations in the United States: Colorado (CO), California (CA) and New York (NY) (Table 2.1). In Colorado, conventional and certified organic broccoli fields were located at the Colorado State University Horticulture Field Research Center, North of Fort Collins, and this location has a semiarid steppe climate (Daly *et al.*, 2011). In California, both certified organic and conventional tomato farming systems were located on the Russell Ranch Sustainable Agriculture Facility belonging to the University of California, Davis, and the climate is Mediterranean (University of California: Agricultural Sustainability Institute, 2016). In New York, organic cabbage farming systems in transition to organic certification and conventional cabbage farming systems were

located at the Homer C. Thompson Vegetable Research Farm, Cornell University, Freeville, and the area has a humid continental climate (Cornell University, 2016).

2.3.2 Experimental design and soil sampling

Soils were sampled in November 2014 at all three locations (California, Colorado, and New York). At each location, a single farm which had both conventional and organic farming systems was utilized for soil sampling. Then, three plots were selected from each farming system in California and Colorado, and four plots were selected from each farming system in New York (Fig. 2.1). Within each plot, soils were sampled from three depths (0-10, 10-20, and 20-30 cm) using a soil probe (2.00 cm diameter). Soil samples were collected randomly from each plot, and combined into one composite sample per plot-depth combination. These soils were bagged, kept in boxes with packs of ice, and transported to the laboratory at Colorado State University within one day. Each soil sample was thoroughly mixed before being separated into sub-samples, for soil property analyses, microbial biomass, and physical fractionation.

2.3.3 Soil property analyses

Soil samples were sieved through a 2 mm sieve for initial soil analyses (Table 2.2). Soil pH and electrical conductivity (EC) were measured in a 1:1 soil to water suspension using a Mettler Toledo pH meter (Thermo Fischer Scientific, Waltham, MA). Cation exchange capacity (CEC) was determined using displacement with sodium acetate (pH 8.2) which was based on the Kellogg soil survey laboratory methods manual (Soil Survey Staff, 2004). Soil texture was determined using the hydrometer method based on Stoke's law (Gee and Bauder, 1979).

2.3.4 Soil microbial biomass

Soil microbial biomass was determined on all soils (n=60) using the chloroform fumigation-extraction method (Horwath and Paul, 1994). Briefly, two 10 g sub-samples of each field moist soil sample were weighed. The non-fumigated soil sample was extracted with 50 mL 0.5M K₂SO₄ immediately, and the fumigated soil sample was fumigated with ethanol-free chloroform for five days. After the fumigation, the soil was extracted with the same method as the non-fumigated soil sample. Dissolved organic C and N in the extracts were analyzed with a Shimadzu analyzer (Model TOC-L, Shimadzu Scientific Instruments, Inc., Kyoto, Japan). The microbial biomass carbon (MBC) was determined using $MBC = E_c / K_c$, where E_c is the difference between organic C extracted from fumigated and non-fumigated soil samples, and K_c is the extractable part of MBC after fumigation which is assumed to be 0.45 (Beck et al., 1997). The microbial biomass nitrogen (MBN) was determined using $MBN = E_n / K_n$, where E_n is the difference between N extracted from fumigated and non-fumigated soil samples, and K_n is the extractable part of MBN after fumigation which is assumed to be 0.68 (Brookes et al., 1985).

2.3.5 Physical fractionation

Soil samples which were kept for aggregate separation were fractionated using the wet-sieving method (Elliott, 1986; Six *et al.*, 2000b). After aggregate separation, there were three size fractions obtained (macroaggregates (>250 μm), free microaggregates (53-250 μm), and free silt and clay fraction (<53 μm)). Then, a microaggregate isolator was used to isolate fractions held within macroaggregates (Six *et al.*, 2000a). In this separation, there were three size fractions obtained (coarse particulate organic matter (cPOM, >250 μm); microaggregates within

macroaggregates (mM, 53-250 μm); and silt plus clay fraction within macroaggregates (S&C-M, <53 μm)).

2.3.6 Carbon and nitrogen analysis

The C and N concentrations were measured with a LECO Tru-SPEC elemental analyzer (Leco Corp., St. Joseph, MI) on all bulk soil samples and physical fractions. All soil samples and fractions were tested to determine the presence of carbonate minerals and carbonate rocks. The test was performed by applying a few drops of 2M HCl onto the samples, and if gas bubbles were visible, the sample was tested for soil inorganic carbon (SIC). Soil inorganic carbon was determined using a modified pressure transducer method (Sherrod *et al.*, 2002). If SIC was present, soil organic carbon (SOC) was calculated by the difference of total C concentration from a LECO Tru-SPEC elemental analyzer and SIC.

2.3.7 Data analysis

Mean weight diameter (MWD) of aggregates was determined based on the aggregate size distribution. The MWD of aggregates is the sum of the proportion of soil weight recovered in the sized fraction after wet-sieving after multiplying by the mean diameter of each size fraction (Hurisso *et al.*, 2013; Lichter *et al.*, 2008). The MWD of aggregates was calculated as: $\text{MWD} = (f_{>250 \mu\text{m}} \times 4.125) + (f_{53-250 \mu\text{m}} \times 0.1515) + (f_{<53 \mu\text{m}} \times 0.0265)$, where f is the proportion of soil weight recovered in the sized fraction after wet-sieving, and the subscript presents the size of the fraction. Mean diameters of each fraction are 4.125, 0.1515, and 0.0265 mm.

Carbon and N stocks were calculated using $\text{C stock (kg m}^{-2}\text{)} = \text{soil depth (m)} \times \text{bulk density (kg m}^{-3}\text{)} \times \% \text{ SOC}$, and $\text{N stock (kg m}^{-2}\text{)} = \text{soil depth (m)} \times \text{bulk density (kg m}^{-3}\text{)} \times \% \text{ TN}$,

respectively (Shrestha *et al.*, 2004). Bulk density (BD) of soil in California and Colorado were collected from the farm databases, and BD of the New York soil was estimated from % clay (USDA-NRCS, 2016).

Physical and chemical protection was estimated as the sum of SOC or N stock in free microaggregates and mM for physical protection and in free silt and clay fractions and S&C-M for chemical protection. Physical protection was estimated from microaggregates because microaggregates protect SOM more than macroaggregates in the long term (Segoli *et al.*, 2013; Six *et al.*, 2004).

All data were analyzed in SAS version 9.3 (SAS Institute Inc., Cary, NC). Some data were transformed before ANOVA analysis based on the results of the normality test. The Proc Mixed procedure in SAS was used for the analysis. Fixed effects included sites (California, Colorado, and New York), farming systems (conventional and organic), sampling depths (0-10, 10-20 and 20-30 cm), and their interactions. The soil cores which represented replication at each site and within each system were included as a random effect. The effect of sites, farming systems, sampling depths, and their interactions on the mean response variables were analyzed (Table 2.2). Then, the data were sorted by site, and the differences in the response variables among levels of each factor (farming systems, sampling depths) were analyzed by the SLICE and PDIFF options, and identified by least square means with a Tukey-Kramer test ($p < 0.05$). In addition, linear correlation using the CORR procedure was performed to evaluate the relationships between aggregate stability, physically and chemically protected C and N, MBC, MBN, and SOC and TN in bulk soil. The stepwise selection method using PROC REG was used to select the best combination of variables including % clay, MBC, MBN, SOC and N in bulk soil, DOC and dissolved N variables to predict aggregate stability. Additionally, % clay, MBC,

DOC and aggregate stability variables were used to predict SOC in bulk soil and % clay, MBN, dissolved N and aggregate stability variables were used to predict N in bulk soil using stepwise selection.

2.4 Results

2.4.1 Whole soil characteristics

The general soil properties of the three sites are as follows: pH ranged from 6.4 to 7.9, EC ranged from 0.2 to 2.2 dS m⁻¹, CEC ranged from 15.0 to 37.0 cmol kg⁻¹, and soil textures of all sites were clayey. Site affected all soil properties, while depth affected pH, EC and % clay, and system affected only EC and CEC (Table 2.2). However, many of the interactions were significant for soil properties, indicating that the impact of a main effect on a soil property was dependent on other main effects (Table 2.2).

In CA, soil pH under the organic farming system was greater than the conventional farming system at all depths, while there were no pH differences between farming systems in either CO or NY. However, at 0-10 cm soil depth in NY, pH was lower in the organic system (Table 2.3). Electrical conductivity under the organic farming system in CO was lower than under the conventional farming system, while there were no differences in EC between farming systems in CA or NY (Table 2.3). The CEC in NY under the organic farming system was greater than under the conventional farming system, whereas no differences in CEC were found between farming systems in CA or CO. Nonetheless, CEC at the 0-20 cm soil depth under the organic farming system was greater than under the conventional farming system in CO (Table 2.3). The differences in % clay between farming systems were, as expected, not significant.

2.4.2 Soil microbial biomass

Across all sites, MBC ranged from 88.3 to 405.2 $\mu\text{g g}^{-1}$, and MBN ranged from 8.5 to 96.8 $\mu\text{g g}^{-1}$. C/N ratio of microbial biomass ranged from 3.7 to 12.2 (Table 2.4). Soil MBC was affected by site, system and depth, while MBN and C/N ratio of microbial biomass were affected by site and system (Table 2.2). Significant interactions were also found in soil microbial biomass (Table 2.2).

Organic farming systems enhanced MBC and MBN, except MBC at 20-30 cm soil depth in CO (Table 2.4). Soil MBC and MBN were significantly greater under the organic farming systems by approximately 97% and 129%, respectively, compared to the conventional farming systems (Table 2.4). However, there were no significant differences in C/N ratio of microbial biomass between farming systems in any site or depth (Table 2.4). Sites differed slightly in the distribution of microbial biomass with depth (Fig. 2.2).

2.4.3 Aggregate size distribution

Macroaggregates ranged from 19 to 70% of the dry soil weight, free microaggregates ranged from 21 to 72% of the dry soil weight, and free silt plus clay fractions ranged from 6 to 11% of the dry soil weight (Table 2.5). Aggregate stability was determined based on MWD which ranged from 0.9 to 2.9 mm (Table 2.5). There were significant site, system, and depth main effects on macroaggregates, free microaggregates, and MWD and significant site and system effects on the free silt plus clay fraction (Table 2.2). Main effect interactions were also detected (Table 2.2).

Organic farming systems promoted macroaggregate structures, approximately 73%, and therefore increased MWD, approximately 66%, in CO and NY, while these effects were not

detected in CA (Table 2.5). However, the change in the free silt and clay fraction between farming systems was not significant, except in CA (Table 2.5). The differences in aggregate distribution among depths were not always consistent among sites (Fig. 2.3).

2.4.4 Fractions within macroaggregates

Coarse particulate organic matter (cPOM) ranged from 1 to 22% of the dry soil weight, mM ranged from 8 to 37% of the dry soil weight, and S&C-M ranged from 6 to 34% of the dry soil weight (Table 2.6). Site, system, and depth main effects affected the abundance of all fractions within macroaggregates, and many main effect interactions were observed (Table 2.2).

There were no significant differences in cPOM between farming systems in CA and CO, but in NY, cPOM under the conventional farming system was about 58% greater relative to the organic farming system (Table 2.6). In CO and NY, mM and S&C-M under the organic farming system were higher by about 156% and 82%, respectively, than the conventional farming system, although no difference was detected in CA (Table 2.6). Comparing among depths within the same system, significant differences were observed in all fractions within macroaggregates in CO (Fig. 2.4).

2.4.5 Soil organic carbon stock and dissolved organic carbon

Overall, SOC stock in bulk soil ranged from 120 to 538 kg m⁻², and C/N ratio in bulk soil ranged from 6 to 12 (Table 2.7). Site, system, and depth main effects affected SOC stock, while only site affected C/N ratio in bulk soil (Table 2.2). Organic farming systems increased SOC stock 59% relative to conventional farming systems across all three sites, and C/N ratio in bulk

soil in all sites was not significantly different between farming systems. However, the differences between farming systems were not consistent among depths (Table 2.7 and Fig. 2.5).

Dissolved organic carbon (DOC) ranged from 50.2 to 318.9 $\mu\text{g g}^{-1}$. Site, system, and depth main effects and their interactions affected DOC (Table 2.2). Organic farming systems increased DOC 103% relative to conventional farming systems across sites in CA and CO, while there were no significant differences between farming systems in NY (Table 2.8). The abundance of DOC was usually greater in the top soil (0-10 cm) than in deeper depths (Fig. 2.6).

Soil organic carbon stock in each aggregate was analyzed. Soil organic carbon stock in macroaggregates ranged from 35 to 445 kg m^{-2} , in free microaggregates ranged from 22 to 119 kg m^{-2} , and in free silt and clay fractions ranged from 10 to 29 kg m^{-2} (Table 2.9). Soil organic carbon stock in cPOM ranged from 1 to 91 kg m^{-2} , in mM ranged from 14 to 192 kg m^{-2} , and in S&C-M ranged from 12 to 65 kg m^{-2} (Table 2.10). Three main effects (site, system, and depth) significantly affected the abundance of SOC stock in macroaggregates, free microaggregates, and cPOM, whereas the change in SOC stock in free silt and clay fractions was affected by site and depth, and the change of SOC stock in mM and S&C-M were affected by site and system. Many interaction effects on the change in SOC stock were also detected (Table 2.2).

Organic farming systems enhanced SOC stock in macroaggregates approximately 153% relative to conventional farming systems across all sites. There were no significant differences between farming systems for SOC stock in free microaggregates in CA and NY, while in CO, conventional farming systems enhanced SOC stock in free microaggregates 47% compared to organic farming systems. No differences in SOC stock in free silt and clay fractions were found between farming systems (Table 2.9). Organic farming systems were higher in SOC stock in cPOM, mM, and S&C-M than in conventional farming systems, but results were not always

consistent among sites (Table 2.10). The abundance of SOC stock in all aggregates among depths depended on aggregate sizes, farming systems, and sites (Fig. 2.8 and 2.9).

2.4.6 Nitrogen stock and dissolved nitrogen

Overall, N stock in bulk soil ranged from 16 to 47 kg m⁻² (Table 2.7), and all three main effects and their interactions were significant (Table 2.2). Organic farming systems increased N stock about 58% relative to conventional farming systems across all sites (Table 2.7), and N stock in bulk soil generally decreased with depth (Fig. 2.5).

Dissolved N ranged from 18.7 to 102.4 µg g⁻¹. Site, system, and depth main effects affected dissolved N, and some interaction effects on the change of dissolved N were also detected (Table 2.2). Organic farming systems increased dissolved N 70% relative to conventional farming systems in NY, while there were no significant differences between farming systems in CA or CO (Table 2.8). The abundance of dissolved N was mostly greater in the top soil (0-10 cm) than the deeper depths (Fig. 2.6).

Nitrogen stock in each aggregate was analyzed. Nitrogen stock in macroaggregates ranged from 4 to 35 kg m⁻², in free microaggregates ranged from 3 to 11 kg m⁻², and in free silt and clay fractions ranged from 1 to 3 kg m⁻² (Table 2.11). Soil organic N stock in cPOM ranged from 0.1 to 3 kg m⁻², in mM ranged from 2 to 20 kg m⁻², and in S&C-M ranged from 1 to 7 kg m⁻² (Table 2.12). Three main effects (site, system, and depth) significantly affected the N stock in macroaggregates, free microaggregates, cPOM, and mM, whereas the change in N stock in free silt and clay fractions was affected by site and depth, and the change in N stock in S&C-M was affected by system. Interaction effects on the change in N stock were also detected (Table 2.2).

Organic farming systems enhanced N stock in macroaggregates by approximately 157% relative to conventional farming systems across all sites. There were no significant differences between farming systems for N stock in free microaggregates in CA and NY, while in CO, conventional farming systems enhanced N stock in free microaggregates by 50% compared to organic farming systems. Nitrogen stocks in cPOM, mM, and S&C-M under organic farming systems were higher than conventional farming systems, but not always consistently among sites (Table 2.12). The N stock in aggregates among depths depended on aggregate size, farming system, and site (Fig. 2.10 and 2.11).

2.4.7 Physical and chemical protection

Physically protected C from all sites ranged from 60 to 288 kg m⁻², and physically protected N ranged from 7 to 29 kg m⁻² (Table 2.13). Chemically protected C from all sites ranged from 25 to 90 kg m⁻², and chemically protected N ranged from 3 to 10 kg m⁻² (Table 2.13). Physically and chemically protected C and physically protected N were affected by all three main effects (site, system, and depth), while chemically protected N was affected by site and system. Many significant interactions were also detected (Table 2.2). Organic farming systems promoted C and N protection in soil about 45% to 72% relative to conventional farming systems, but significant differences were not observed at all sites (Table 2.13). In CO, the organic farming system did not significantly increase physically protected C and N, and in CA, the organic farming system did not significantly increase chemically protected C (Table 2.13). The change of physically and chemically protected C and N among depths depended on sites and farming systems (Fig. 2.11 and 2.12).

2.4.8 Correlations of soil microbial biomass, aggregate stability, and carbon and nitrogen protection

The correlation coefficient (r) between MBC and aggregate stability was 0.3559 and the p -value was 0.0057, indicating that the correlation was significant (Table 2.14). As MBC increased, aggregate stability increased. The correlation coefficient between MBN and aggregate stability was similar ($r = 0.3449$, $p = 0.0075$). The correlations between MBC and physically protected C stock ($r = 0.7362$, $p = <.0001$), MBC and chemically protected C ($r = 0.6930$, $p = <.0001$), MBN and physically protected N stock ($r = 0.9245$, $p = <.0001$), and MBN and chemically protected N ($r = 0.8008$, $p = <.0001$) were all significant and positive. Although the correlation coefficients were not high, they provided conclusive evidence for the association between microbes and SOM protection.

A stepwise selection model (Table 2.15) showed that MBN and dissolved N could be used to predict MWD ($r^2 = 0.27$, $p = 0.0001$). In addition, % clay and MBC could be used to predict SOC stock in bulk soil ($r^2 = 0.80$, $p < 0.0001$), and MBN and dissolved N could be used to predict N stock in bulk soil ($r^2 = 0.93$, $p < 0.0001$).

2.5 Discussion

This study was conducted to assess the effect of farming system (conventional and organic practices) in vegetable farms on microbial biomass, aggregate stability, and physical and chemical protection of C and N in soil. Without samples from the initiation of these farming systems, we cannot conclude how much the dependent variables changed over time. However, within each farm's history and environment, our results do indicate that SOM, microbial biomass, MWD, and protected SOM are positively correlated.

2.5.1 Farming system effect on microbial biomass

Higher MBC and MBN were observed in organic farming systems compared to conventional farming systems in this study; however, the difference in MBC varied with depth (Table 2.4). In relation to our finding, Kushwaha *et al.* (2001) stated that OM addition accelerated an increase in microbial biomass. Melero *et al.* (2006) and Tu *et al.* (2006) also observed that the microbial biomass under organic management was significantly higher than under conventional management in different crop rotation systems (broad bean-melon-watermelon and sweet potato-wheat-cabbage). Wang *et al.* (2010) reported that the changes in the size of soil microbial biomass were likely to be influenced by the added crop residues and native soil organic matter. In our study, organic farming systems adopted cover crop management and organic fertilizer application (Table 2.1) resulting in higher organic matter applied to the soil relative to conventional farming systems. This additional organic matter may serve as both a C and nutrient source that supports microbial growth.

2.5.2 Aggregate stability

Organic farming systems increased the proportion of macroaggregates, as well as aggregate stability based on MWD in CO and NY (Table 2.5). The lack of difference in macroaggregates or aggregate stability between farming systems in CA may be due to the effect of tillage, because only in CA, soil samples were collected about one week after tillage. Additionally, the macroaggregate proportion and aggregate stability were positively correlated to MBC and MBN, while the microaggregates were negatively correlated to MBC and MBN (Table 2.14). Although cause and effect cannot be proven, these relationships may be explained by microbial aggregate formation. Extracellular polysaccharides from microbial activities bind

residue and soil particles including free microaggregates, to form macroaggregates (Banwart *et al.*, 2015; Six *et al.*, 2000a; Six *et al.*, 1998; Tisdall and Oades, 1982), and new microaggregates can be formed within macroaggregates (Six *et al.*, 2000a; Six *et al.*, 1998). In our study, the increases in MBC, MBN, and SOC and TN in bulk soil under organic farming systems may have enhanced polysaccharides for binding free microaggregates to form macroaggregates and resulted in increased aggregate stability.

2.5.3 Soil organic carbon and nitrogen stocks

Evidence for stable SOC and TN accrual were found in organic farming systems in this study (Table 2.7). As compared to conventional farming systems, organic farming systems increased SOC and TN stocks approximately 30% and 41% in CA, approximately 43% and 37% in CO, and approximately 103% and 95% in NY. These results at all three sites indicate that within the same environment and tillage management, organic farming systems had greater SOC and TN stocks than conventional farming systems. This may be due to cover crop management and organic fertilizer application under organic farming systems that increased C inputs and SOM. Organic matter can serve as a C source for microorganisms, and the high C input in organic farming can increase soil microbial biomass (Santos *et al.*, 2012; Wang *et al.*, 2010). Although SOC and TN stocks in macroaggregates under organic farming systems were typically greater than under conventional farming systems, these differences were generally not detected in free microaggregates or free silt and clay fractions. Therefore, SOC and TN stocks in macroaggregates were most influential on SOC and TN sequestration in organic farming systems in this study. Additionally, inside macroaggregates, SOC and TN stocks in cPOM, mM and

S&C-M were also important and may influence the capacity of macroaggregates to retain SOC and TN.

Based on the higher OM and the increase in microbial biomass, organic farming systems have the potential to promote C and N sequestration. Greater microbial biomass and available C sources allowed the microbial activity to accelerate, resulting in the production of binding agents. The binding agents helped to form macroaggregates and created a stable environment for the formation of cPOM and mM and for mineral bonding on S&C-M (Fig. 2.13) (Cui *et al.*, 2014; Six *et al.*, 2002). Consequently, the organic farming systems had more macroaggregates to store more SOC and TN than conventional farming systems. Therefore, the observed SOC and TN stocks in macroaggregates and fractions within macroaggregates under organic farming systems were greater than conventional farming systems in our study. It appeared that organic farming systems promoted C and N sequestration, while sustaining microbial activity.

2.5.4 Soil organic matter stabilization

In this study, SOM stabilization was defined as physical and chemical protection of SOM. The magnitude of SOM stabilization depended on the SOM input and protection in soil (Fig. 2.13). Physically protected SOM is protected by aggregates that reduce the accessibility of decomposers (Six *et al.*, 2002; von Lützow *et al.*, 2008). Chemically protected SOM is protected through the interaction with minerals (Six *et al.*, 2002). These physical (free microaggregates and mM) and chemical (free silt and clay fractions and S&C-M) forms of SOM protection will assure long-term stability of the SOM accrued in organic farming systems, despite macroaggregate susceptibility to breakdown after disturbances, such as tillage (Grandy and Robertson, 2007; Six *et al.*, 1998). Physically and chemically protected C and N were generally greater under organic

farming systems than conventional farming systems in our study. This may be explained by the amount of SOC and TN in soil, the proportion of free microaggregates and mM for physical protection, and the proportion of free silt and clay fractions and S&C-M for chemical protection. However, the proportions of free microaggregates and free silt and clay fractions under organic farming systems were less than or not different from the conventional farming systems. Therefore, the higher physically and chemically protected C and N under organic farming systems in this study were likely influenced by the higher SOC, TN, and proportions of mM and S&C-M under organic farming systems. Physically protected C and N was greater than chemically protected C and N by about three times. Physically protected C and N within aggregates is important and may influence the capacity for SOM stabilization. However, chemically protected C and N through mineral association is also important and may influence the persistence of SOM over long-term periods since mineral-associated OM is not easily separated from mineral particles (<53 μm) and consists of inert OM; therefore, adsorbed OM on mineral surfaces has a longer turnover time than physically protected OM (Segoli et al., 2013).

2.6 Conclusions

Soil organic C and total N in bulk soil, physically and chemically protected C and N, microbial biomass, macroaggregates, and aggregate stability were generally greater under organic farming systems than under conventional farming systems at all sites and depths. The results of this study suggest that (i) the addition of OM to organic farming systems can increase SOC and total N resulting in increased soil microbial biomass, and soil microbial biomass yields organic binding agents from decomposition of SOM, resulting in increased aggregate stability, (ii) farms adopting organic practices have higher C inputs which can result in higher amounts of

physically and chemically protected C and N and more stable SOM in soil, and (iii) the variation of aggregate stability and SOM stabilization and persistence among soil depths depended on sites and farming systems. Therefore, our hypotheses were confirmed. According to the higher levels of SOC, TN, physically protected C and N, and aggregate stability under organic farming systems, we suggest that organic farming practices have a greater potential to sequester C and N and promote soil aggregate stability in vegetable systems compared to conventional farming practices.

TABLES

Table 2.1. Details regarding the conventional (Con) and organic (Org) vegetable farming systems in Colorado, California, and New York in 2014.

Experimental design	Colorado		California		New York	
	Con	Org	Con	Org	Con	Org
Latitude	40° 39' 6"N	40° 39' 6"N	38° 32' 41"N	38° 32' 41"N	42° 31' 12"N	42° 31' 28"N
Longitude	104° 59' 57"W	104° 59' 57"W	121° 52' 42"W	121° 52' 42"W	76° 19' 59"W	76° 19' 33"W
Soil series	Nunn clay loam	Nunn clay loam	Yolo silty clay loam/Rincon clay loam	Yolo silty clay loam/Rincon clay loam	Howard gravelly loam	Rhinebeck silt loam
Slope (%)	1-3	1-3	0	0	0-3	0-3
Year of organic certification	-	2001	-	2011	-	2015
Plot area (m ²)	87	16	4047	4047	2023	2023
Crop	Broccoli	Broccoli	Tomato	Tomato	Cabbage	Cabbage
Cover crop management	None	Rye or hairy vetch tilled into soil	None	Winter legume cover crop tilled into soil	Rye tilled into soil	Sorghum sudan grass tilled into soil
Pesticide or herbicide application	Yes (Glyphosate)	None	Yes (Treflan 4D, Radiant SC, Parallel PCS, Cabrio EG Fungicide, Belt SC Insecticide, ActiSpred, Bifen 2 AG Gold, ABBA 0.15 EC)	Yes (Pyganic, Kumulus, and Deliver (BT))	Yes (Gramoxone and Dual II Magnum)	None

Table 2.1. Details regarding the conventional (Con) and organic (Org) vegetable farming systems in Colorado, California, and New York in 2014 (Cont.).

Experimental design	Colorado		California		New York	
	Con	Org	Con	Org	Con	Org
Fertilizer application	Urea	Fish emulsion (55 gal/acre)	Liquid starter (N=24.6 lbs/acre P ₂ O ₅ =73.8 lbs/acre K ₂ O = 18.5 lbs/acre Zinc chelate = 1.54 lbs/acre) and UAN32	Chicken manure compost (4 tons/acre)	20-10-10 and 34-0-0	Certified organic compost (12 tons/acre)
Tillage management	Roto-till once per year (15 cm)	Roto-till once per year (15 cm)	Finish disk (30 cm) and stubble disk (35 cm) after tomato harvest, lister (15 cm), incorporator (7.5 cm) and mulcher (2.5 cm) to form beds; once per year after previous crop harvest, subsoil to 45 cm once per year after harvest	Finish disk (30 cm) and stubble disk (35 cm) after tomato harvest, lister (15 cm), incorporator (7.5 cm) and mulcher (2.5 cm) to form beds; once per year after previous crop harvest, subsoil to 45 cm once per year after harvest	Plowed to about 20 cm, disked then harrowed	Plowed to about 20 cm, disked then harrowed
Irrigation	Furrow/Gated pipe	Drip and solid set	Furrow	Furrow	None	None
Annual precipitation (mm)*	323.9	464.6	866.7			
Average temperature (°C)*		8.6		17.6		7.2

* Weather data were taken from <http://169.237.140.1/calludt.cgi/WXDATAREPORT> for California, http://www.coagmet.colostate.edu/rawdata_form.php for Colorado, and <http://newa.cornell.edu/index.php?page=all-weather-data> for New York.

Table 2.2. Statistical results from the analysis of variance of the effects of site, farming system (sys), and depth and their interactions on the studied soil variables: pH, electrical conductivity (EC), cation exchange capacity (CEC), percentage of clay in soil (% clay), microbial biomass (MB), microbial biomass carbon (MBC), nitrogen (MBN), C/N ratio of microbial biomass, aggregate size distribution, mean weight diameter (MWD), aggregate size distribution from microaggregate isolation, C/N ratio of bulk soil, soil organic carbon (SOC) and nitrogen (N) stock in bulk soil and each aggregate size, physically and chemically protected C and nitrogen N.

Dependent variable	Independent variable						
	Site	Sys	Depth	Site x Sys	Site x Depth	Sys x Depth	Site x Sys x Depth
pH	*	NS	*	NS	NS	*	*
EC	*	*	*	*	*	NS	*
CEC	*	*	NS	*	*	NS	*
% Clay	*	NS	*	NS	*	NS	NS
MBC	*	*	*	*	*	NS	*
MBN	*	*	NS	*	*	NS	NS
C/N ratio of MB	*	*	NS	NS	*	NS	NS
Macroaggregates	*	*	*	NS	*	*	NS
Free microaggregates	*	*	*	NS	*	NS	NS
Free silt and clay	*	*	NS	NS	*	NS	*
MWD	*	*	*	NS	*	NS	NS
cPOM	*	*	*	*	*	NS	NS
mM	*	*	*	*	*	NS	NS
S&C-M	*	*	*	NS	*	NS	NS
SOC stock in bulk soil	*	*	*	*	*	*	*
N stock in bulk soil	*	*	*	*	*	*	*
C/N ratio of bulk soil	*	NS	NS	NS	NS	NS	NS
Dissolved SOC	*	*	*	*	*	*	*
Dissolved N	*	*	*	NS	*	NS	*
SOC stock in macroaggregates	*	*	*	*	*	NS	NS
SOC stock in free microaggregates	*	*	*	NS	*	NS	NS
SOC stock in free silt and clay fractions	*	NS	*	NS	NS	NS	NS
SOC stock in cPOM	*	*	*	NS	*	NS	NS
SOC stock in mM	*	*	NS	*	*	NS	NS
SOC stock in S&C-M	*	*	NS	*	*	NS	NS
N stock in macroaggregates	*	*	*	*	*	NS	*
N stock in free microaggregates	*	*	*	NS	*	NS	NS
N stock in free silt and clay fractions	*	NS	*	NS	NS	NS	*
N stock in cPOM	*	*	*	*	*	NS	*
N stock in mM	*	*	*	*	*	NS	NS
N stock in S&C-M	NS	*	NS	*	*	NS	NS
Phy-protected C	*	*	*	*	*	NS	NS
Phy-protected N	*	*	*	*	*	NS	NS
Chem-protected C	*	*	*	NS	*	NS	*
Chem-protected N	*	*	NS	NS	*	NS	*

* Significant at the 0.05 probability level. NS is not significant.

Table 2.3. Soil properties at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth (cm)	Soil properties (Mean (SE))			
			pH [‡]	Electrical conductivity [‡] dS m ⁻¹	Cation exchange capacity [†] cmol kg ⁻¹	Clay [§] %
CA	Con	0-10	7.37 (0.04)b ^{††}	0.91 (0.14)a	36.96 (5.39)a	52.33 (4.33)a
		10-20	7.33 (0.07)b	0.76 (0.07)a	29.91 (2.18)a	51.00 (2.52)a
		20-30	7.26 (0.06)b	0.52 (0.01)a	28.21 (1.81)a	50.00 (2.00)a
		Average	7.32 (0.03)B ^{‡‡}	0.73 (0.07)A	31.69 (2.21)A	51.11 (1.59)A
	Org	0-10	7.57 (0.01)a	1.02 (0.02)a	30.36 (0.06)a	52.67 (4.37)a
		10-20	7.53 (0.03)a	0.84 (0.04)a	31.66 (0.51)a	52.00 (3.06)a
		20-30	7.52 (0.02)a	0.49 (0.06)a	32.34 (0.51)a	54.67 (2.40)a
		Average	7.54 (0.01)A	0.78 (0.08)A	31.45 (0.36)A	53.11 (1.74)A
CO	Con	0-10	7.82 (0.06)a	2.22 (0.13)a	24.96 (0.21)b	57.33 (0.67)a
		10-20	7.87 (.006)a	1.38 (0.07)a	25.88 (0.34)b	57.33 (0.67)a
		20-30	7.72 (0.05)a	2.09 (0.07)a	30.62 (0.92)a	65.33 (0.67)a
		Average	7.80 (0.04)A	1.90 (0.14)A	27.15 (0.92)A	60.00 (1.37)A
	Org	0-10	7.87 (0.04)a	1.29 (0.32)b	30.36 (2.26)a	60.00 (3.46)a
		10-20	7.79 (0.03)a	0.86 (0.19)b	31.27 (1.72)a	61.33 (2.91)a
		20-30	7.81 (0.02)a	0.70 (0.07)b	31.70 (1.37)a	65.33 (3.53)a
		Average	7.82 (0.02)A	0.95 (0.14)B	31.11 (0.93)A	62.22 (1.84)A
NY	Con	0-10	6.98 (0.12)a	0.25 (0.04)a	15.30 (0.43)b	43.00 (1.22)a
		10-20	6.68 (0.22)a	0.23 (0.03)a	15.02 (0.62)b	41.25 (0.25)a
		20-30	6.45 (0.14)a	0.19 (0.02)a	15.53 (0.77)b	41.25 (0.25)a
		Average	6.70 (0.11)A	0.22 (0.02)A	15.28 (0.33)B	41.83 (0.46)A
	Org	0-10	6.29 (0.20)b	0.24 (0.03)a	23.65 (0.88)a	37.00 (4.36)a
		10-20	6.44 (0.17)a	0.19 (0.01)a	23.66 (1.45)a	36.00 (4.69)a
		20-30	6.52 (0.14)a	0.23 (0.01)a	23.32 (1.50)a	37.50 (5.50)a
		Average	6.41 (0.09)A	0.22 (0.01)A	23.54 (0.69)A	36.83 (2.55)A

* Significant at the 0.05 probability level. NS is not significant.

[‡] 1:1 soil:water extraction; [†] Displacement with sodium acetate (pH 8.2); and [§] Hydrometer.

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.4. Microbial biomass carbon (MBC) and nitrogen (MBN) and CN ratio of microbial biomass at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth (cm)	Microbial biomass (Mean (SE))		
			MBC [‡]	MBN [‡]	CN ratio
			-----µg g ⁻¹ -----		
CA	Con	0-10	88.27 (7.03)b ^{††}	8.49 (0.21)b	10.38 (0.57)a
		10-20	124.43 (6.28)b	10.25 (0.68)b	12.16 (0.19)a
		20-30	107.14 (10.85)b	15.56 (2.13)b	7.3 (1.52)a
		Average	108.90 (6.85)B ^{‡‡}	11.80 (1.35)B	9.89 (0.95)A
	Org	0-10	141.83 (22.33)a	21.07 (2.07)a	6.67 (0.51)a
		10-20	196.73 (13.60)a	25.07 (4.47)a	8.27 (1.21)a
		20-30	157.70 (13.99)a	23.76 (2.70)a	6.76 (0.73)a
		Average	165.42 (11.82)A	23.30 (1.72)A	7.23 (0.51)A
CO	Con	0-10	200.27 (5.14)b	29.15 (2.43)b	6.94 (0.46)a
		10-20	180.13 (7.43)b	20.76 (5.18)b	10.12 (2.88)a
		20-30	188.72 (7.52)a	18.98 (1.22)b	9.98 (0.35)a
		Average	189.71 (4.48)B	22.96 (2.30)B	9.01 (0.99)A
	Org	0-10	343.37 (24.27)a	49.79 (4.20)a	7.03 (0.93)a
		10-20	316.60 (29.81)a	44.73 (5.43)a	7.14 (0.43)a
		20-30	243.21 (31.70)a	30.92 (2.17)a	7.81 (0.5)a
		Average	301.06 (20.76)A	41.81 (3.50)A	7.33 (0.35)A
NY	Con	0-10	166.29 (14.89)b	34.1 (5.41)b	5.46 (1.39)a
		10-20	140.16 (6.98)b	27.77 (2.28)b	5.12 (0.33)a
		20-30	111.03 (8.53)b	29.94 (1.92)b	3.70 (0.07)a
		Average	139.16 (8.8)B	30.60 (2.02)B	4.76 (0.49)A
	Org	0-10	405.19 (25.21)a	96.83 (7.47)a	4.20 (0.06)a
		10-20	376.12 (25.49)a	86.11 (3.71)a	4.36 (0.16)a
		20-30	357.47 (15.83)a	81.99 (2.31)a	4.36 (0.19)a
		Average	379.59 (13.22)A	88.31 (3.22)A	4.31 (0.08)A

* Significant at the 0.05 probability level. NS is not significant.

[‡] Fumigation-extraction.

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.5. Aggregate size distribution and mean weight diameter (MWD) at three depths in conventional (Con) and organic (Org) farming systems in Colorado (CO), California (CA) and New York (NY). Aggregate sizes include macroaggregates (> 250 μm), free microaggregates (53-250 μm), and free silt and clay fractions (< 53 μm).

Site	System	Depth cm	Aggregate size distribution (Mean (SE))			MWD mm
			> 250 μm	53-250 μm	< 53 μm	
			-----g 100 g ⁻¹ soil-----			
CA	Con	0-10	50.01 (6.68)a ^{††}	37.77 (6.03)a	11.41 (1.09)a	2.12 (0.27)a
		10-20	50.95 (8.38)a	37.47 (7.97)a	10.58 (0.72)a	2.16 (0.33)a
		20-30	53.28 (8.75)a	35.45 (7.98)a	10.60 (1.10)a	2.25 (0.35)a
		Average	51.41 (4.02)A ^{‡‡}	36.90 (3.71)A	10.86 (0.51)A	2.18 (0.16)A
	Org	0-10	69.60 (0.42)a	21.11 (0.76)a	8.31 (0.52)b	2.90 (0.02)a
		10-20	66.30 (3.87)a	22.90 (2.98)a	10.06 (0.9)a	2.77 (0.15)a
		20-30	69.83 (3.38)a	20.90 (3.52)a	8.46 (0.33)a	2.91 (0.13)a
		Average	68.57 (1.59)A	21.64 (1.39)A	8.94 (0.42)B	2.86 (0.06)A
CO	Con	0-10	18.88 (1.06)b	71.92 (0.45)a	7.21 (0.96)a	0.89 (0.04)b
		10-20	35.93 (0.93)b	53.57 (1.93)a	7.68 (1.29)a	1.57 (0.04)b
		20-30	38.41 (2.68)b	50.14 (3.08)a	9.94 (0.53)a	1.66 (0.11)b
		Average	31.07 (3.19)B	58.55 (3.54)A	8.28 (0.65)A	1.37 (0.13)B
	Org	0-10	47.87 (7.42)a	42.99 (7.02)b	7.67 (1.00)a	2.04 (0.30)a
		10-20	61.75 (4.43)a	30.12 (4.00)b	6.95 (0.24)a	2.59 (0.18)a
		20-30	65.94 (1.82)a	26.49 (1.28)b	6.24 (0.30)b	2.76 (0.07)a
		Average	58.52 (3.73)A	33.20 (3.44)B	6.95 (0.37)A	2.47 (0.15)A
NY	Con	0-10	41.65 (3.83)b	39.61 (1.29)a	8.96 (0.53)a	1.78 (0.16)b
		10-20	42.18 (1.59)b	42.11 (1.24)a	7.04 (0.51)a	1.81 (0.06)b
		20-30	48.35 (2.55)b	36.22 (2.17)a	5.94 (0.52)a	2.05 (0.10)b
		Average	44.06 (1.73)B	39.31 (1.12)A	7.32 (0.47)A	1.88 (0.07)B
	Org	0-10	62.83 (3.17)a	24.92 (2.53)b	6.92 (0.75)a	2.63 (0.13)a
		10-20	65.12 (1.42)a	23.73 (1.64)b	6.45 (0.54)a	2.72 (0.06)a
		20-30	66.66 (4.27)a	23.49 (3.89)b	6.29 (1.25)a	2.79 (0.17)a
		Average	64.87 (1.73)A	24.05 (1.50)B	6.55 (0.48)A	2.71 (0.07)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.6. Aggregate size distribution from microaggregate isolation at three depths in conventional (Con) and organic (Org) farming systems in Colorado (CO), California (CA) and New York (NY). Aggregate sizes include coarse particulate organic matter (cPOM), microaggregates within macroaggregates (mM), and silt and clay fractions within macroaggregates (S&C-M).

Site	System	Depth	Aggregate size distribution (Mean (SE))		
			cPOM	mM	S&C-M
		cm	-----g 100 g ⁻¹ soil-----		
CA	Con	0-10	1.47 (0.42)a ^{††}	26.09 (3.43)a	22.48 (3.40)a
		10-20	1.42 (0.38)a	26.29 (3.94)a	22.86 (4.95)a
		20-30	1.41 (0.36)a	27.25 (4.42)a	24.57 (4.86)a
		Average	1.44 (0.19)A ^{‡‡}	26.55 (1.98)A	23.30 (2.25)A
	Org	0-10	1.29 (0.21)a	34.93 (1.06)a	33.48 (1.38)a
		10-20	1.23 (0.15)a	33.43 (1.49)a	31.34 (2.91)a
		20-30	0.95 (0.17)a	35.74 (1.24)a	32.73 (2.10)a
		Average	1.16 (0.10)A	34.7 (0.72)A	32.51 (1.15)A
CO	Con	0-10	3.37 (0.14)a	8.37 (0.81)b	6.79 (0.02)b
		10-20	2.56 (0.12)a	18.38 (1.22)b	13.83 (0.12)b
		20-30	1.35 (0.06)a	20.01 (1.75)b	16.32 (0.69)b
		Average	2.43 (0.30)A	15.59 (1.93)B	12.31 (1.44)B
	Org	0-10	2.95 (0.71)a	24.26 (5.63)a	18.91 (2.16)a
		10-20	2.85 (0.94)a	34.78 (2.89)a	22.07 (3.12)a
		20-30	1.81 (0.65)a	37.23 (1.58)a	24.48 (1.31)a
		Average	2.54 (0.43)A	32.09 (2.74)A	21.82 (1.41)A
NY	Con	0-10	21.57 (0.66)a	9.83 (2.28)b	6.85 (1.73)b
		10-20	21.18 (0.61)a	9.19 (1.18)b	5.66 (0.97)b
		20-30	20.88 (1.06)a	13.78 (2.32)b	7.58 (1.38)b
		Average	21.21 (0.43)A	10.94 (1.21)B	6.70 (0.77)B
	Org	0-10	12.83 (0.98)b	31.81 (2.33)a	12.74 (0.48)a
		10-20	13.89 (0.96)b	33.56 (1.66)a	11.91 (0.74)a
		20-30	13.55 (0.28)b	35.17 (3.91)a	12.74 (0.91)a
		Average	13.42 (0.44)B	33.51 (1.52)A	12.46 (0.40)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.7. Soil organic carbon (SOC) and nitrogen (N) stock in bulk soil and CN ratio of bulk soil at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth cm	Bulk soil (Mean (SE))		
			SOC stock -----kg m ⁻² -----	N stock	CN ratio
CA	Con	0-10	148.74 (6.33)b ^{††}	18.42 (0.41)b	8.08 (0.30)a
		10-20	141.21 (4.87)b	17.88 (1.04)b	7.95 (0.50)a
		20-30	118.75 (6.85)a	15.58 (0.35)b	7.62 (0.36)a
		Average	136.24 (5.43)B ^{††}	17.29 (0.55)B	7.88 (0.21)A
	Org	0-10	206.71 (6.33)a	27.93 (0.43)a	7.40 (0.17)a
		10-20	188.85 (13.50)a	26.29 (1.31)a	7.17 (0.17)a
		20-30	134.99 (8.09)a	19.09 (0.83)a	7.06 (0.18)a
		Average	176.85 (11.84)A	24.44 (1.44)A	7.21 (0.10)A
CO	Con	0-10	127.53 (2.88)b	19.89 (0.28)b	6.41 (0.05)b
		10-20	120.31 (3.03)b	18.39 (0.49)b	6.56 (0.33)a
		20-30	111.53 (0.94)a	17.98 (0.42)a	6.21 (0.17)a
		Average	119.79 (2.62)B	18.75 (0.36)B	6.39 (0.12)A
	Org	0-10	205.35 (23.82)a	29.03 (3.06)a	7.06 (0.11)a
		10-20	170.17 (16.02)a	26.12 (2.19)a	6.50 (0.07)a
		20-30	136.63 (14.23)a	21.79 (1.74)a	6.25 (0.17)a
		Average	170.72 (13.56)A	25.64 (1.59)A	6.60 (0.13)A
NY	Con	0-10	261.40 (22.38)b	24.30 (1.01)b	10.71 (0.53)a
		10-20	264.89 (21.21)b	24.04 (0.67)b	10.98 (0.67)a
		20-30	255.18 (21.02)b	23.46 (0.44)b	10.87 (0.86)a
		Average	260.49 (11.32)B	23.93 (0.4)B	10.86 (0.37)A
	Org	0-10	524.85 (34.71)a	46.94 (2.94)a	11.18 (0.23)a
		10-20	527.71 (42.77)a	46.51 (1.88)a	11.29 (0.47)a
		20-30	537.54 (37.47)a	46.64 (2.44)a	11.50 (0.34)a
		Average	530.03 (20.15)A	46.7 (1.29)A	11.33 (0.19)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

[#] The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.8. Dissolved soil organic carbon and nitrogen in bulk soil at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth cm	Dissolved SOC ----- $\mu\text{g g}^{-1}$ -----	Dissolved N
CA	Con	0-10	128.08 (3.76)a ^{††}	73.38 (5.33)a
		10-20	126.53 (4.91)a	74.33 (7.15)a
		20-30	50.24 (13.76)b	29.9 (5.42)a
		Average	98.31 (14.88)B ^{‡‡}	57.43 (8.64)A
	Org	0-10	318.94 (11.92)a	102.39 (5.16)a
		10-20	219.47 (2.25)a	78.80 (1.88)a
		20-30	50.98 (6.28)a	19.70 (2.65)b
		Average	196.46 (39.30)A	66.97 (12.42)A
CO	Con	0-10	123.08 (13.22)b	24.93 (4.46)a
		10-20	79.07 (6.38)b	18.67 (6.73)a
		20-30	71.14 (2.49)b	18.84 (4.17)a
		Average	91.09 (9.15)B	20.81 (2.82)A
	Org	0-10	277.90 (35.57)a	65.45 (24.13)a
		10-20	171.56 (18.43)a	37.84 (10.22)a
		20-30	115.95 (12.24)a	32.02 (13.88)a
		Average	188.47 (26.66)A	45.11 (9.99)A
NY	Con	0-10	180.75 (7.91)b	40.01 (5.87)a
		10-20	136.33 (4.62)a	26.33 (1.83)b
		20-30	131.46 (6.72)a	19.31 (1.15)b
		Average	149.51 (7.51)A	28.55 (3.21)B
	Org	0-10	195.51 (12.43)a	46.65 (5.30)a
		10-20	174.76 (9.24)a	52.48 (4.27)a
		20-30	168.35 (20.72)a	46.72 (6.26)a
		Average	179.54 (8.55)A	48.61 (2.91)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.9. Soil organic carbon stock in macroaggregates (> 250 µm), free microaggregates (53-250 µm), and free silt and clay fractions (< 53 µm) at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth cm	Soil organic carbon stock (Mean (SE))		
			> 250 µm	53-250 µm	< 53 µm
			-----kg m ⁻² -----		
CA	Con	0-10	65.69 (8.13)b ^{††}	40.80 (6.78)a	15.46 (1.76)a
		10-20	63.97 (12.56)b	37.38 (6.77)a	14.47 (0.83)a
		20-30	58.79 (12.78)a	31.87 (6.26)a	13.41 (1.33)a
		Average	62.82 (5.77)B ^{‡‡}	36.68 (3.55)A	14.44 (0.74)A
	Org	0-10	113.61 (3.51)a	29.67 (0.13)a	13.04 (0.66)a
		10-20	108.32 (10.10)a	31.43 (3.21)a	15.86 (2.03)a
		20-30	87.60 (7.30)a	22.12 (2.60)a	11.07 (0.45)a
		Average	103.17 (5.45)A	27.74 (1.86)A	13.32 (0.94)A
CO	Con	0-10	35.45 (4.75)b	87.24 (0.58)a	12.73 (1.59)a
		10-20	54.73 (2.40)b	55.68 (1.83)a	11.65 (1.69)a
		20-30	51.97 (4.25)b	51.26 (3.15)a	11.05 (0.47)a
		Average	47.38 (3.60)B	64.73 (5.76)A	11.81 (0.73)A
	Org	0-10	105.78 (24.79)a	64.37 (5.74)b	15.19 (1.64)a
		10-20	122.44 (20.46)a	39.54 (2.07)b	12.48 (0.61)a
		20-30	103.50 (11.84)a	28.08 (2.89)b	10.46 (0.50)a
		Average	110.57 (10.33)A	44.00 (5.70)B	12.71 (0.86)A
NY	Con	0-10	105.67 (28.44)b	110.80 (1.10)a	28.73 (1.73)a
		10-20	100.04 (16.63)b	119.09 (4.10)a	23.36 (1.75)a
		20-30	107.54 (19.33)b	100.18 (7.00)a	19.54 (2.20)a
		Average	104.42 (11.56)B	110.02 (3.40)A	23.88 (1.51)A
	Org	0-10	304.60 (10.38)a	104.67 (16.99)a	26.03 (2.44)a
		10-20	378.40 (38.94)a	93.52 (9.13)a	22.03 (0.48)a
		20-30	444.93 (10.96)a	95.96 (19.41)a	22.44 (2.89)a
		Average	375.98 (21.38)A	98.05 (8.38)A	23.50 (1.27)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

[#] The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.10. Soil organic carbon stock in coarse particulate organic matter (cPOM), microaggregates within macroaggregates (mM), and silt and clay fractions within macroaggregates (S&C-M) at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth	Soil organic carbon stock (Mean (SE))			
			cPOM	mM	S&C-M	
		cm	-----kg m ⁻² -----			
CA	Con	0-10	1.19 (0.47)b ^{††}	33.34 (4.60)b	28.95 (3.75)b	
		10-20	1.60 (0.44)a	31.95 (6.67)b	28.33 (5.79)b	
		20-30	0.69 (0.08)a	28.49 (7.27)a	26.66 (5.17)a	
		Average	1.16 (0.23)B ^{‡‡}	31.26 (3.22)B	27.98 (2.51)A	
	Org	0-10	3.22 (0.54)a	57.49 (2.96)a	46.57 (2.17)a	
		10-20	2.88 (0.29)a	52.22 (4.56)a	43.49 (4.19)a	
		20-30	0.89 (0.16)a	43.59 (3.43)a	38.65 (3.5)a	
		Average	2.33 (0.41)A	51.10 (2.75)A	42.91 (2.05)A	
	CO	Con	0-10	3.98 (0.53)a	14.24 (1.23)b	12 (0.48)b
			10-20	0.80 (0.17)b	27.34 (1.62)b	20.86 (0.39)b
			20-30	0.94 (0.61)a	27.82 (2.82)b	18.69 (0.7)b
			Average	1.91 (0.57)A	23.13 (2.44)B	17.18 (1.36)B
Org		0-10	14.55 (4.27)a	56.51 (13.95)a	36.5 (4.7)a	
		10-20	4.17 (1.48)a	64.08 (8.37)a	39.2 (7.04)a	
		20-30	2.50 (1.37)a	55.68 (7.74)a	34.18 (4.4)a	
		Average	7.07 (2.33)A	58.76 (5.37)A	36.63 (2.85)A	
NY		Con	0-10	42.26 (16.94)b	28.88 (8.29)b	24.79 (6.46)b
			10-20	33.70 (11.59)b	25.12 (4.22)b	19.8 (3.17)b
			20-30	34.38 (13.35)b	36.46 (7.59)b	25.95 (4.81)b
			Average	36.78 (7.47)B	30.15 (3.89)B	23.51 (2.73)B
	Org	0-10	81.46 (7.39)a	168.47 (1.19)a	63.92 (1.68)a	
		10-20	90.93 (9.30)a	188.95 (8.61)a	60.14 (4.15)a	
		20-30	85.60 (10.87)a	192.44 (15.73)a	64.67 (4.6)a	
		Average	86.00 (4.99)A	183.29 (6.29)A	62.91 (2.02)A	

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.11. Nitrogen stock in macroaggregates (> 250 µm), free microaggregates (53-250 µm), and free silt and clay fractions (< 53 µm) at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth	Nitrogen stock (Mean (SE))		
			> 250 µm	53-250 µm	< 53 µm
			-----kg m ⁻² -----		
			cm		
CA	Con	0-10	7.53 (0.90)b ^{††}	5.12 (1.37)a	1.64 (0.18)a
		10-20	7.65 (1.31)b	4.39 (0.72)a	1.49 (0.06)a
		20-30	7.24 (1.50)b	3.70 (0.82)a	1.45 (0.14)a
		Average	7.47 (0.64)B ^{††}	4.4 (0.54)A	1.53 (0.07)A
	Org	0-10	14.10 (0.50)a	3.69 (0.09)a	1.50 (0.04)a
		10-20	13.93 (1.13)a	3.97 (0.40)a	1.81 (0.24)a
		20-30	11.25 (0.84)a	2.60 (0.44)a	1.17 (0.09)a
		Average	13.09 (0.63)A	3.42 (0.27)A	1.49 (0.12)A
CO	Con	0-10	3.88 (0.24)b	11.21 (0.11)a	1.50 (0.18)a
		10-20	7.28 (0.33)b	7.52 (0.90)a	1.38 (0.18)a
		20-30	7.24 (0.56)b	6.76 (0.39)a	1.44 (0.08)a
		Average	6.14 (0.60)B	8.5 (0.74)A	1.44 (0.08)A
	Org	0-10	13.51 (3.09)a	8.24 (0.74)b	1.87 (0.23)a
		10-20	15.56 (1.57)a	4.93 (0.24)b	1.49 (0.07)a
		20-30	14.26 (1.40)a	3.86 (0.41)b	1.17 (0.04)a
		Average	14.44 (1.12)A	5.67 (0.71)B	1.51 (0.12)A
NY	Con	0-10	8.81 (1.41)b	10.56 (0.51)a	2.80 (0.17)a
		10-20	8.36 (0.84)b	10.84 (0.11)a	2.29 (0.18)a
		20-30	9.43 (1.21)b	9.24 (0.51)a	1.85 (0.21)a
		Average	8.87 (0.63)B	10.21 (0.31)A	2.31 (0.15)A
	Org	0-10	28.25 (0.12)a	10.20 (1.68)a	2.87 (0.28)a
		10-20	32.97 (1.38)a	9.03 (0.83)a	2.40 (0.08)a
		20-30	34.48 (2.51)a	8.94 (1.97)a	2.47 (0.36)a
		Average	31.90 (1.18)A	9.39 (0.84)A	2.58 (0.15)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

[#] The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.12. Nitrogen stock in coarse particulate organic matter (cPOM), microaggregates within macroaggregates (mM), and silt and clay fractions within macroaggregates (S&C-M) at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth cm	Nitrogen stock (Mean (SE))		
			cPOM	mM	S&C-M
			-----kg m ⁻² -----		
CA	Con	0-10	0.12 (0.04)b ^{††}	4.33 (0.73)b	3.25 (0.35)b
		10-20	0.14 (0.04)b	4.26 (1.07)a	2.88 (0.55)b
		20-30	0.08 (0.01)a	3.75 (1)a	2.99 (0.63)a
		Average	0.11 (0.02)B ^{‡‡}	4.11 (0.48)A	3.04 (0.27)B
	Org	0-10	0.34 (0.04)a	7.17 (0.26)a	5.72 (0.26)a
		10-20	0.31 (0.05)a	6.15 (0.18)a	5.31 (0.56)a
		20-30	0.09 (0.01)a	5.27 (0.5)a	4.37 (0.29)a
		Average	0.25 (0.04)A	6.2 (0.32)A	5.14 (0.28)A
CO	Con	0-10	0.25 (0.02)b	1.71 (0.16)b	1.45 (0.03)b
		10-20	0.08 (0.01)b	3.4 (0.21)b	2.73 (0.05)b
		20-30	0.05 (0.001)b	3.82 (0.26)b	2.49 (0.13)b
		Average	0.12 (0.03)B	2.98 (0.34)B	2.22 (0.2)B
	Org	0-10	1.37 (0.37)a	6.9 (1.58)a	4.56 (0.59)a
		10-20	0.49 (0.13)a	8.22 (1.05)a	5.16 (0.82)a
		20-30	0.27 (0.12)a	7.32 (1.04)a	4.63 (0.53)a
		Average	0.71 (0.21)A	7.48 (0.65)A	4.78 (0.34)A
NY	Con	0-10	2.51 (0.42)a	2.52 (0.72)b	2.54 (0.62)b
		10-20	2.01 (0.22)b	2.27 (0.39)b	2.05 (0.33)b
		20-30	1.98 (0.29)b	3.42 (0.67)b	2.63 (0.47)b
		Average	2.17 (0.18)A	2.74 (0.35)B	2.41 (0.27)B
	Org	0-10	2.68 (0.15)a	16.91 (0.25)a	6.95 (0.22)a
		10-20	3.12 (0.16)a	18.83 (0.91)a	6.36 (0.41)a
		20-30	2.88 (0.34)a	19.7 (1.7)a	6.95 (0.45)a
		Average	2.90 (0.13)A	18.48 (0.68)A	6.75 (0.21)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

^{‡‡} The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.13. Physically and chemically protected carbon (C) and nitrogen (N) at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Site	System	Depth	Physical protection (Mean (SE))		Chemical protection (Mean (SE))	
			C	N	C	N
			-----kg m ⁻² -----			
			cm			
CA	Con	0-10	74.14 (4.02)b ^{††}	9.45 (0.65)b	44.41 (2.70)b	4.88 (0.29)b
		10-20	69.33 (1.51)b	8.65 (0.35)b	42.80 (4.97)b	4.37 (0.49)b
		20-30	60.35 (1.30)a	7.45 (0.30)a	40.07 (4.07)a	4.44 (0.52)a
		Average	67.94 (2.40)B ^{††}	8.52 (0.37)B	42.42 (2.11)A	4.57 (0.24)B
	Org	0-10	87.16 (3.06)a	10.85 (0.28)a	59.62 (2.67)a	7.21 (0.23)a
		10-20	83.65 (3.93)a	10.12 (0.51)a	59.34 (4.08)a	7.13 (0.50)a
		20-30	65.71 (1.01)a	7.86 (0.18)a	49.72 (3.91)a	5.54 (0.29)a
		Average	78.84 (3.63)A	9.61 (0.48)A	56.23 (2.43)A	6.63 (0.33)A
CO	Con	0-10	101.48 (0.83)a	12.92 (0.17)a	24.73 (1.53)b	2.95 (0.21)b
		10-20	83.02 (0.72)a	10.92 (0.90)a	32.51 (2.05)b	4.11 (0.22)b
		20-30	79.08 (2.60)a	10.58 (0.17)a	29.74 (1.17)b	3.93 (0.19)b
		Average	87.86 (3.55)A	11.47 (0.45)A	28.99 (1.4)B	3.66 (0.21)B
	Org	0-10	120.88 (8.27)a	15.13 (0.84)a	51.69 (4.88)a	6.43 (0.61)a
		10-20	103.62 (8.16)a	13.15 (1.01)a	51.67 (7.51)a	6.65 (0.85)a
		20-30	83.76 (10.62)a	11.18 (1.42)a	44.65 (4.31)a	5.80 (0.56)a
		Average	102.75 (7.03)A	13.15 (0.80)A	49.34 (3.1)A	6.29 (0.37)A
NY	Con	0-10	139.68 (9.26)b	13.08 (1.09)b	53.52 (6.66)b	5.34 (0.62)b
		10-20	144.21 (7.14)b	13.11 (0.45)b	43.16 (3.15)b	4.34 (0.31)b
		20-30	136.63 (10.03)b	12.66 (0.61)b	45.48 (3.06)b	4.48 (0.32)b
		Average	140.17 (4.74)B	12.95 (0.41)B	47.39 (2.75)B	4.72 (0.27)B
	Org	0-10	273.15 (17.98)a	27.11 (1.61)a	89.95 (1.62)a	9.82 (0.21)a
		10-20	282.47 (15.25)a	27.87 (1.29)a	82.17 (4.60)a	8.76 (0.45)a
		20-30	288.40 (15.39)a	28.64 (1.43)a	87.11 (3.46)a	9.41 (0.32)a
		Average	281.34 (8.70)A	27.87 (0.78)A	86.41 (2.05)A	9.33 (0.22)A

^{††} The same lowercase letter indicates that the parameter within the same site and depth was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

[#] The same uppercase letter indicates that the parameter within the same site was not significantly different among systems according to the least square means with a Tukey-Kramer test ($p < 0.05$).

Table 2.14. Correlation results between soil variables and microbial biomass (n=59).

Parameters			Correlation coefficient (r)	p-value
MBC	vs	Macroaggregates	0.35490	0.0058
MBC	vs	Microaggregates	-0.30761	0.0178
MBC	vs	Aggregate stability	0.35591	0.0057
MBC	vs	Physically protected C stock	0.73617	<0.0001
MBC	vs	Chemically protected C stock	0.69299	<0.0001
MBC	vs	SOC in bulk soil	0.70238	<0.0001
MBN	vs	Macroaggregates	0.34606	0.0073
MBN	vs	Microaggregates	-0.35877	0.0053
MBN	vs	Aggregate stability	0.34489	0.0075
MBN	vs	Physically protected N stock	0.92445	<0.0001
MBN	vs	Chemically protected N stock	0.80080	<0.0001
MBN	vs	N in bulk soil	0.92244	<0.0001
SOC in bulk soil	vs	Aggregate stability	0.38719	0.0024
SOC in bulk soil	vs	Macroaggregates	0.38861	0.0024
SOC in bulk soil	vs	Microaggregates	-0.40936	0.0013
N in bulk soil	vs	Aggregate stability	0.47009	0.0002
N in bulk soil	vs	Macroaggregates	0.47040	0.0002
N in bulk soil	vs	Microaggregates	-0.45839	0.0003
DOC	vs	Macroaggregates	0.33427	0.0097
DOC	vs	Microaggregates	-0.34410	0.0076
DOC	vs	Aggregate stability	0.33350	0.0098
DOC	vs	Physically protected C stock	0.16477	NS
DOC	vs	Chemically protected C stock	0.26975	0.0388
Dissolved N	vs	Macroaggregates	0.40189	0.0016
Dissolved N	vs	Microaggregates	-0.39203	0.0021
Dissolved N	vs	Aggregate stability	0.40215	0.0016
Dissolved N	vs	Physically protected N stock	-0.02247	NS
Dissolved N	vs	Chemically protected N stock	0.22171	NS

Note: Microbial biomass carbon (MBC); microbial biomass nitrogen (MBN); soil organic carbon (SOC); carbon (C); nitrogen (N); dissolved organic carbon (DOC); and NS is not significant.

Table 2.15. A stepwise regression model predicting aggregate stability, soil organic carbon and nitrogen stock in bulk soil.

Dependent variable	Model	r ²	p-value
Aggregate stability	1.56 + 0.007(MNB) + 0.008(DN)	0.27	0.0001
SOC stock in bulk soil	433.26 – 7.76(% clay) + 0.92(MBC)	0.80	<0.0001
SOC stock in bulk soil	506.97 – 8.55(% clay) + 75.07(MWD)	0.49	<0.0001
N stock in bulk soil	8.47 + 0.36(MBN) + 0.10(DN)	0.93	<0.0001
N stock in bulk soil	33.55 – 0.45 (% clay) + 7.20(MWD)	0.42	<0.0001

Note: All variables left in the model were significant at the 0.05 level.

Microbial biomass carbon (MBC); microbial biomass nitrogen (MBN); soil organic carbon (SOC); nitrogen (N); dissolved nitrogen (DN); mean weight diameter (MWD).

FIGURES

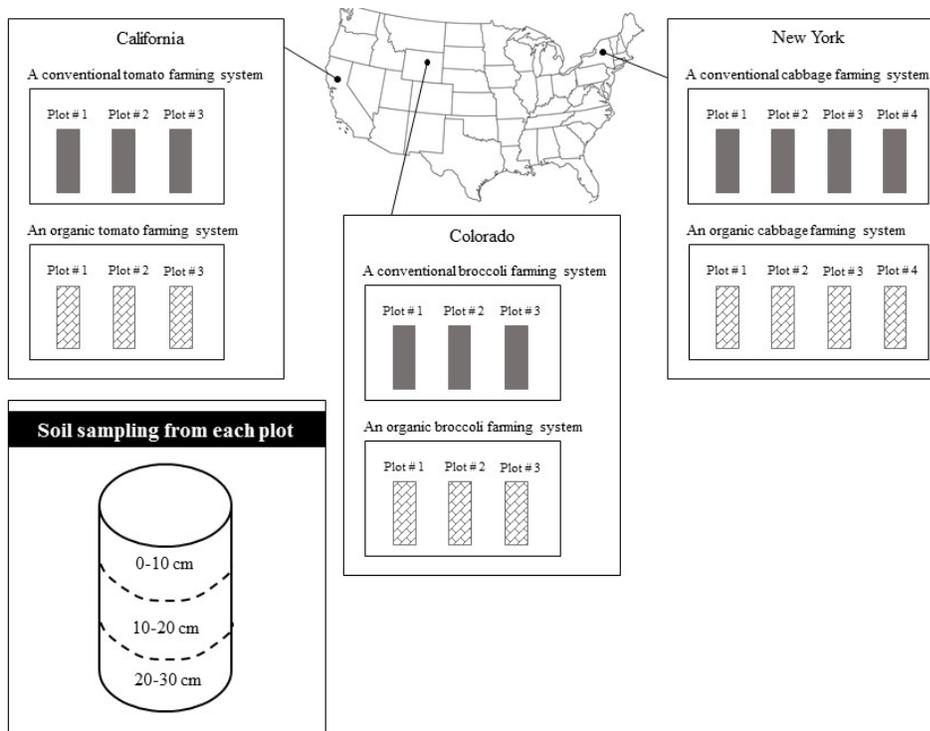


Fig. 2.1. Experimental design.

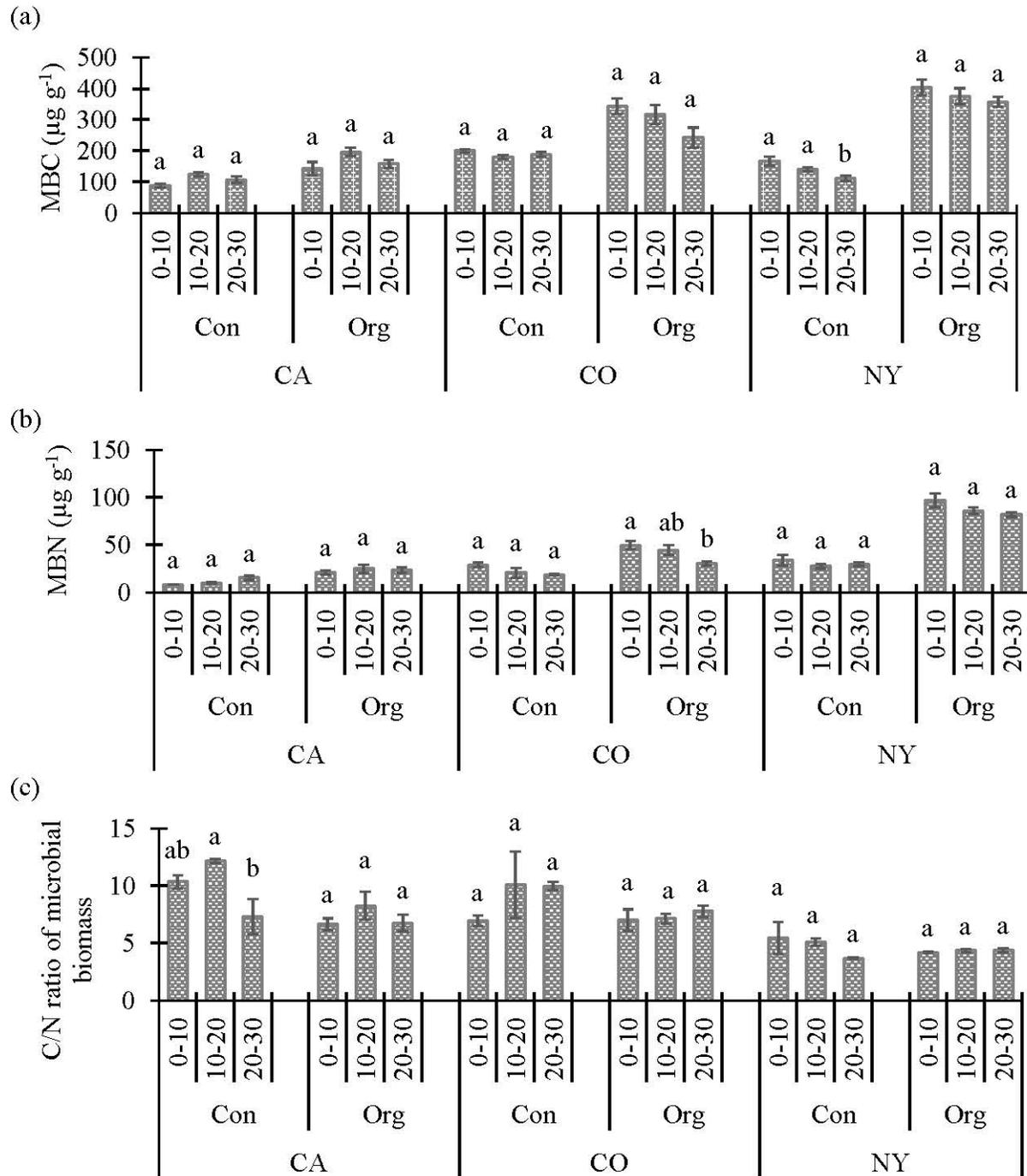


Fig. 2.2. Microbial biomass and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) Microbial biomass carbon (MBC); and Microbial biomass nitrogen (MBN); and (c) C/N ratio of microbial biomass.

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

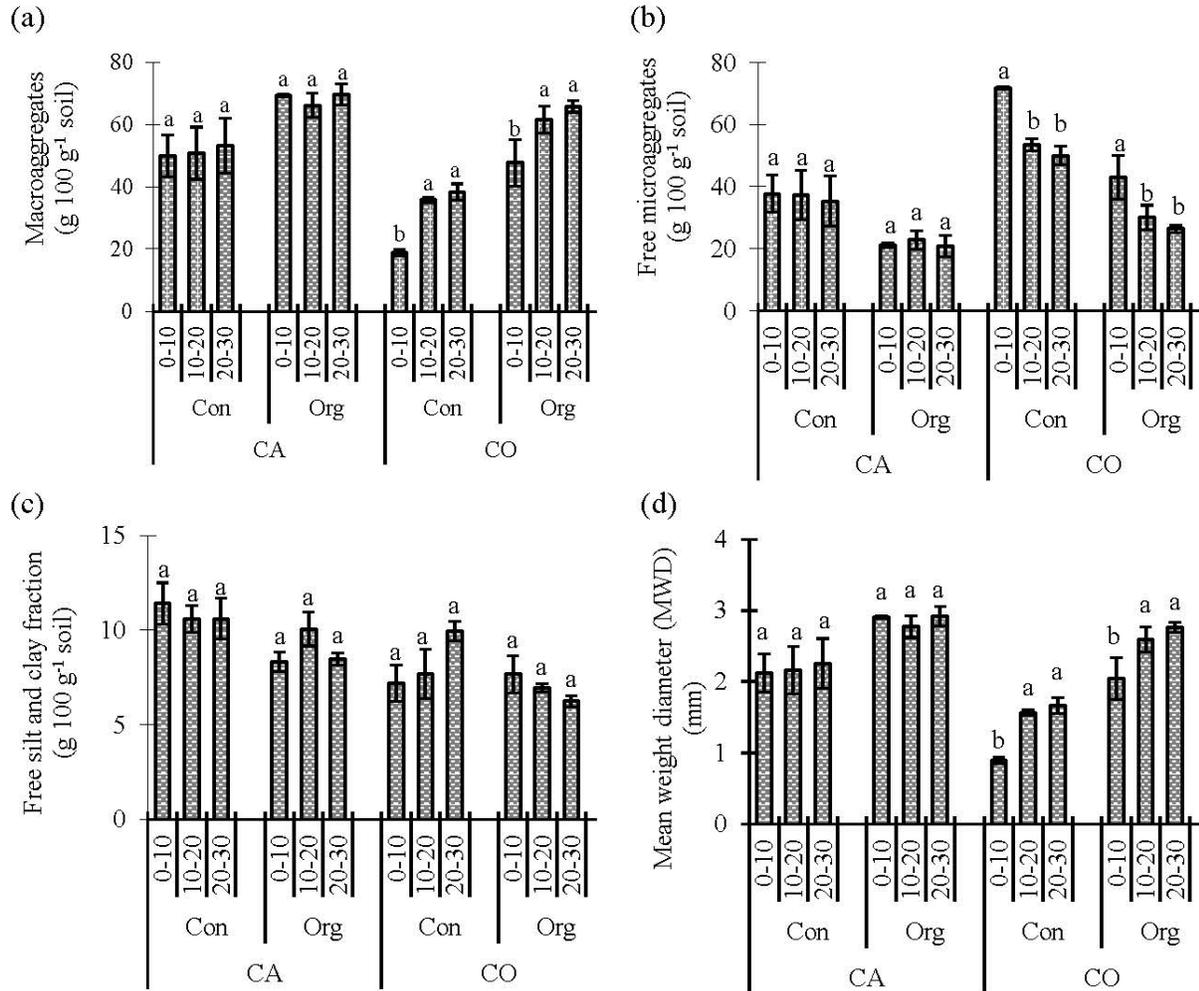


Fig. 2.3. Aggregation size distribution, mean weight diameter, and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) Macroaggregates; (b) Free microaggregates; (c) Free silt and clay fraction; and (d) Mean weight diameter (MWD).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

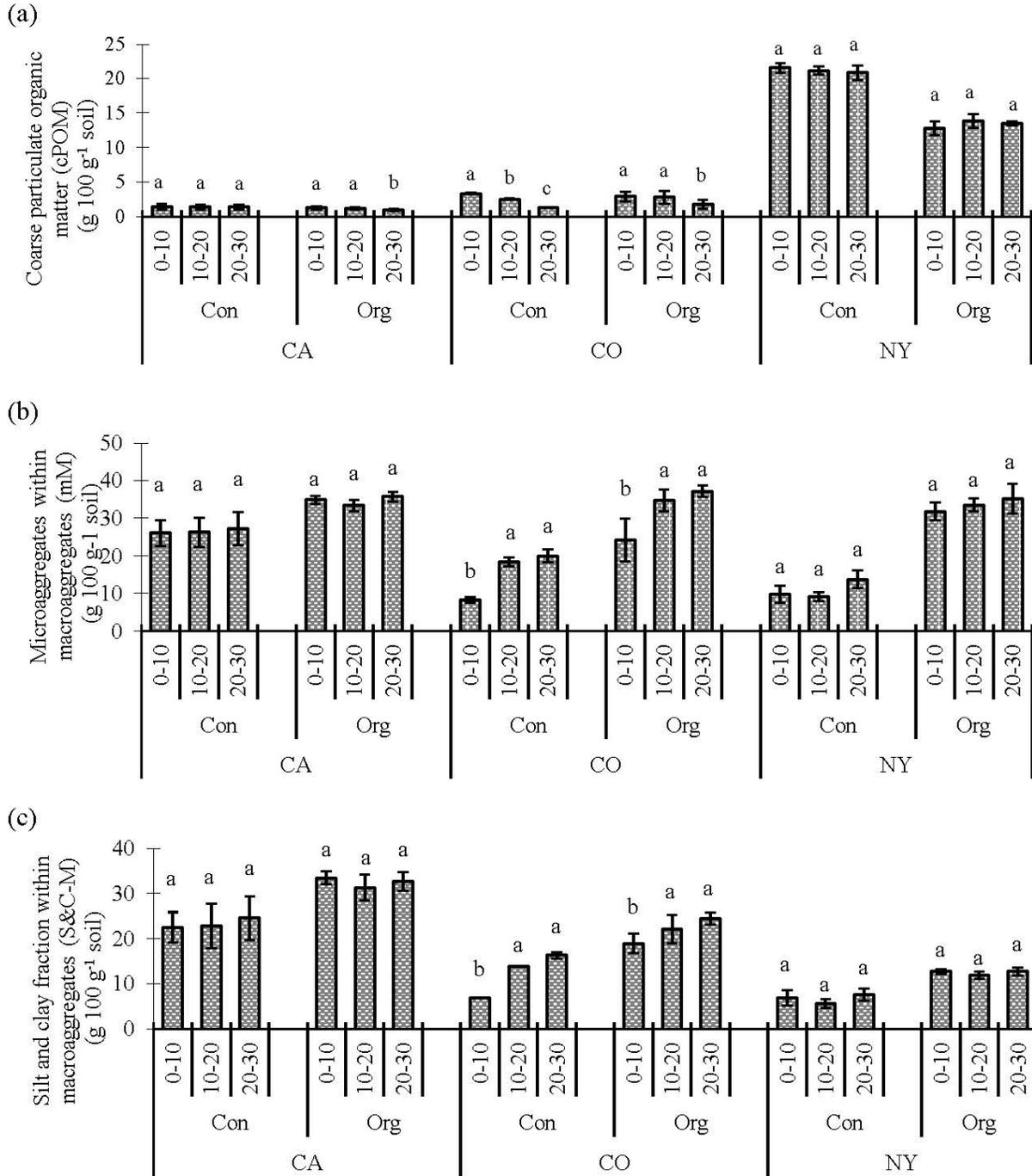


Fig. 2.4. Aggregation size distribution within macroaggregates and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) Coarse particulate organic matter (cPOM); (b) Microaggregates within macroaggregates (mM); and (c) Silt and clay fraction within macroaggregates (S&C-M).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

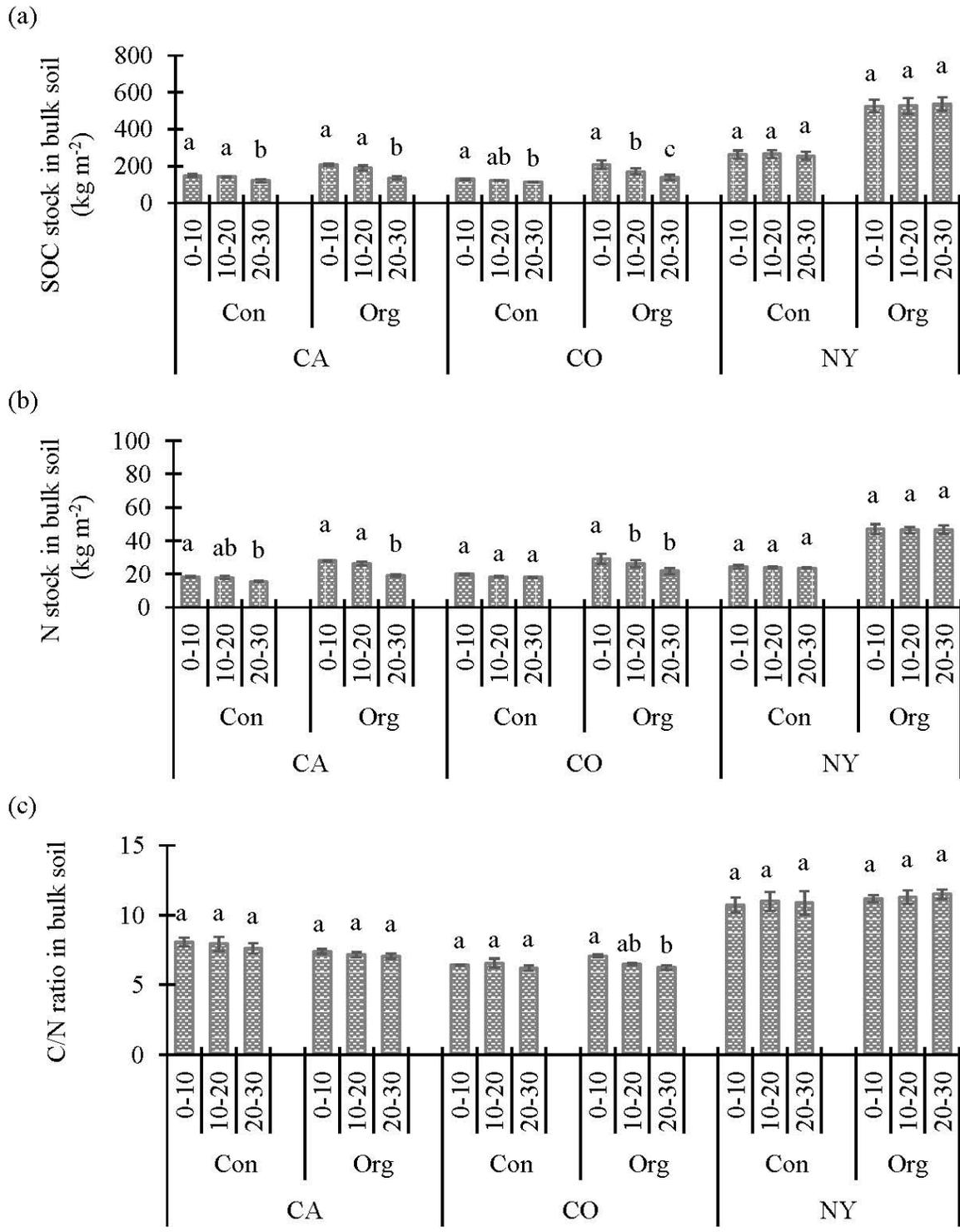


Fig. 2.5. Soil organic carbon stock (a) and N stock (b) in bulk, C/N ratio of bulk soil (c), and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

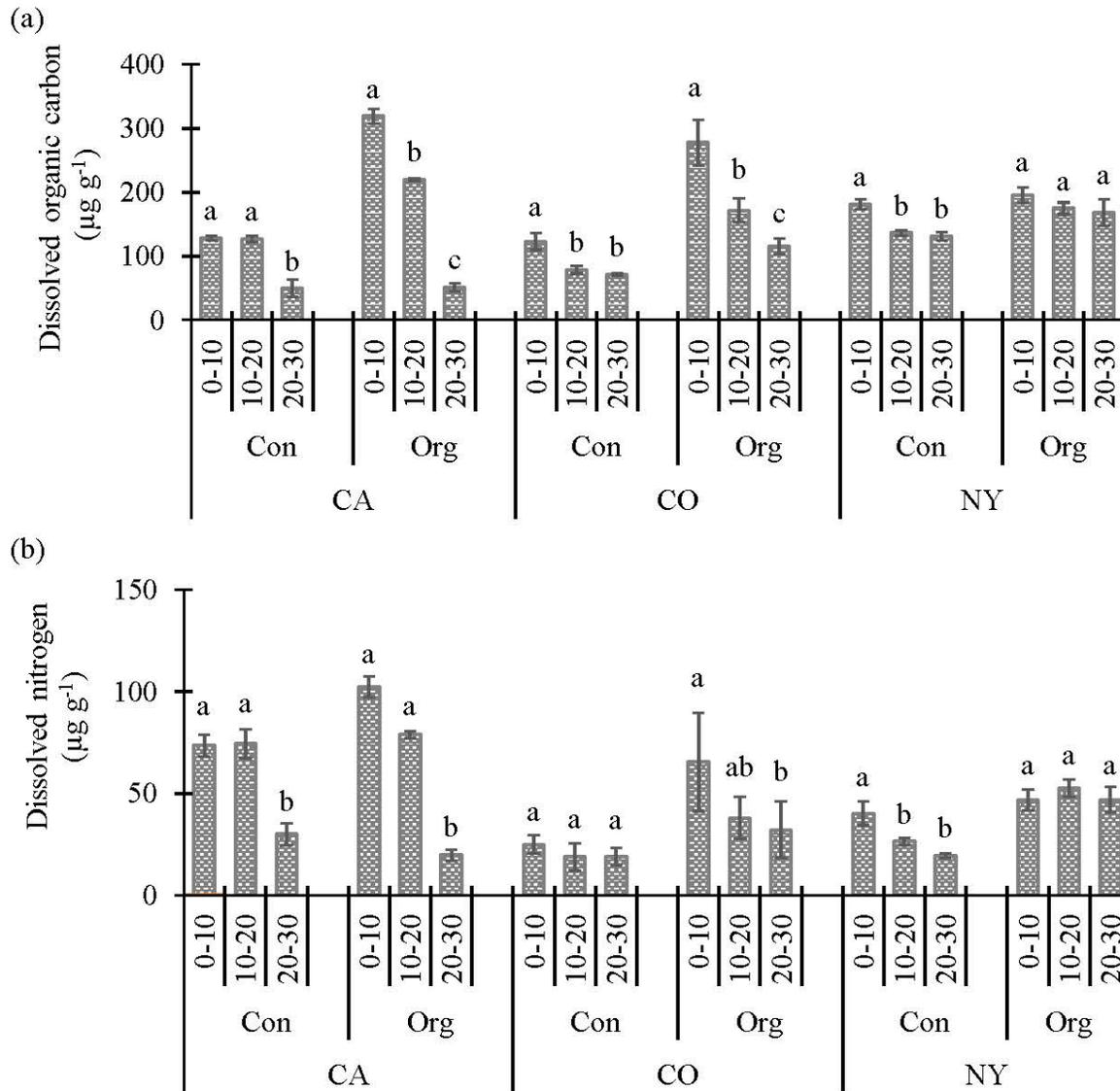


Fig. 2.6. Dissolved soil organic carbon (a) and N (b) in bulk and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

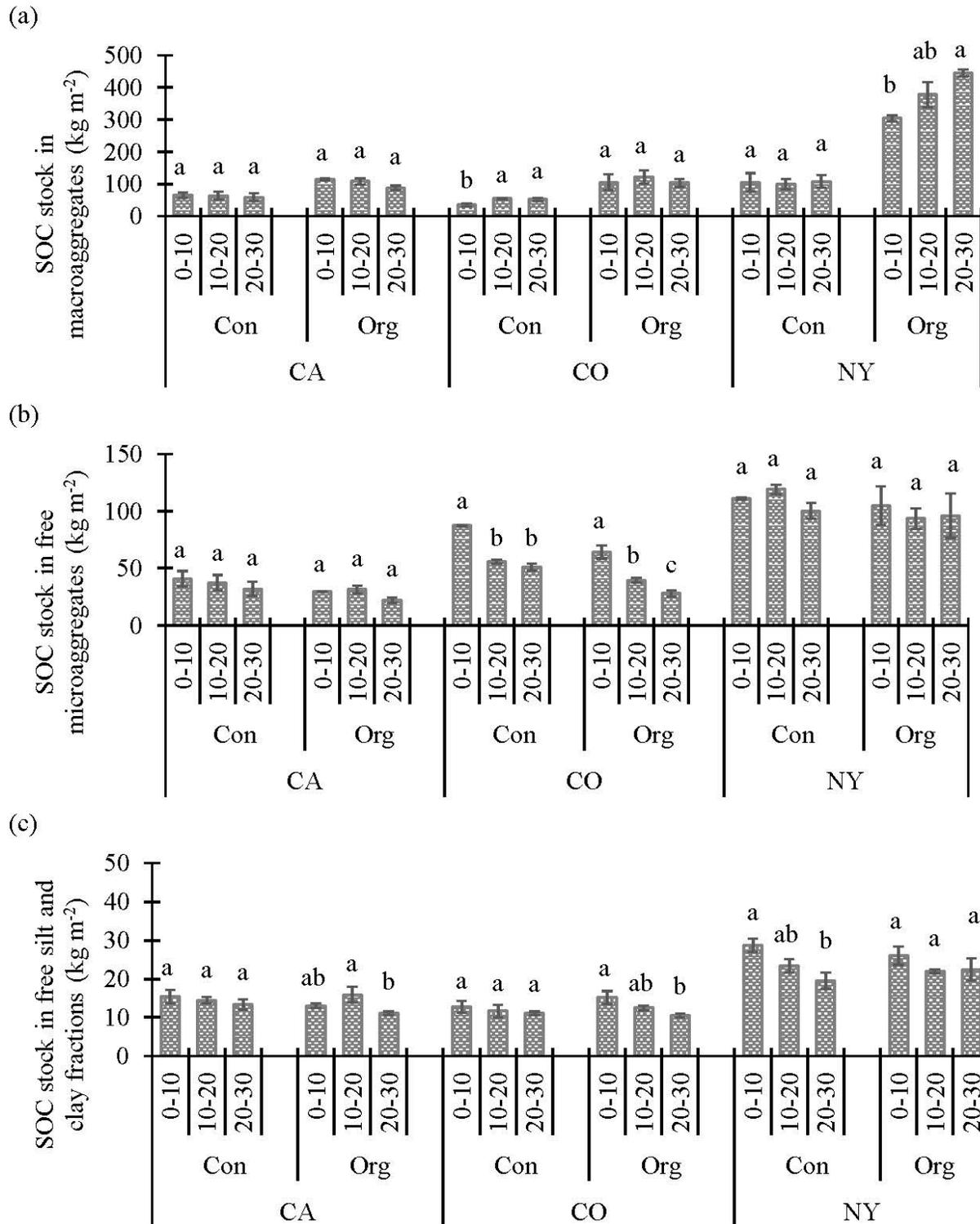


Fig. 2.7. Soil organic carbon stock in macroaggregates (a), free microaggregates (b), free silt and clay fraction (c), and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

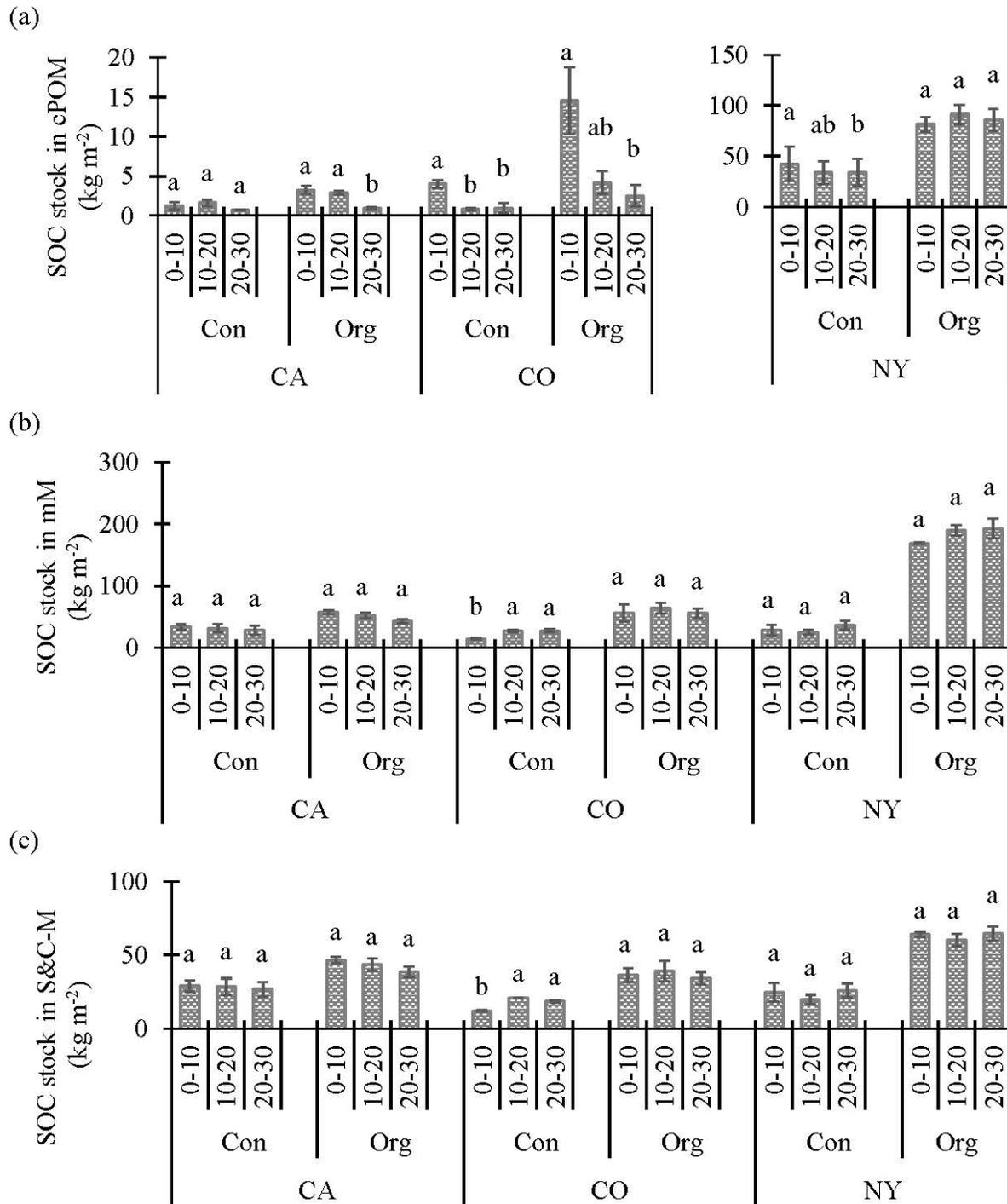


Fig. 2.8. Soil organic carbon (SOC) stock in aggregates within macroaggregates and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) SOC stock in coarse particulate organic matter (cPOM); (b) SOC stock in microaggregates within macroaggregates (mM); and (c) SOC stock in silt and clay fraction within macroaggregates (S&C-M).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

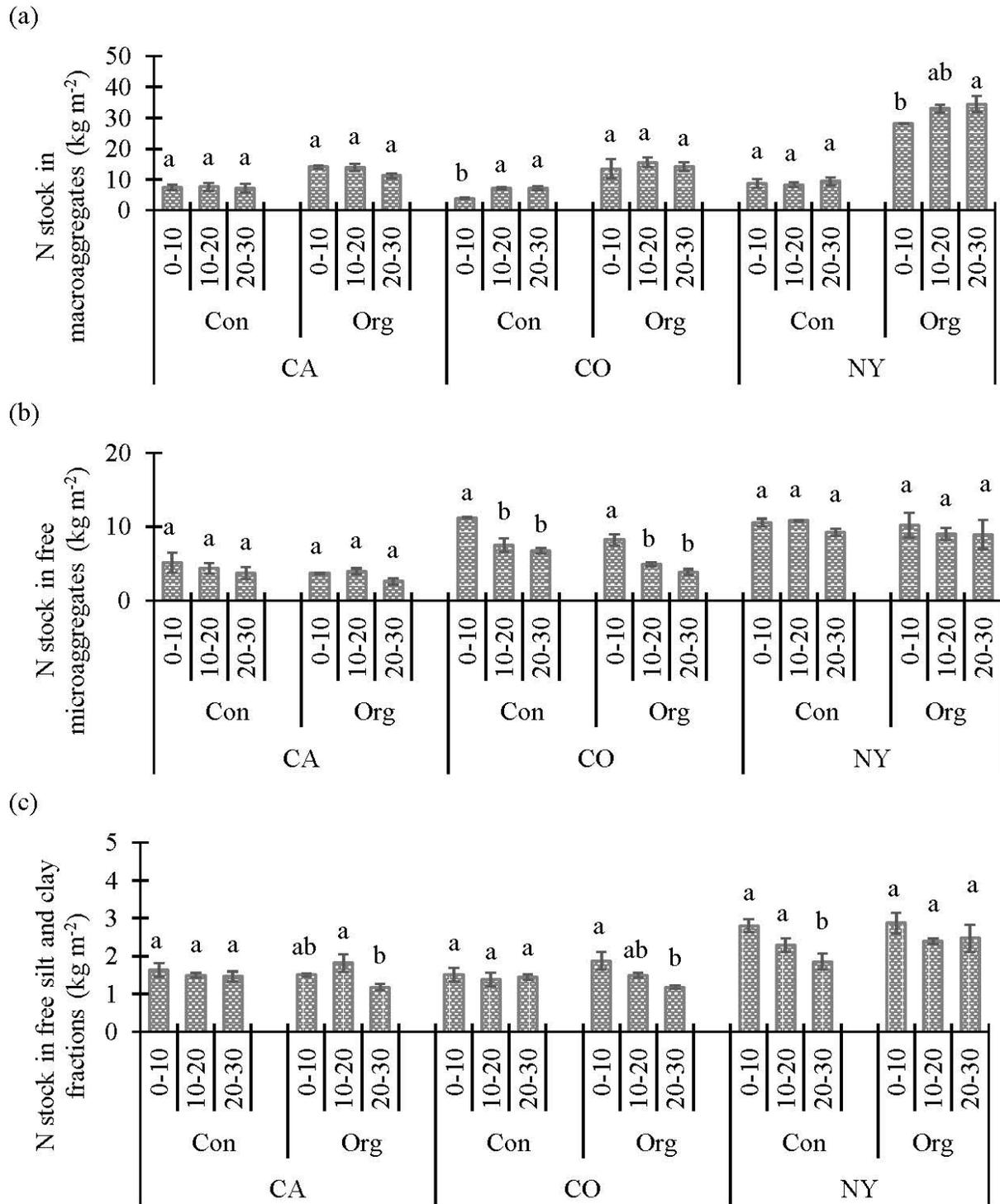


Fig. 2.9. Nitrogen stock in macroaggregates (a), free microaggregates (b), free silt and clay fraction (c), and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY).

Note: A common indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

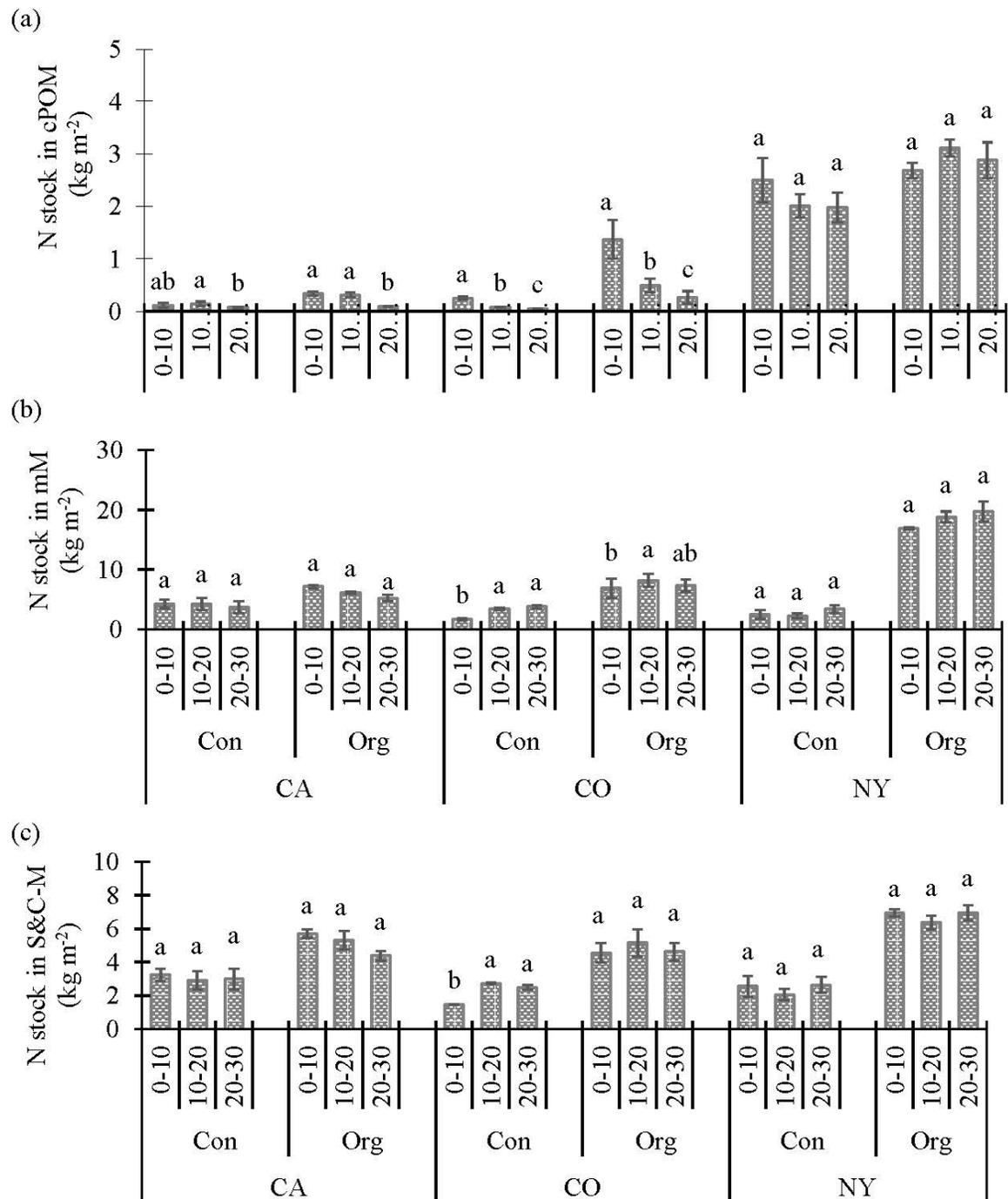


Fig. 2.10. Nitrogen (N) stock in aggregates within macroaggregates and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) N stock in coarse particulate organic matter (cPOM); (b) N stock in microaggregates within macroaggregates (mM); and (c) N stock in silt and clay fraction within macroaggregates (S&C-M).

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

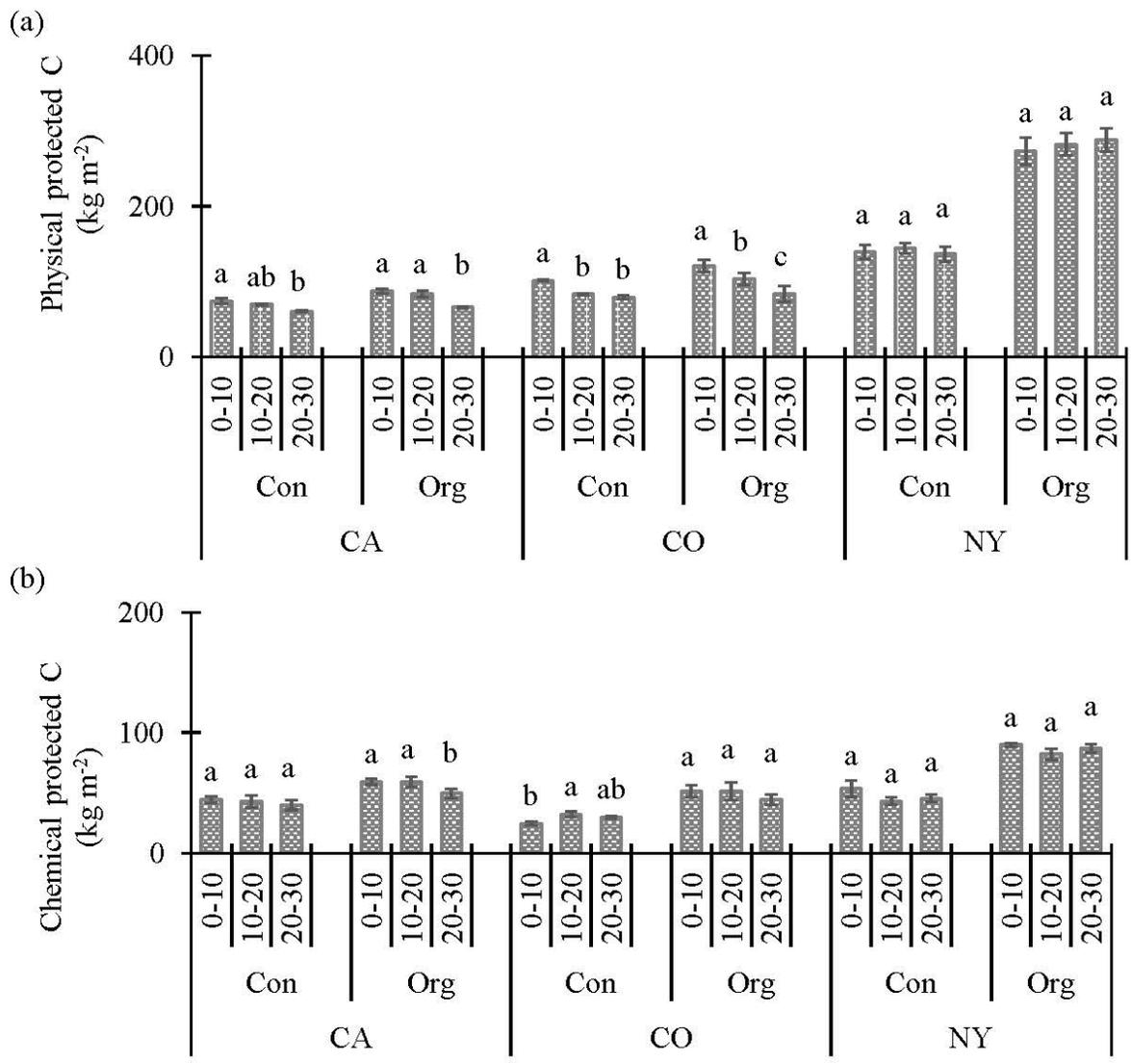


Fig. 2.11. Physically and chemically protected carbon and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) Physically protected carbon; and (b) Chemically protected carbon.

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

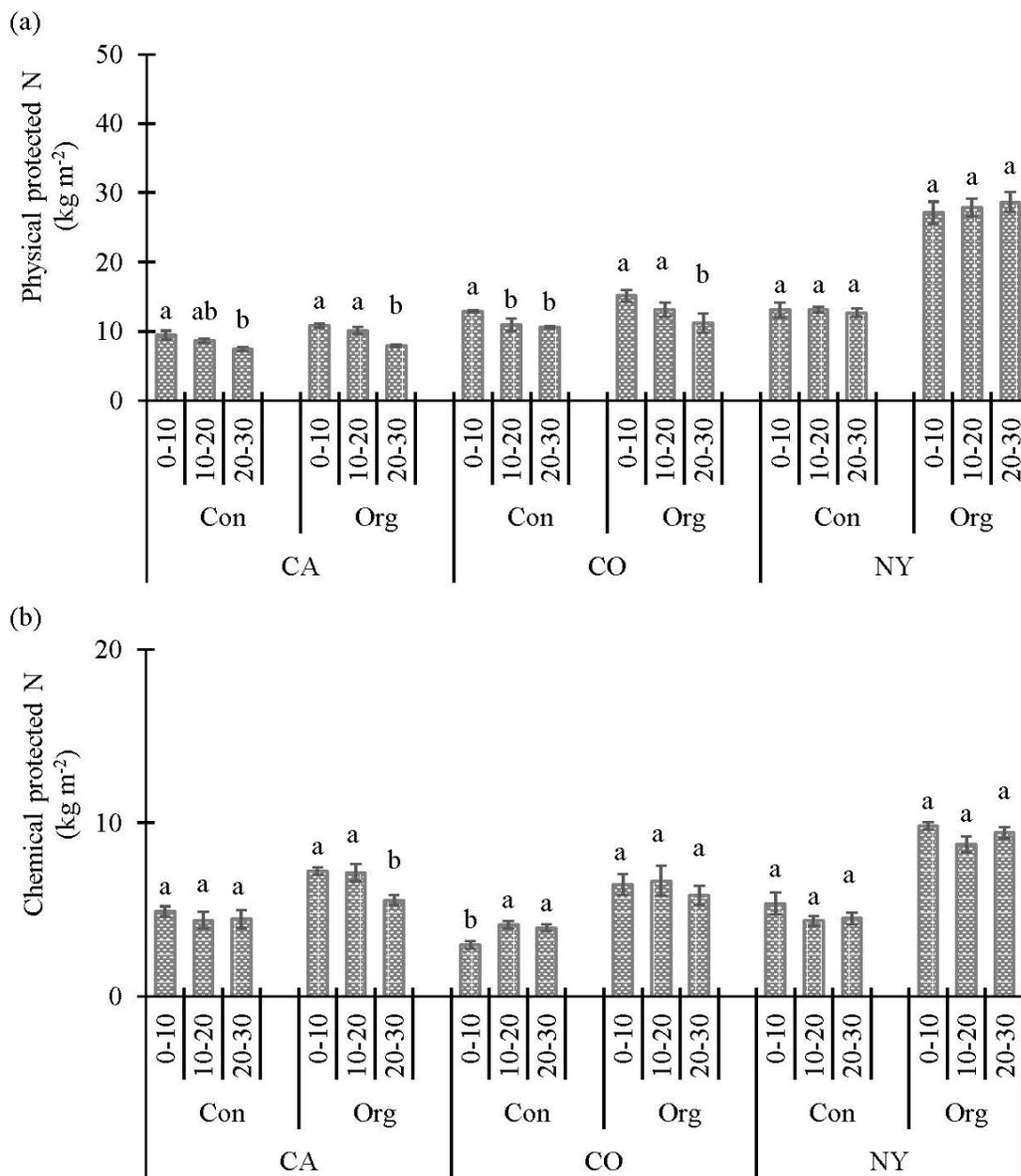


Fig. 2.12. Physically and chemically protected nitrogen and standard error at three depths in conventional (Con) and organic (Org) farming systems in California (CA), Colorado (CO) and New York (NY). (a) Physically protected nitrogen; and (b) Chemically protected nitrogen.

Note: A common letter indicates that the parameter within the same site and system was not significantly different among depths according to the least square means with a Tukey-Kramer test ($p < 0.05$).

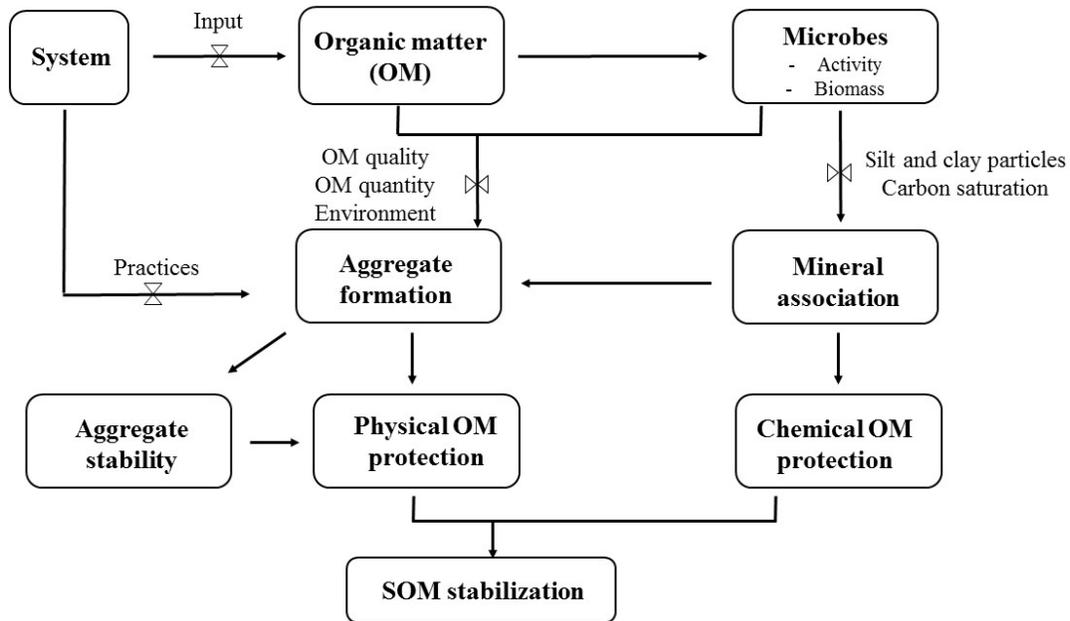


Fig. 2.13. Conceptual diagram of the effect of farming system on soil organic matter protection and aggregate stability.

REFERENCES

- Amézketa, E. 1999. Soil aggregate stability: a review. *Journal of Sustainable Agriculture* 14: 83-151. doi:10.1300/J064v14n02_08
- Banwart, S.A., H. Black, Z. Cai, P.T. Gicheru, H. Joosten, R.L. Victoria, E. Milne, E. Noellemeyer, and U. Pascual. 2015. The global challenge for soil carbon In: S.A. Banwart et al., editors, *Soil carbon: science, management and policy for multiple benefits*. CAB International. p. 1-9.
- Beck, T., R.G. Joergensen, E. Kandeler, F. Makeschin, E. Nuss, H.R. Oberholzer, and S. Scheu. 1997. An inter laboratory comparison of ten different ways of measuring soil microbial biomass C. *Soil BioI. Biochem.* 29:1023-1032.
- Brookes, P.C., A. Landman, G. Pruden, and D.S. Jenkinson. 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil BioI. Biochem.* 17:837-842.
- Bulluck III, L.R., M. Brosius, G.K. Evanylo, and J.B. Ristaino. 2002. Organic and synthetic fertility amendments influence soil microbial, physical and chemical properties on organic and conventional farms. *Applied Soil Ecology* 19: 147-160. doi:10.1016/S0929-1393(01)00187-1
- Castellano, M., K. Mueller, D. Olk, J. Sawyer, and J. Six. 2015. Integrating plant litter quality, soil organic matter stabilization, and the carbon saturation concept. *Global change biology* 21: 3200-3209.
- Cornell University. 2016. All weather data. <http://newa.cornell.edu/index.php?page=all-weather-data> (accessed 20 Feb 2016).

- Cotrufo, M.F., M. Wallenstein, C. Boot, K. Deneff, and E. Paul. 2013. The Microbial Efficiency-Matrix Stabilization (MEMS) framework integrates plant litter decomposition with soil organic matter stabilization: do labile plant inputs form stable soil organic matter? *Global change biology* 19: 988-995.
- Cui, J., Z. Li, Z. Liu, B. Ge, C. Fang, C. Zhou, and B. Tang. 2014. Physical and chemical stabilization of soil organic carbon along a 500-year cultivated soil chronosequence originating from estuarine wetlands: Temporal patterns and land use effects. *Agriculture, Ecosystems & Environment* 196: 10-20. doi:10.1016/j.agee.2014.06.013
- Daly, C., G.H. Taylor, D.B. Hannaway, and H.A. Fribourg. 2011. Climate and agro-ecological zones. In: H.A.F. David B. Hannaway, editor *Country pasture/forage resource profile for the United States of America*. AGP, FAO, Rome.
- Elliott, E. 1986. Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. *Soil science society of America journal* 50: 627-633.
- Eswaran, H., P.F. Reich, J.M. Kimble, F.H. Beinroth, E. Padmanabhan, and P. Moncharoen. 2000. Global carbon stocks. In: R. Lal et al., editors, *Global climate change and pedogenic carbonates*. CRC/Lewis, Boca Raton, FL. p. 15-25.
- FAO. 2011. *Organic agriculture and climate change mitigation: a report of the round table on organic agriculture and climate change*. FAO, Rome, Italy
- Gee, G.W., and J.W. Bauder. 1979. Particle Size Analysis by Hydrometer: A Simplified Method for Routine Textural Analysis and a Sensitivity Test of Measurement Parameters¹. *Soil Science Society of America Journal* 43: 1004.
- Grandy, A.S., and G.P. Robertson. 2007. Land-Use Intensity Effects on Soil Organic Carbon Accumulation Rates and Mechanisms. *Ecosystems* 10: 59-74.

- Green, V.S., M. Cavigelli, T. Dao, and D. Flanagan. 2005. Soil physical properties and aggregate-associated C, N, and P distributions in organic and conventional cropping systems. *Soil science* 170: 822-831.
- Herencia, J.F., J.C. Ruiz-Porras, S. Melero, P.A. Garcia-Galavis, E. Morillo, and C. Maqueda. 2007. Comparison between organic and mineral fertilization for soil fertility levels, crop macronutrient concentrations, and yield. *Agronomy Journal* 99: 973-983.
doi:10.2134/agronj2006.0168
- Horwath, W., and E. Paul. 1994. Microbial biomass. *Methods of Soil Analysis: Part 2—Microbiological and Biochemical Properties*: 753-773.
- Hurisso, T.T., J.G. Davis, J.E. Brummer, M.E. Stromberger, M.M. Mikha, M.L. Haddix, M.R. Booher, and E.A. Paul. 2013. Rapid changes in microbial biomass and aggregate size distribution in response to changes in organic matter management in grass pasture. *Geoderma* 193: 68-75.
- Król, A., J. Lipiec, M. Turski, and J. Kuś. 2013. Effects of organic and conventional management on physical properties of soil aggregates. *International Agrophysics* 27: 15-21. doi:10.2478/v10247-012-0063-1
- Kushwaha, C., S. Tripathi, and K. Singh. 2001. Soil organic matter and water-stable aggregates under different tillage and residue conditions in a tropical dryland agroecosystem. *Applied Soil Ecology* 16: 229-241.
- Lal, R. 2004. Soil carbon sequestration to mitigate climate change. *Geoderma* 123: 1-22.
- Lehmann, J., J. Kinyangi, and D. Solomon. 2007. Organic matter stabilization in soil microaggregates: implications from spatial heterogeneity of organic carbon contents and carbon forms. *Biogeochemistry* 85: 45-57.

- Lehmann, J., and M. Kleber. 2015. The contentious nature of soil organic matter. *Nature* 528: 60-68. doi:10.1038/nature16069
- Lichter, K., B. Govaerts, J. Six, K.D. Sayre, J. Deckers, and L. Dendooven. 2008. Aggregation and C and N contents of soil organic matter fractions in a permanent raised-bed planting system in the Highlands of Central Mexico. *Plant and Soil* 305: 237-252.
- Lutzow, M.v., I. Kogel Knabner, K. Ekschmitt, E. Matzner, G. Guggenberger, B. Marschner, and H. Flessa. 2006. Stabilization of organic matter in temperate soils: mechanisms and their relevance under different soil conditions - a review. *European journal of soil science* 57: 426-445.
- Melero, S., J.C.R. Porras, J.F. Herencia, and E. Madejon. 2006. Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil and Tillage Research* 90: 162-170.
- Mikha, M., G. Hergert, J. Benjamin, J. Jabro, and R. Nielsen. 2015. Long-term manure impacts on soil aggregates and aggregate-associated carbon and nitrogen. *Soil Science Society of America Journal* 79: 626-636. doi:10.2136/sssaj2014.09.0348
- Mikha, M.M., and C.W. Rice. 2004. Tillage and manure effects on soil and aggregate-associated carbon and nitrogen. *Soil Science Society of America Journal* 68: 809-816.
- Miltner, A., P. Bombach, B. Schmidt-Brücken, and M. Kästner. 2012. SOM genesis: microbial biomass as a significant source. *Biogeochemistry* 111: 41-55. doi:10.1007/s10533-011-9658-z
- Olson, K., M. Al Kaysi, R. Lal, and B. Lowery. 2014. Experimental consideration, treatments, and methods in determining soil organic carbon sequestration rates. *Soil Science Society of America Journal* 78: 348.

- Paustian, K., J. Lehmann, S. Ogle, D. Reay, G.P. Robertson, and P. Smith. 2016. Climate-smart soils. *Nature* 532: 49-57. doi:10.1038/nature17174
- Plante, A., R. Conant, C. Stewart, K. Paustian, and J. Six. 2006. Impact of soil texture on the distribution of soil organic matter in physical and chemical fractions. *Soil Science Society of America Journal* 70: 287-296. doi:10.2136/sssaj2004.0363
- Santos, V.B., A.S. Araújo, L.F. Leite, L.A. Nunes, and W.J. Melo. 2012. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma* 170: 227-231.
- Segoli, M., S. De Gryze, F. Dou, J. Lee, W.M. Post, K. Denef, and J. Six. 2013. AggModel: a soil organic matter model with measurable pools for use in incubation studies. *Ecological Modelling* 263: 1-9. doi:10.1016/j.ecolmodel.2013.04.010
- Semenov, V.M., L.A. Ivannikova, N.A. Semenova, A.K. Khodzhaeva, and S.N. Udal'tsov. 2010. Organic matter mineralization in different soil aggregate fractions. *Eurasian Soil Science* 43: 141-148. doi:10.1134/s1064229310020031
- Sherrod, L., G. Dunn, G. Peterson, and R. Kolberg. 2002. Inorganic carbon analysis by modified pressure-calciometer method. *Soil Science Society of America Journal* 66: 299-305.
- Shrestha, B., B. Sitaula, B. Singh, and R. Bajracharya. 2004. Soil organic carbon stocks in soil aggregates under different land use systems in Nepal. *Nutrient cycling in Agroecosystems* 70: 201-213.
- Six, J., H. Bossuyt, S. Degryze, and K. Denef. 2004. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. *Soil and Tillage Research* 79: 7-31. doi:10.1016/j.still.2004.03.008

- Six, J., R.T. Conant, E.A. Paul, and K. Paustian. 2002. Stabilization mechanisms of soil organic matter: Implications for C-saturation of soils. *Plant and Soil* 241: 155-176.
doi:10.1023/a:1016125726789
- Six, J., E.T. Elliott, and K. Paustian. 2000a. Soil macroaggregate turnover and microaggregate formation: a mechanism for C sequestration under no-tillage agriculture. *Soil Biology and Biochemistry* 32: 2099-2103. doi:10.1016/S0038-0717(00)00179-6
- Six, J., E.T. Elliott, K. Paustian, and J.W. Doran. 1998. Aggregation and soil organic matter accumulation in cultivated and native grassland soils. *Soil Science Society of America Journal* 62: 1367-1377.
- Six, J., K. Paustian, E.T. Elliott, and C. Combrink. 2000b. Soil Structure and Organic Matter I. Distribution of aggregate-size classes and aggregate-associated Carbon. *Soil Science Society of America Journal* 64: 681-689. doi:10.2136/sssaj2000.642681x
- Smith, P., M.F. Cotrufo, C. Rumpel, K. Paustian, P.J. Kuikman, J.A. Elliott, R. McDowell, R.I. Griffiths, S. Asakawa, M. Bustamante, J.I. House, J. Sobocká, R. Harper, G. Pan, P.C. West, J.S. Gerber, J.M. Clark, T. Adhya, R.J. Scholes, and M.C. Scholes. 2015. Biogeochemical cycles and biodiversity as key drivers of ecosystem services provided by soils. *SOIL* 1: 665-685. doi:10.5194/soil-1-665-2015
- Soil Survey Staff. 2004. Method 4B4b. In: R. Burt, editor, *Soil survey laboratory methods manual*. Soil Survey Investigations Report No. 42. Version 4.0. Soil Survey Staff, USDA-NRCS, Washington, DC. p. 202.
- Tisdall, J., and J.M. Oades. 1982. Organic matter and water-stable aggregates in soils. *Journal of soil science* 33: 141-163.

- Tu, C., F.J. Louws, N.G. Creamer, J.P. Mueller, C. Brownie, K. Fager, M. Bell, and S. Hu. 2006. Responses of soil microbial biomass and N availability to transition strategies from conventional to organic farming systems. *Agriculture, ecosystems & environment* 113: 206-215.
- University of California: Agricultural Sustainability Institute. 2016. Russell ranch sustainable agriculture facility <http://asi.ucdavis.edu/programs/rr> (accessed 02 Feb 2016).
- USDA-NRCS. 2016. Estimating moist bulk density by texture. https://www.nrcs.usda.gov/wps/portal/nrcs/detail/soils/survey/office/ssr10/tr/?cid=nrcs144p2_074844 (accessed 11 Nov 2016).
- Van Oost, K., T.A. Quine, G. Govers, S. De Gryze, J. Six, J.W. Harden, J.C. Ritchie, G.W. McCarty, G. Heckrath, C. Kosmas, J.V. Giraldez, J.R.M. da Silva, and R. Merckx. 2007. The Impact of Agricultural Soil Erosion on the Global Carbon Cycle. *Science* 318: 626-629.
- von Lützw, M., I. Kögel Knabner, B. Ludwig, E. Matzner, H. Flessa, K. Ekschmitt, G. Guggenberger, B. Marschner, and K. Kalbitz. 2008. Stabilization mechanisms of organic matter in four temperate soils: Development and application of a conceptual model. *Journal of plant nutrition and soil science* 171: 111-124.
- Wang, Y., L. Zhou, J. Wu, C.R. Butterly, C. Tang, and J. Xu. 2010. Soil Microbial Biomass and pH as Affected by the Addition of Plant Residues. *Molecular Environmental Soil Science at the Interfaces in the Earth's Critical Zone*. Springer. p. 320-322.
- Wells, A., K. Chan, and P. Cornish. 2000. Comparison of conventional and alternative vegetable farming systems on the properties of a yellow earth in New South Wales. *Agriculture, Ecosystems & Environment* 80: 47-60.

CHAPTER 3. SIMULATED GREENHOUSE GAS EMISSIONS FROM SOIL-APPLIED SOLID AND LIQUID ORGANIC FERTILIZERS USING THE DAYCENT MODEL³

3.1 Summary

Organic fertilizer application can affect nitrous oxide (N₂O) and carbon dioxide (CO₂) emissions from soil by influencing nitrification, denitrification and microbial decomposition. DAYCENT, a widely used biogeochemical model, has been extensively tested for major commodity crops but not for specialty crops. The objectives of this study were to compare simulated and measured N₂O and CO₂ emissions from irrigated lettuce plots from different organic fertilizer treatments. The N₂O and CO₂ emissions were measured during 2014 from a lettuce field (*Lactuca sativa* L.) with nine treatments: four organic fertilizers (feather meal: FM, blood meal: BM, fish emulsion: FE and cyano-fertilizer: CF) applied at different nitrogen (N) rates (28 and 56 kg N ha⁻¹) and an unfertilized control. The measured data from the low N rate treatments were used for calibration, and the data from the high N rate treatments were used for validation. Comparison of daily N₂O and CO₂ emissions simulated by DAYCENT and measured from the field yielded coefficients of determination (r²) of 0.0004 to 0.48 and 0.002 to 0.65, respectively. DAYCENT simulated the effect of BM and FM (single application) on both N₂O and CO₂ emissions better than for FE and CF (multiple applications). Cumulative emissions from DAYCENT were overestimated except for cumulative N₂O emissions from FM and BM treatments. The effect of single organic fertilizer applications on N₂O and CO₂ emissions were

³ In preparation for submission to Journal of Environmental Quality with Stephen J. Del Grosso and Jessica G. Davis

simulated well by DAYCENT, and daily N and C mineralization rates could further improve the performance of the single application simulations.

3.2 Introduction

As food demand increases, the productivity of farms will become more crucial (FAO, 2011; Grant *et al.*, 2016). Fertilizer use is a major factor in increasing farm productivity. However, excessive and improper application of nitrogen (N) fertilizer can release nitrous oxide (N₂O) to the environment (Toonsiri *et al.*, 2016) resulting in the increase of greenhouse gas (GHG) emissions to the atmosphere. In the United States, total GHG emissions were about 6870 million t CO₂ equivalent in 2014 (an increase of 1% from 2013), and GHG emissions derived from agriculture accounted for 8.3% of total GHG emissions, with agricultural activities such as fertilizer application being a primary source of N₂O emissions (USEPA, 2016).

Nitrogen fertilizer provides a N source for denitrification and nitrification in soil, and N supply can influence these two processes and enhance N₂O fluxes to the atmosphere (Amos *et al.*, 2005). With concern increasing regarding N₂O emissions from agriculture, organic fertilizer application has been promoted as an alternative option to minimize N₂O emissions because of lower N₂O emissions from organic relative to synthetic fertilizers (Aguilera *et al.*, 2013; Vallejo *et al.*, 2006). The organic food market and certified organic acreage have been growing in the United States (USDA-ERS, 2016), and these trends imply a concurrent increase in organic fertilizer utilization. Therefore, the effect of organic fertilizer forms and application methods on N₂O emissions in the present and future is important to quantify. Several studies have observed the effect of organic fertilizers on N₂O emissions in the field (Aguilera *et al.*, 2013). However, the ability of models to simulate N₂O emissions from organic fertilizers (with the exception of

livestock manure) has not been extensively investigated. Similarly, the ability of models such as DAYCENT to simulate plant/soil processes that contribute to GHG emissions has been well validated for commodity crops, but there has been little model evaluation for specialty crops. After models are properly validated, they can be applied to compare management options with less cost than field studies, and model results can be used to inform choices regarding organic fertilizers to achieve economic and environmental goals. The objectives of this study were (i) to simulate the effect of four organic fertilizers (feather meal, blood meal, fish emulsion, and cyano-fertilizer) with differing applications (single or multiple applications) on N₂O and CO₂ emissions, and (ii) to determine the performance of the DAYCENT simulation by comparing simulated data with measured data.

3.3 Materials and Methods

3.3.1 Field and experimental data

The experimental site was situated on certified organic land at the Colorado State University (CSU) Horticulture Field Research Center near Fort Collins, CO (40°36'39.78" N, 104°59'48.25" W, altitude 1540 m). The soil type is Nunn clay loam (fine, smectitic, mesic Aridic Argiustoll) (NRCS, 1980), and the study site has a semiarid steppe climate (Daly *et al.*, 2011).

The experimental data and N₂O and CO₂ emissions collected in 2014 by Toonsiri *et al.* (2016) were used to perform DAYCENT simulations in parameter modification and validation. Briefly, data were collected from a lettuce field receiving nine treatments. The nine treatments included four organic fertilizers (feather meal, blood meal, fish emulsion, and cyano-fertilizers) applied at different nitrogen (N) rates (28 and 56 kg N ha⁻¹) plus an unfertilized treatment.

Feather meal and blood meal fertilizers were applied by subsurface banding at the full rate (single application) before transplanting, while fish emulsion and cyano-fertilizers were applied five times (multiple applications) after transplanting.

Gas samples were collected using a closed static chamber method (Hutchinson and Mosier, 1981). Chambers were placed between lettuce plants, covering the fertilizer band, and the dripline for fertigation crossed over the chambers. Both the fertilizer band and the dripline emitters were placed directly in the middle of each chamber. Soil water content and soil temperature were measured by 5TM and 5EC-TM probes (Decagon Devices, Inc.) at 10 cm depth, and weather data were collected from CoAgMet at the Colorado State University Agricultural Research, Development and Education Center (ARDEC) which is located within 6 km of the site.

3.3.2 DAYCENT model description

DAYCENT (Daily CENTURY) is a daily time-step biogeochemical model based on the CENTURY model which was developed to simulate more realistic trace gas emissions (Del Grosso *et al.*, 2005; Parton *et al.*, 1998). DAYCENT is used to simulate flows of carbon (C), nutrients, and trace gases among the atmosphere, soil, and vegetation for terrestrial ecosystems such as crop, grassland, forest, and savanna ecosystems (Del Grosso *et al.*, 2005; Del Grosso *et al.*, 2011; Parton *et al.*, 1998). The DAYCENT simulation is conditional on management practices, location, and environment. Within DAYCENT, there are main submodels for plant productivity, decomposition of plant material and soil organic matter (SOM), soil water and temperature dynamics, nitrogen (N) gas fluxes, and methane (CH₄) oxidation (Del Grosso *et al.*, 2005; Del Grosso *et al.*, 2011). The DAYCENT inputs require site latitude, soil properties,

current and historical land use, soil management, disturbance events, vegetation cover, and daily weather data for the simulation process (Campbell, 2015; Del Grosso *et al.*, 2011). Nitrous oxide emissions from DAYCENT were simulated from nitrification and denitrification in the N gas flux submodel. Also, CO₂ emissions from DAYCENT were simulated from heterotrophic respiration in the decomposition submodel (Del Grosso *et al.*, 2011).

3.3.3 DAYCENT application

DAYCENT 2016 version was used for this study. The inputs to DAYCENT included five main data types: (i) daily weather data, (ii) site information, (iii) soil properties, (iv) vegetation, and (v) management practices for site characterization.

Twenty-two year (1993 to 2014) daily weather data from CoAgMet at ARDEC was used to create a daily weather file for this study. The weather data included maximum and minimum temperature (°C) and precipitation (cm) values. Mean actual evapotranspiration for calculating N input from atmospheric deposition and asymbiotic fixation was calculated from these weather values (Del Grosso *et al.*, 2011).

Latitude, 40.61, and longitude, -104.99, of the site was used in the model. This latitude, along with day of year, was used to calculate solar radiation in the plant growth submodel (Del Grosso *et al.*, 2011). The model also required soil properties for the processes of plant growth, water and nutrient flows, and decomposition (Del Grosso *et al.*, 2011). In this study, soil texture (fraction of sand: 0.46; silt: 0.23; clay: 0.31), bulk density (1.29 g cm⁻³), and pH (7.5) were input to the model to conduct the simulation.

DAYCENT was conducted to initialize SOC pools to equilibrium by growing native grass from year 1 to 1899 with moderate grazing and fire disturbance events. Then, a simulation

was implemented based on land use history to represent the field before the experiment began. Five types of land use were represented for the base simulation: (1) grass with low intensity grazing (1900-1949); (2) irrigated conventional corn (1950-1997); (3) cover crop and organic matter addition (1998-2001); (4) irrigated organic vegetables and organic matter addition (2002-2004); and (5) irrigated organic vegetable production (2005-2012). The experimental simulation started in 2013 with a summer irrigated cover crop grown until 2014 which was killed by plowing before lettuce was planted. Lettuce (*Lactuca sativa*) starters were transplanted into the field on day 160, 2014. Note that irrigation was summed with precipitation because the model does not distinguish water inputs from irrigation vs. precipitation (Zhang, 2012).

Organic fertilizer contained organic N and C and mineral N (Toonsiri *et al.*, 2016). Two sets of simulations were conducted for comparison. These simulations were 1) a simulation which obtained mineral N from mineralization of the added organic fertilizers, and 2) a simulation which obtained mineral N from the mineralization of the added organic fertilizers and extra N added in the mineral form. The daily mineral N addition was calculated based on the N mineralization rate of each organic fertilizer. Amount of N availability of feather meal is 64% of organic N at 28 days after application, blood meal is 60% of organic N at 14 days after application, fish emulsion is 16% of organic N at 28 days after fertilization, and cyano-fertilizer is 8% of organic N at 56 days after fertilization (Hartz and Johnstone, 2006; Sukor, 2013). For the simulation with low N rate, N rate addition of feather meal and blood meal were assumed to be $10 \text{ kg N ha}^{-1} \text{ day}^{-1}$, N rate addition of fish emulsion was assumed as $2.5 \text{ kg N ha}^{-1} \text{ day}^{-1}$, and $1 \text{ kg N ha}^{-1} \text{ day}^{-1}$ for cyano-fertilizer based on mineralization rates. At the high N rate, N rate addition was double that of the low N rate.

In this study, N₂O and CO₂ emissions measured in 2014 by Toonsiri *et al.* (2016) were used to calibrate and test the performance of DAYCENT. The measured N₂O and CO₂ emissions from four organic fertilizers at 28 kg N ha⁻¹ (low N rate) and unfertilized treatments were used for calibration, and the measured N₂O and CO₂ emissions from four organic fertilizers at 56 kg N ha⁻¹ (high N rate) treatments were used for validation. However, measured N₂O and CO₂ emissions could be overestimated because of the placement of chambers over fertilizer bands. Therefore, the N rates of 28 and 56 kg N ha⁻¹ were recalculated to 140 and 280 kg N ha⁻¹ based on the amount of fertilizer applied within the gas sampling chamber (excluding the unfertilized interrow area).

The base simulation was run, and model reliability was evaluated by comparing simulated N₂O and CO₂ emissions and measured values. The model was calibrated to better represent the N₂O and CO₂ emissions from organic lettuce fields receiving organic fertilizers by adjusting parameters in FILE100 to improve the model before conducting the validation. Then, the simulations of N₂O and CO₂ emissions from four organic fertilizers at high and low N rates treatments were performed, and the comparison of simulated and measured values were evaluated using the coefficient of determination (r²), root mean square error (RMSE), normalized RMSE (NRMSE), and model efficiency (ME) (Bista *et al.*, 2016; Campbell, 2015; Del Grosso *et al.*, 2005; Karhu *et al.*, 2012; Necpálová *et al.*, 2015) as follows:

$$r^2 = \left\{ \frac{\sum_{i=1}^n (Mi - \bar{M})(Si - \bar{S})}{\left[\sum_{i=1}^n (Mi - \bar{M})^2 \right]^{1/2} \left[\sum_{i=1}^n (Si - \bar{S})^2 \right]^{1/2}} \right\}^2$$

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^n (Mi - Si)^2}$$

$$\text{NRMSE} = \frac{\sqrt{\frac{1}{n} \sum_{i=1}^n (Mi - Si)^2} \times 100}{\bar{M}}$$

$$\text{ME} = \frac{[\sum_{i=1}^n (Mi - \bar{M})^2 - \sum_{i=1}^n (Si - Mi)^2]}{[\sum_{i=1}^n (Mi - \bar{M})^2]}$$

where Mi is measured value, Si is simulated value, \bar{M} is the mean of the measured data, \bar{S} is the mean of the simulated data, n is the number of measurements.

The coefficient of determination (r^2) was used to estimate the accuracy of the simulation compared to the measurement, RMSE was used to measure the mean error between values from simulation and measurement, NRMSE was used to express the RMSE as % of the measured mean (\bar{M}), and ME was used to evaluate the efficiency of the model simulation compared to the mean of measured data (Bista *et al.*, 2016; Karhu *et al.*, 2012). The simulated data describes the measured data better than the measured mean when the ME yields a positive value, while the simulated data describes the measured data more poorly than the measured mean when the ME yields a negative value, and the model performs well when the ME value is close to 1 (Bista *et al.*, 2016; Karhu *et al.*, 2012).

3.4 Results

Measured data from organic fertilizer treatments at the low N rate (28 kg N ha⁻¹ or 140 kg N ha⁻¹ after recalculation) in 2014 were used to calibrated the DAYCENT model, and measured data from the organic fertilizer treatments at the high N rate (56 kg N ha⁻¹ or 280 kg N ha⁻¹ after recalculation) in 2014 were used to validate the DAYCENT model. The simulations were conducted based on two different scenarios including with and without extra mineral N addition. Generally, the performance of these models was poor based on negative ME values. These

results showed that the model did not reasonably capture the variations with time. However, DAYCENT performance over the validation was similar to the performance over the calibration, and both scenarios resulted in similar performance for simulated N₂O and CO₂ emissions, soil water content and soil temperature at 10 cm depth, and yield.

3.4.1 N₂O emission

DAYCENT simulated lower N₂O emissions for blood meal and feather meal (single application) treatments and higher N₂O emissions for fish emulsion and cyano-fertilizer (multiple application) treatments compared to measured data in both scenarios (Table 3.1 and Fig. 3.1 and 3.2). Seasonal simulated N₂O emissions from single application treatments were higher than multiple application treatments in both scenarios, which showed the same trend as measured values. Seasonal simulated N₂O emissions for multiple application treatments from both scenarios were the same, while seasonal simulated N₂O emissions for single application treatments from the scenario with extra mineral N addition were about 58% higher than from the scenario without extra mineral N addition (Table 3.1). The model performance within each scenario showed that the model of single application treatments resulted in a good fit to the measured data, better than the model of multiple application treatments according to higher r² and lower NRMSE (Table 3.1). Considering both scenarios, the model performances of multiple application treatments were the same, but the model performances of single application treatments were not. The model performances of single application treatments from a scenario with extra mineral N addition fit the measured data better than from a scenario without extra mineral N addition based on lower RMSE and NRMSE (Table 3.1).

3.4.2 CO₂ emission

DAYCENT simulated higher CO₂ emissions than measured data in both scenarios (Table 3.1 and Fig. 3.3). Seasonal simulated CO₂ emissions from single application treatments were higher than from multiple application treatments in both scenarios, the same trend as seen in the measured values. Seasonal simulated CO₂ emissions for multiple application treatments from both scenarios were the same, while seasonal simulated CO₂ emissions for single application treatments from the simulation without extra mineral N addition were slightly higher than those from the simulation with extra mineral N addition (Table 3.1 and Fig. 3.3). Model performance within each scenario showed that the model of single application treatments had a better fit to the measured data than the model of multiple application treatments according to higher r^2 and lower NRMSE (Table 3.1). Between scenarios, the model performance for multiple application treatments was the same, whereas the model performance of single application treatments was slightly different. The model performance for single application treatments in the scenario with extra mineral N addition fit the measured data better than the scenario without extra mineral N addition based on lower RMSE and NRMSE (Table 3.1).

3.4.3 Soil water content and soil temperature

Measured soil water content, 31%, and measured soil temperature, 19 °C, values of each treatment were the same value because we use soil water content and soil temperature values based on averaged values for each measurement day. The probes for measuring the soil water content and soil temperature were installed randomly around the experimental site. For each measurement day, the data from all probes were averaged to represent daily soil water content and soil temperature for the experimental site. Due to probe installation at 10 cm soil depth,

DAYCENT simulated daily soil water content and soil temperature values in soil layer 3 (5-10 cm depth) and soil layer 4 (10-20 cm depth) for consideration.

DAYCENT simulated daily soil water content in both soil layers for both scenarios were lower compared to measured values and did not correlate to measured values (Table 3.2 and Fig. 3.4). Simulated daily soil water content from multiple application treatments were generally greater than simulated daily soil water content from single application (feather meal and blood meal) treatments in both scenarios, and the model for multiple application treatments resulted in a better fit than the model for single application treatments based on lower RMSE and NRMSE (Table 3.2).

DAYCENT simulated daily soil temperature for both scenarios in layer 3 (5-10 cm soil depth) were higher, while in layer 4 (10-20 cm soil depth) they were lower, compared to measured values. However, there were some treatments with significant correlation to measured values (Table 3.2). Simulated daily soil temperature values from the single application treatments varied according to fertilizer type and scenario, but simulated daily soil temperature values from the multiple application treatments were the same (Table 3.2). In layer 3 (5-10 cm depth), simulated daily soil temperature values from the feather meal treatment with extra mineral N addition and multiple application treatments in both scenarios showed significant correlation to the measured values. In layer 4 (10-20 cm depth), simulated daily soil temperature values from the feather meal treatment in both scenarios showed significant correlation to the measured values. However, the model for the feather meal treatment in layer 3 from the scenario of extra mineral N addition and in layer 4 from both scenarios resulted in a better fit than the other fertilizer treatments under the same scenario according to lower RMSE and NRMSE (Table 3.2).

3.4.4 Yield

Simulated yields from all treatments and all scenarios were 1 to 190% higher than measured yields (Table 3.1). DAYCENT simulated substantially higher yields for the single application (feather meal and blood meal) treatments, whereas DAYCENT simulated only slightly higher yields for the multiple application (fish emulsion and cyano-fertilizer) treatments relative to measured yields. Simulated yields from single application treatments were twice as high as simulated yields from multiple application treatments, although measured yields were not significantly different (Sukor, 2016). Yield of single application treatments from the simulation with extra mineral N addition was about 30% more than from the scenario without extra mineral N addition, while simulated yield of multiple application treatments from both scenarios were similar (Table 3.1).

3.5 Discussion

3.5.1 N₂O emission

Comparisons of DAYCENT simulation with data from irrigated organic lettuce fields showed that the variation in simulated N₂O emissions for single application treatments (blood meal and feather meal) were associated with the variation in measured N₂O emissions ($p < 0.05$), although DAYCENT underestimated the N₂O emissions for single application treatments (Fig. 3.1 and 3.2). The DAYCENT simulation without extra mineral N addition explained 38% and 46% of the variation in measured N₂O emissions from blood meal and feather meal treatments, respectively. The DAYCENT simulation with extra mineral N addition explained 48% of the variation in measured N₂O emissions from blood meal, while the variation in measured N₂O emissions from feather meal was not significantly predicted by the simulation. In the simulation,

the organic fertilizer delivered mineral N to the system through mineralization. The simulation without extra mineral N addition failed to simulate the peak of N₂O emissions for single application treatments, while the simulation with extra mineral N addition did predict the peak in N₂O emissions. Results indicate that mineral N from organic fertilizer mineralization was inadequate to simulate the peak after fertilization; therefore, the extra mineral N was added based on mineralization rate (Hartz and Johnstone, 2006; Sukor, 2013) to simulate the peak after fertilization. However, the magnitude of the peak in the scenario with extra mineral N addition was lower than measured data (Fig. 3.1). There was no difference between scenarios in the simulation of multiple application treatments (fish emulsion and cyano-fertilizer) because the extra mineral N was added into the simulation after the growing season based on the mineralization rate (Hartz and Johnstone, 2006; Sukor, 2013). However, the model overestimated N₂O emissions, even if the extra mineral N was not added during the growing season in the simulation (Fig. 3.1 and 3.2). The less predictive simulation of multiple application treatments may be due to N₂O overestimation and excessive N mineralization rate for fish emulsion and cyano-fertilizer (Table 3.1 and Fig. 3.1 and 3.2).

Comparing cumulative N₂O emissions from different simulations with actual measurements (Fig. 3.2), underestimation was found in single application treatments, while overestimation was found in multiple application treatments. As compared to cumulative N₂O emissions from the IPCC default of 1.0% (IPCC, 2006; Millar *et al.*, 2010), cumulative N₂O emissions from all single application treatments were lower than simulated emissions, except the blood meal treatment with extra mineral N addition. In the blood meal treatment, the DAYCENT simulation with extra mineral N addition simulated cumulative N₂O emissions well as compared to measurements; however, the cumulative N₂O emissions from the DAYCENT simulation with

extra mineral N addition were slightly higher than the cumulative N₂O emissions from the IPCC default of 1.0%. High measured cumulative N₂O emissions from single application treatments may be due to the chamber placement over fertilizer bands. In multiple application treatments, DAYCENT simulated cumulative N₂O emissions better than the IPCC default of 1.0%. However, these measured cumulative N₂O emissions were close to the result from the DAYCENT simulation for the unfertilized treatment (Fig. 3.2). This may imply that N availability in the DAYCENT simulation or the N mineralization rate of fish emulsion and cyano-fertilizer were overestimated.

Nitrogen availability is an essential input to N₂O production (Guzman *et al.*, 2015). Therefore, knowing the daily N availability after fertilizer application may help to adjust the mineralization rate of organic fertilizers or adjust the amount of daily available N in the model. This may improve the ability of the model to simulate the magnitude and pattern of N₂O emissions.

3.5.2 CO₂ emission

Comparisons of DAYCENT simulation with measured data from irrigated organic lettuce fields showed that the variation in simulated CO₂ emissions for single application (blood meal and feather meal) treatments were associated with the variation in measured CO₂ emissions, although DAYCENT overestimated CO₂ emissions (Fig. 3.2 and 3.3). The DAYCENT simulation with and without extra mineral N addition explained about 22% and 65% of the variation in measured CO₂ emissions from blood meal and feather meal treatments, respectively. The simulated CO₂ emissions from both scenarios yielded almost the same values. This implied that extra mineral N addition had very little influence on the CO₂ production. Carbon dioxide

emissions from soil are related to soil respiration by plant roots and soil microorganisms (Al-Kaisi *et al.*, 2008; Sainju *et al.*, 2008). However, CO₂ emissions from DAYCENT were a result of microbial respiration or decomposition (Del Grosso *et al.*, 2011; Metherell *et al.*, 1994). Cumulative simulated CO₂ emissions from a scenario without extra mineral N additions were reduced by less than 1% for single application treatments when extra mineral N was included in the simulation. This relates to the study of Al-Kaisi *et al.* (2008) that showed that greater N application reduced CO₂ emissions.

The potential decomposition rate from soil depends on plant growth, C substrate, and environmental factors such as soil water and temperature that control microbial activity (Al-Kaisi *et al.*, 2008; Guzman *et al.*, 2015; Metherell *et al.*, 1994). Peak CO₂ emission was observed at the beginning of the season. This may be influenced by the high C substrate after single application fertilizer treatments or the high C substrate from cover crop management in multiple application treatments coincident with the high precipitation on that day. Soil CO₂ emissions are sensitive to rapid changes in soil water content following a dry period (Ryals *et al.*, 2015) resulting in large CO₂ emissions (Fierer and Schimel, 2002). The magnitude of the peak for single application treatments was greater than for multiple application treatments because the C substrate in single application treatments was derived from organic fertilizer and organic residue, while C substrate in multiple application treatments was derived from organic residue only.

Decomposition releases CO₂ to the atmosphere (Metherell *et al.*, 1994); therefore, knowing the daily decomposition rate in soil after applying organic fertilizer may help to adjust the mineralization rate of organic fertilizers or adjust the amount of daily C substrate in the model. This may improve the model's ability to simulate CO₂ emissions.

3.5.3 Soil water content and soil temperature

Simulated soil water content changes were not associated with changes in measured soil water (Table 3.2 and Fig. 3.4). DAYCENT slightly underestimated soil water content, approximately 30%, relative to measured values of 31% (Table 3.2 and Fig. 3.4). There were slight differences between scenarios. However, simulated soil water contents did not correlate with measured soil water contents (Table 3.2). Proper simulation of soil water content was found in previous DAYCENT applications (Del Grosso *et al.*, 2001) such as in irrigated corn (Del Grosso *et al.*, 2008a), irrigated turfgrass (Zhang, 2012), and a shortgrass steppe (Kelly *et al.*, 2000). However, poorer simulation was observed in this study for soil water content in an irrigated organic lettuce field (Table 3.1). Since soil water content affects N₂O emission rates, unreliable simulation of soil water content may contribute to errors in simulation of N₂O emission (Del Grosso *et al.*, 2008b; Del Grosso *et al.*, 2008c).

Variation in simulated soil temperature in soil layer 4 (10-20 cm depth) in both scenarios was associated with the variation in measured soil temperature at 10 cm depth only in the feather meal treatment ($r^2 = 0.14$ and $p < 0.05$), whereas in soil layer 3 (5-10 cm depth), variation in simulated soil temperature from the feather meal treatment with extra mineral N addition and multiple application treatments in both scenarios were associated with the variation in measured soil temperature at 10 cm depth (Table 3.2). Therefore, the simulated soil temperature in soil layer 3 (5-10 cm depth) was more reliable than the simulated soil temperature in soil layer 4 (10-20 cm depth). The variation in measured soil temperature at 10 cm depth also was associated with the variation in air temperature ($r^2 = 0.45$ and $p < 0.05$), similar to the findings reported by Zhang (2012). Simulated average daily soil temperature in soil layer 3 (5-10 cm depth), 23 °C, was overestimated relative to average measured daily soil temperature of 19 °C (Table 3.2 and

Fig. 3.5). However, the simulation of soil temperature in soil layer 3 (5-10 cm depth) was poor, with low r^2 and unsatisfied ME (Table 3.2 and Fig. 3.5). The model performance was slightly different in both scenarios under all treatments. Previous studies suggest that modifying the damping factor coefficient for calculating soil temperature can improve the simulation (Necpálová *et al.*, 2015; Zhang, 2012).

3.5.4 Yield

Nitrogen is an essential nutrient for plants, including lettuce, typically resulting in increased yield with increased N application, but excessive N application can have a negative impact on lettuce quality (Hoque *et al.*, 2010; Ouzounidou *et al.*, 2013). In DAYCENT, plant productivity is controlled by soil moisture, temperature, nutrients, shading and genetic potential (Metherell *et al.*, 1994). Therefore, simulated yield will increase with increased N levels, but the maximum simulated yield will be limited by the genetic potential. In our study, simulated yield with extra mineral N addition for single application treatments was higher than simulated yield without extra mineral N addition. Simulated yield from both scenarios for multiple application treatments were similar because extra mineral N was added after harvest based on the mineralization rates determined by Sukor (2013). In each scenario, yield depended on fertilizer type. The highest simulated yield was from the blood meal treatment. This may be due to higher N availability during the growing season as compared to other treatments. Conversely, cyano-fertilizer provided lower N availability than fish emulsion (Sukor, 2013); therefore, the yield from cyano-fertilizer treatment should be lower than from the fish emulsion treatment (if N is limiting), but the simulation showed higher N availability and yield for the cyano-fertilizer treatment relative to the fish emulsion treatment. Therefore, known daily N availability of the

experimental site may improve the model's ability to simulate yield. Also, DAYCENT tended to overestimate yields in all treatments, possibly since the model did not consider the effect of disease or environmental damage. In our field study, the lettuce was damaged by a hailstorm on 22 June 2014, resulting in a setback and longer growing period.

Overall, the DAYCENT simulation for an irrigated organic lettuce field showed that both N scenarios yielded similar performance. The DAYCENT simulation predicted N₂O and CO₂ emissions better from single application treatments than multiple application treatments under both scenarios. Varying the amount of N availability in the simulations did not appreciably affect CO₂ emissions, soil water content or soil temperature (Table 3.1 and 3.2). However, a scenario without extra mineral N addition better simulated N₂O emissions for the feather meal treatment than for other fertilizer treatments. The scenario without extra mineral N addition overestimated yields less for single application treatments (feather meal and blood meal) than in the scenario with extra mineral N addition. Additionally, a scenario without extra mineral N addition failed to simulate the spike in N₂O emissions from single application treatments immediately after applying fertilizers. A scenario with extra mineral N addition simulated the spike in N₂O emissions from single application treatments better, but the excessive mineral N resulted in excessively high simulated yields. Therefore, to avoid over addition of N to the system and to simulate the spike in N₂O emissions after fertilizer application, the daily N availability of the site should be determined and used to adjust the mineralization rate of organic fertilizer or the amount of N availability in the system. This may improve the performance of DAYCENT in organic vegetable farming systems.

3.6 Conclusion

The DAYCENT model with and without extra mineral N addition has a reasonable potential to simulate the effect of single application organic fertilizer treatments (feather meal and blood meal) on N₂O and CO₂ emissions, whereas DAYCENT is considered to have low potential to simulate the effect of multiple application organic fertilizer treatments (fish emulsion and cyano-fertilizer) on N₂O and CO₂ emissions. Cumulative simulated N₂O and CO₂ emissions of all treatments were higher than measured emissions except cumulative simulated N₂O emissions from single application treatments. We suggest that the daily N availability and daily decomposition rate of organic fertilizer in a site should be determined and used to calibrate the model to improve the performance of DAYCENT in simulating the effect of organic fertilizer applications on N₂O and CO₂ emissions.

TABLES

Table 3.1. DAYCENT validation results of N₂O and CO₂ emissions and yield for organic fertilizer treatments at high N rate (56 kg N ha⁻¹ or 280 kg N ha⁻¹ after recalculation) including feather meal (FM), blood meal (BM), fish emulsion (FE), cyano-fertilizer (CF) with different scenarios, and validation performance with coefficient of determination (r²), root mean square error (RMSE), normalized RMSE (NRMSE), and model efficiency (ME).

Parameter	Without extra mineral N addition				With extra mineral N addition			
	BM	FM	FE	CF	BM	FM	FE	CF
<i>N₂O emission (kg N ha⁻¹)</i>								
Seasonal measured data	6.53	4.57	0.37	0.19	6.53	4.57	0.37	0.19
Seasonal simulated data	1.79	1.71	1.09	1.12	2.84	2.67	1.09	1.12
r ²	0.38 [‡]	0.46 [‡]	0.004	0.0004	0.48 [‡]	0.11	0.004	0.0004
RMSE	116.02	78.85	17.49	20.26	94.20	69.80	17.49	20.26
NRMSE	102	100	287	657	83	89	287	657
ME	-0.99	-0.52	-18.88	-135.93	-0.31	-0.19	-18.88	-135.93
<i>CO₂ emission (kg C ha⁻¹)</i>								
Measured data	1299.48	1473.81	836.76	525.64	1299.48	1473.81	836.76	525.64
Simulated data	2241.00	2232.78	1423.79	1391.58	2215.77	2223.30	1423.79	1391.58
r ²	0.21 [‡]	0.65 [‡]	0.01	0.002	0.22 [‡]	0.65 [‡]	0.01	0.002
RMSE	13767.45	9328.74	10730.56	13512.00	13494.91	9242.88	10730.56	13512.00
NRMSE	67	40	84	171	66	40	84	171
ME	-1.73	0.10	-2.20	-8.16	-1.62	0.12	-2.20	-8.16
<i>Yield (g C m⁻²)</i>								
Measured data (Sukor, 2016)	61.39	66.21	43.07	56.57	61.39	66.21	43.07	56.57
Simulated data	126.89	122.41	56.64	57.07	175.50	157.02	56.64	57.07

[‡]r² is significant at the 0.05 probability level.

Table 3.2. DAYCENT validation results of soil water content and soil temperature for organic fertilizer treatments at high N rate (56 kg N ha⁻¹ or 280 kg N ha⁻¹ after recalculation) including feather meal (FM), blood meal (BM), fish emulsion (FE), cyano-fertilizer (CF) with different scenarios, and validation performance with coefficient of determination (r²), root mean square error (RMSE), normalized RMSE (NRMSE), and model efficiency (ME).

Parameter	Without extra mineral N addition				With extra mineral N addition			
	BM	FM	FE	CF	BM	FM	FE	CF
<i>Soil water content at 5-10 cm depth (%)</i>								
Average daily measured data	31	31	31	31	31	31	31	31
Average daily simulated data	29	29	29	29	29	29	29	29
r ²	0.02	0.03	0.04	0.04	0.02	0.02	0.04	0.04
RMSE	5.67	5.49	4.42	4.42	5.26	5.21	4.40	4.42
NRMSE	17	17	14	14	18	18	14	14
ME	-3.17	-3.09	-1.93	-1.94	-3.85	-3.54	-1.94	-1.94
<i>Soil water content at 10-20 cm depth (%)</i>								
Average daily measured data	31	31	31	31	31	31	31	31
Average daily simulated data	29	30	30	30	29	29	30	30
r ²	0.09	0.10	0.11	0.11	0.09	0.10	0.11	0.11
RMSE	3.70	3.60	3.40	3.40	3.60	3.59	3.40	3.40
NRMSE	12	12	11	11	12	12	11	11
ME	-0.95	-0.94	-0.74	-0.74	-1.06	-0.96	-0.74	-0.74
<i>Soil temperature at 5-10 cm depth (°C)</i>								
Average daily measured data	18.88	18.88	18.885	18.88	18.88	18.88	18.88	18.88
Average daily simulated data	23.33	23.43	23.41	23.41	22.59	23.17	23.41	23.41
r ²	0.14	0.15 [†]	0.24 [†]	0.24 [†]	0.07	0.12	0.24 [†]	0.24 [†]
RMSE	5.09	5.19	5.47	5.47	4.43	4.93	5.47	5.47
NRMSE	27	27	29	29	23	26	29	29
ME	-6.73	-7.02	-7.94	-7.93	-4.85	-6.23	-7.94	-7.93
<i>Soil temperature at 10-20 cm depth (°C)</i>								
Average daily measured data	18.88	18.88	18.88	18.88	18.88	18.88	18.88	18.88
Average daily simulated data	17.20	17.25	17.04	17.04	16.78	17.13	17.04	17.04
r ²	0.14	0.14 [†]	0.12	0.12	0.12	0.14 [†]	0.12	0.12
RMSE	2.70	2.69	3.02	3.02	2.84	2.70	3.02	3.02
NRMSE	14	14	16	16	15	14	16	16
ME	-1.17	-1.15	-1.72	-1.72	-1.41	-1.17	-1.72	-1.72

[†]r² is significant at the 0.05 probability level.

FIGURES

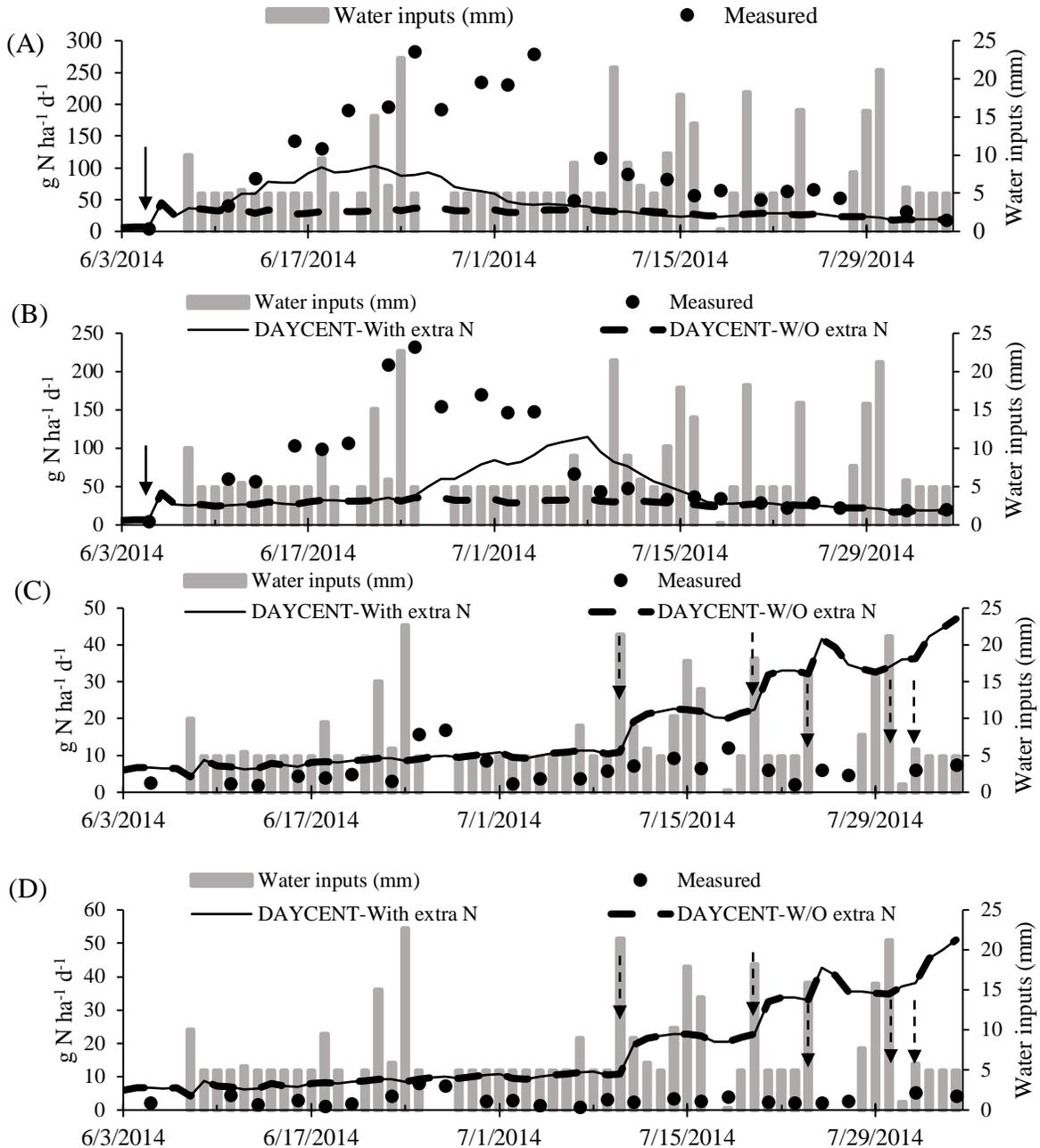


Fig. 3.1. Daily N_2O emissions from measurement and DAYCENT simulation with and without extra mineral N addition during the 2014 growing season from organic lettuce plots applied (A) blood meal, (B) feather meal, (C) fish emulsion, and (D) cyano-fertilizer at 56 kg N ha^{-1} or 280 kg N ha^{-1} after recalculating N rate. Solid arrows indicate timing of solid fertilizer application (blood meal and feather meal) and dashed arrows indicate timing of liquid fertilizer application (fish emulsion and cyano-fertilizer).

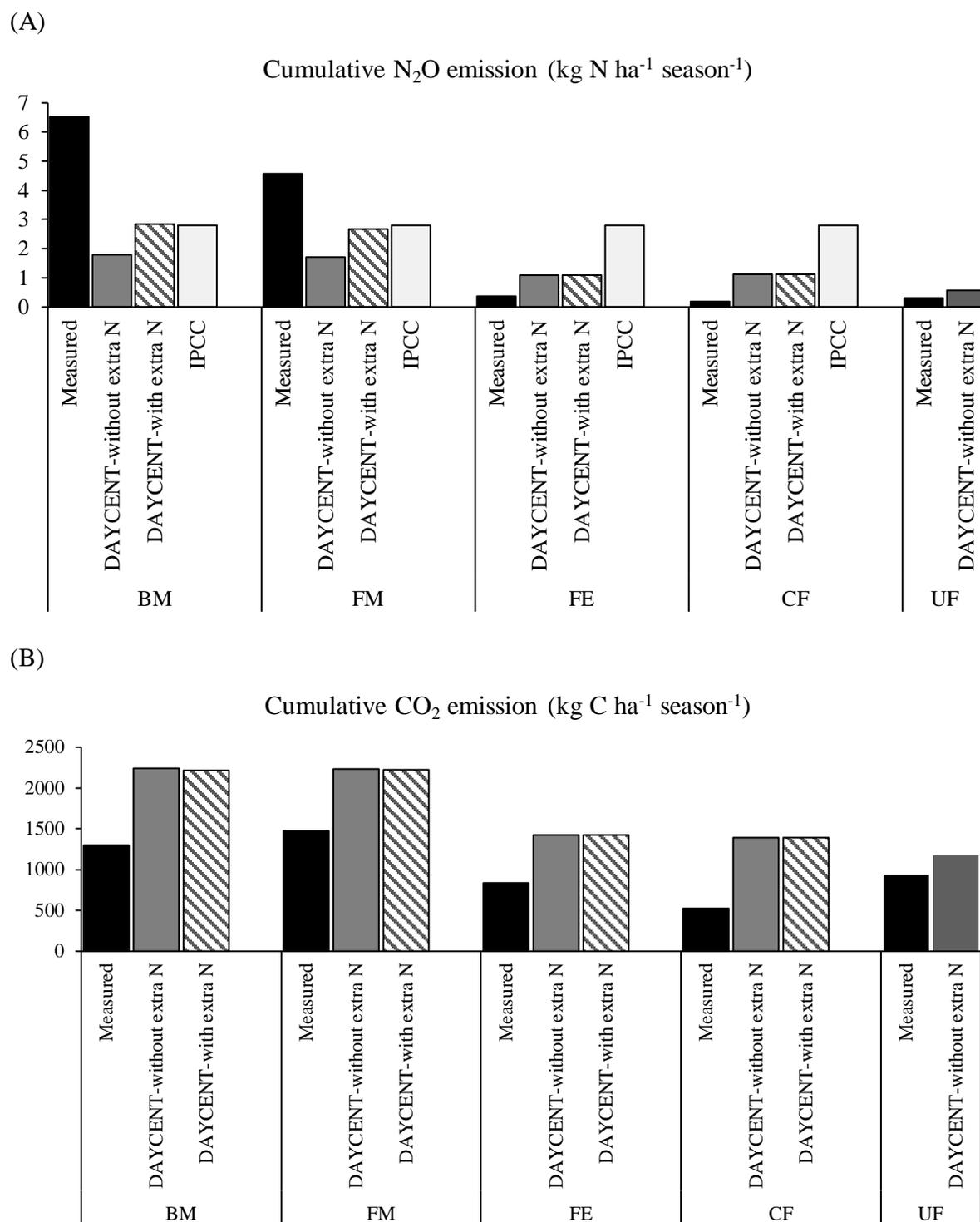


Fig. 3.2. Cumulative gaseous emissions from measurement, DAYCENT simulation with and without extra mineral N addition, and the IPCC default of 1.0%. (A) cumulative N₂O emission and (B) cumulative CO₂ emission during the 2014 growing season from organic lettuce plots applied blood meal (BM), feather meal (FM), fish emulsion (FE), and cyano-fertilizer (CF) at 56 kg N ha⁻¹ or 280 kg N ha⁻¹ after recalculating N rate and an unfertilized treatment (UF).

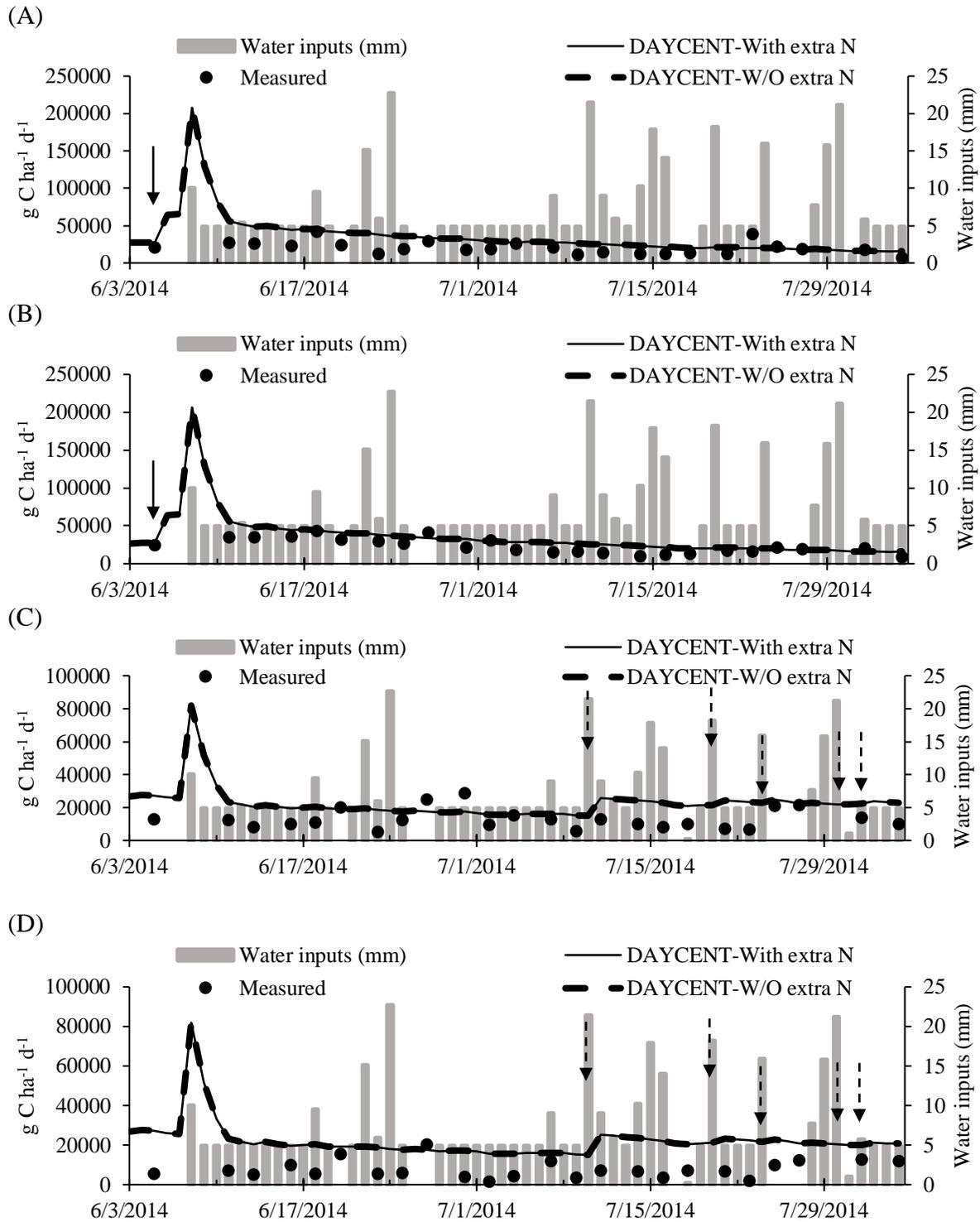


Fig. 3.3. Daily CO₂ emissions from measurement and DAYCENT simulation with and without extra mineral N addition during the 2014 growing season from organic lettuce plots applied (A) blood meal, (B) feather meal, (C) fish emulsion, and (D) cyano-fertilizer at 56 kg N ha⁻¹ or 280 kg N ha⁻¹ after recalculating N rate. Solid arrows indicate timing of solid fertilizer application (blood

meal and feather meal) and dashed arrows indicate timing of liquid fertilizer application (fish emulsion and cyano-fertilizer).

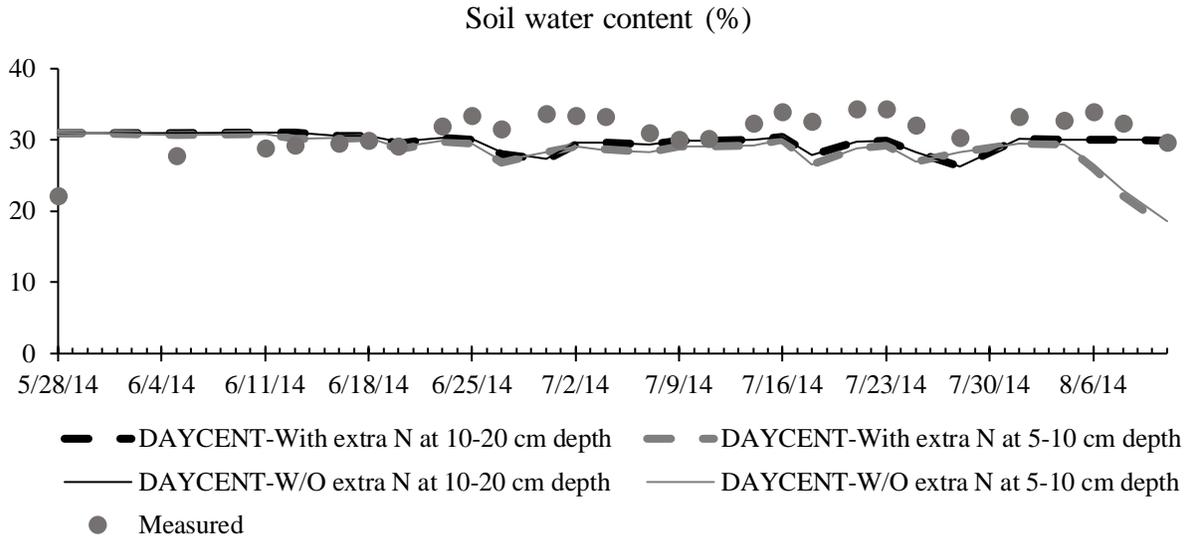


Fig. 3.4. Daily soil water content during the 2014 growing season from measurement and averaged DAYCENT simulation with and without extra mineral N addition at 5-10 cm depth ($r^2 = 0.03$, $p > 0.05$; $r^2 = 0.03$, $p > 0.05$, respectively) and at 10-20 cm depth ($r^2 = 0.10$, $p > 0.05$; $r^2 = 0.10$, $p > 0.05$, respectively).

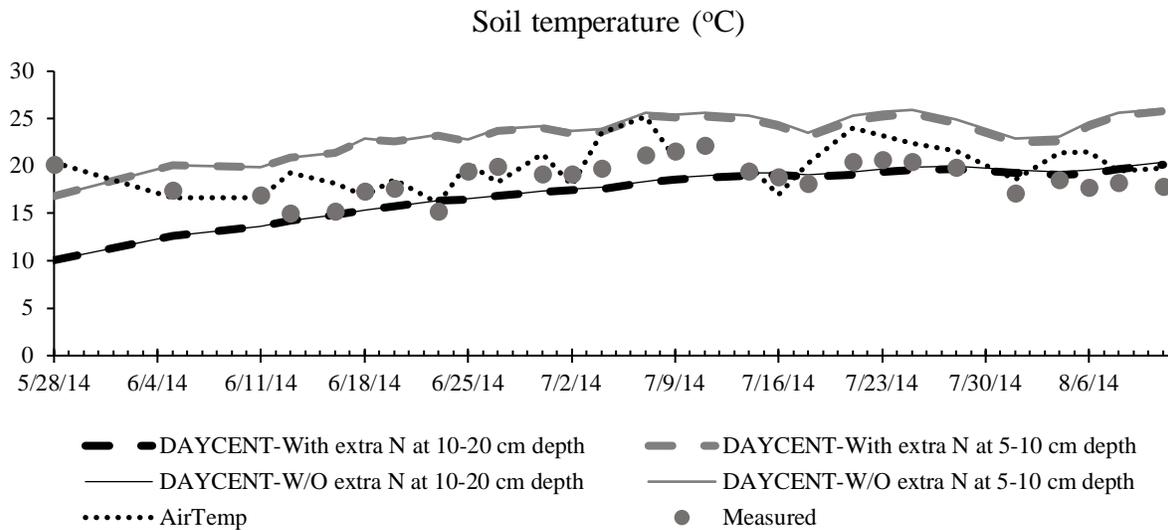


Fig. 3.5. Daily soil temperature during the 2014 growing season from measurement, from averaged DAYCENT simulation with and without extra mineral N addition at 5-10 cm depth ($r^2 = 0.19$, $p < 0.05$; $r^2 = 0.21$, $p < 0.05$, respectively) and at 10-20 cm depth ($r^2 = 0.13$, $p > 0.05$; $r^2 = 0.13$, $p > 0.05$, respectively), and from air temperature ($r^2 = 0.45$, $p < 0.05$).

REFERENCES

- Aguilera, E., L. Lassaletta, A. Sanz-Cobena, J. Garnier, and A. Vallejo. 2013. The potential of organic fertilizers and water management to reduce N₂O emissions in Mediterranean climate cropping systems: a review. *Agriculture, Ecosystems & Environment* 164: 32-52. doi:10.1016/j.agee.2012.09.006
- Al-Kaisi, M.M., M.L. Kruse, and J.E. Sawyer. 2008. Effect of nitrogen fertilizer application on growing season soil carbon dioxide emission in a corn–soybean rotation. *Journal of environmental quality* 37: 325-332. doi:10.2134/jeq2007.0240
- Amos, B., T.J. Arkebauer, and J.W. Doran. 2005. Soil surface fluxes of greenhouse gases in an irrigated maize-based agroecosystem. *Soil Science Society of America Journal* 69: 387-395. doi:10.2136/sssaj2005.0387
- Bista, P., S. Machado, R. Ghimire, S. Del Grosso, and M. Reyes Fox. 2016. Simulating soil organic carbon in a wheat–fallow system using the DAYCENT model. *Agronomy Journal* 108: 2554. doi:10.2134/agronj2016.04.0202
- Campbell, E.E. 2015. Modeling soil organic matter: theory, development, and applications in bioenergy cropping systems. Colorado State Univ., Fort Collins.
- Daly, C., G.H. Taylor, D.B. Hannaway, and H.A. Fribourg. 2011. Climate and agro-ecological zones. In: H.A.F. David B. Hannaway, editor Country pasture/forage resource profile for the United States of America. AGP, FAO, Rome.
- Del Grosso, S.J., A.D. Halvorson, and W.J. Parton. 2008a. Testing DAYCENT Model Simulations of Corn Yields and Nitrous Oxide Emissions in Irrigated Tillage Systems in Colorado All rights reserved. No part of this periodical may be reproduced or transmitted

in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. *Journal of Environmental Quality* 37: 1383-1389.

doi:10.2134/jeq2007.0292

Del Grosso, S.J., A.R. Mosier, W.J. Parton, and D.S. Ojima. 2005. DAYCENT model analysis of past and contemporary soil NO and net greenhouse gas flux for major crops in the USA.

Soil & tillage research 83: 9-24. doi:10.1016/j.still.2005.02.007

Del Grosso, S.J., W.J. Parton, C.A. Keough, and M. Reyes Fox. 2011. Special features of the DayCent modeling package and additional procedures for parameterization, calibration, validation, and applications. In: L. Ahuja et al., editors, *Methods of introducing system models into agricultural research*. ASA, CSSA, SSSA, Madison, WI, USA. p. 155-176.

Del Grosso, S.J., W.J. Parton, A.R. Mosier, M.D. Hartman, J. Brenner, D.S. Ojima, and D.S. Schimel. 2001. Simulated interaction of carbon dynamics and nitrogen trace gas fluxes using the DAYCENT model. *Modeling carbon and nitrogen dynamics for soil management*: 303-332.

Del Grosso, S.J., W.J. Parton, D.S. Ojima, C.A. Keough, T.H. Riley, and A.R. Mosier. 2008b. DAYCENT simulated effects of land use and climate on county level N loss vectors in the USA. *Publications from USDA-ARS/UNL Faculty*: 571-594.

Del Grosso, S.J., W.J. Parton, D.S. Ojima, C.A. Keough, T.H. Riley, and A.R. Mosier. 2008c. DAYCENT simulated effects of land use and climate on county level N loss vectors in the USA. *Publications from USDA-ARS/UNL Faculty*: 571-595.

FAO. 2011. *Organic agriculture and climate change mitigation: a report of the round table on organic agriculture and climate change*. FAO, Rome, Italy

- Fierer, N., and J.P. Schimel. 2002. Effects of drying–rewetting frequency on soil carbon and nitrogen transformations. *Soil Biology and Biochemistry* 34: 777-787.
doi:10.1016/S0038-0717(02)00007-X
- Grant, B.B., W.N. Smith, C.A. Campbell, R.L. Desjardins, R.L. Lemke, R. Kröbel, B.G. McConkey, E.G. Smith, and G.P. Lafond. 2016. Comparison of DayCent and DNDC models: case studies using data from long-term experiments on the Canadian prairies. *Synthesis and Modeling of Greenhouse Gas Emissions and Carbon Storage in Agricultural and Forest Systems to Guide Mitigation and Adaptation*: 21-58.
doi:10.2134/advagricysystem6.2013.0035
- Guzman, J., M. Al-Kaisi, and T. Parkin. 2015. Greenhouse gas emissions dynamics as influenced by corn residue removal in continuous corn system. *Soil Science Society of America Journal* 79: 612-625. doi:10.2136/sssaj2014.07.0298
- Hartz, T., and P. Johnstone. 2006. Nitrogen availability from high-nitrogen-containing organic fertilizers. *HortTechnology* 16: 39-42.
- Hoque, M.M., H. Ajwa, M. Othman, R. Smith, and M. Cahn. 2010. Yield and postharvest quality of lettuce in response to nitrogen, phosphorus, and potassium fertilizers. *HortScience* 45: 1539-1544.
- Hutchinson, G., and A. Mosier. 1981. Improved soil cover method for field measurement of nitrous oxide fluxes. *Soil Science Society of America Journal* 45: 311-316.
doi:10.2136/sssaj1981.03615995004500020017x
- IPCC. 2006. Vol. 4: Agriculture, forestry and other land use. In: S. Eggleston et al., editors, 2006 IPCC guidelines for national greenhouse gas inventories. IPCC, Geneva, Switzerland. p. 11.11-11.54.

- Karhu, K., A.I. Gärdenäs, J. Heikkinen, P. Vanhala, M. Tuomi, and J. Liski. 2012. Impacts of organic amendments on carbon stocks of an agricultural soil — Comparison of model-simulations to measurements. *Geoderma* 189–190: 606-616.
doi:10.1016/j.geoderma.2012.06.007
- Kelly, R.H., W.J. Parton, M.D. Hartman, L.K. Stretch, D.S. Ojima, and D.S. Schimel. 2000. Intra-annual and interannual variability of ecosystem processes in shortgrass steppe. *Journal of Geophysical Research: Oceans* 105: 20093-20100. doi:10.1029/2000JD900259
- Metherell, A., L. Harding, C. Cole, and W. Parton. 1994. CENTURY soil organic matter model environment, technical documentation, agroecosystem version 4.0 GPSR Technical Report No. 4. Great Plains System Research Unit, USDA-ARS, Fort Collins, CO.
- Millar, N., G.P. Robertson, P.R. Grace, R.J. Gehl, and J.P. Hoben. 2010. Nitrogen fertilizer management for nitrous oxide (N₂O) mitigation in intensive corn (Maize) production: an emissions reduction protocol for US Midwest agriculture. *Mitigation and Adaptation Strategies for Global Change* 15: 185-204. doi:10.1007/s11027-010-9212-7
- Necpálová, M., R.P. Anex, M.N. Fienen, S.J. Del Grosso, M.J. Castellano, J.E. Sawyer, J. Iqbal, J.L. Pantoja, and D.W. Barker. 2015. Understanding the DayCent model: calibration, sensitivity, and identifiability through inverse modeling. *Environmental Modelling & Software* 66: 110-130. doi:10.1016/j.envsoft.2014.12.011
- NRCS. 1980. Soil survey of Larimer County area, Colorado.
http://www.nrcs.usda.gov/Internet/FSE_MANUSCRIPTS/colorado/larimerCO1980/Larimer.pdf (accessed 6 May 2015).

- Ouzounidou, G., C. Paschalidis, D. Petropoulos, A. Koriki, P. Zamanidis, and A. Petridis. 2013. Interaction of soil moisture and excess of boron and nitrogen on lettuce growth and quality. *Horticultural Science, Slezská, Czech Republic* 40: 119-125.
- Parton, W.J., M. Hartman, D. Ojima, and D. Schimel. 1998. DAYCENT and its land surface submodel: description and testing. *Global and Planetary Change* 19: 35-48.
- Ryals, R., M.D. Hartman, W.J. Parton, M.S. DeLonge, and W.L. Silver. 2015. Long-term climate change mitigation potential with organic matter management on grasslands. *Ecological Applications* 25: 531-545.
- Sainju, U.M., J.D. Jabro, and W.B. Stevens. 2008. Soil carbon dioxide emission and carbon content as affected by irrigation, tillage, cropping system, and nitrogen fertilization. *Journal of Environmental Quality* 37: 98-106. doi:10.2134/jeq2006.0392
- Sukor, A. 2013. Effects of cyanobacterial fertilizers compared to commonly-used organic fertilizers on nitrogen availability, lettuce growth, and nitrogen use efficiency on different soil textures. Thesis, Colorado State Univ., Fort Collins.
- Sukor, A. 2016. Organic nitrogen fertilizers influence nutritional value, water use efficiency, and nitrogen dynamics of drip irrigated lettuce and sweet corn. Ph.D. dissertation, Colorado State Univ., Fort Collins.
- Toonsiri, P., S. Del Grosso, A. Sukor, and J. Davis. 2016. Greenhouse gas emissions from solid and liquid organic fertilizers applied to lettuce. *Journal of Environmental Quality* 45: 1812-1821. doi:10.2134/jeq2015.12.0623
- USDA-ERS. 2016. Organic market overview. USDA, Washington, DC.
<https://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture/organic-market-overview.aspx> (accessed 22 Jan 2017).

USEPA. 2016. Inventory of U.S. greenhouse gas emissions and sinks: 1990-2014.

<https://www.epa.gov/sites/production/files/2016-04/documents/us-ghg-inventory-2016-main-text.pdf> (accessed 8 Feb 2016).

Vallejo, A., U.M. Skiba, L. García-Torres, A. Arce, S. López-Fernández, and L. Sánchez-Martín.

2006. Nitrogen oxides emission from soils bearing a potato crop as influenced by fertilization with treated pig slurries and composts. *Soil Biology and Biochemistry* 38: 2782-2793. doi:10.1016/j.soilbio.2006.04.040

Zhang, Y. 2012. Simulated carbon and nitrogen dynamics in turfgrass systems using the DAYCENT model. Thesis, Colorado State Univ., Fort Collins.

OVERALL CONCLUSIONS

The primary objectives of my dissertation were to understand how organic farming management influences greenhouse gases (GHG) in terms of both sources and sinks, and how GHG emissions under organic farming management can be estimated to optimize future mitigation planning.

In chapter one, the study focused on organic fertilizers as a source of GHG due to their provision of N and C substrates for N_2O and CO_2 production. Four different types of organic fertilizers including blood meal, feather meal, fish emulsion, and cyano-fertilizer application in an organic lettuce field were studied for two years. Solid fertilizers (blood meal and feather meal) applied pre-plant had greater cumulative N_2O emissions than liquid fertilizers (fish emulsion and cyano-fertilizer) with multiple applications. Greater cumulative CO_2 emissions were found in the blood meal, feather meal, and fish emulsion treatments compared to the unfertilized treatment. Interestingly, the cumulative N_2O and CO_2 emissions from cyano-fertilizer application were not higher than the unfertilized treatment. Therefore, cyano-fertilizer with multiple applications could be a strategy for reducing GHG emissions from organic lettuce cropping systems.

In chapter two, the study focused on soil as a sink for GHGs. Carbon dioxide in the atmosphere can be transferred to C in biomass of plants through photosynthesis. Plant residues can be stored and protected in soil as stable soil organic matter (SOM), and agricultural management is an important factor in the stabilization and persistence of SOM. Therefore, conventional and organic vegetable farming systems in California, Colorado, and New York sites were studied. The results showed that organic farming management can promote the soil's

capacity to be a sink for GHGs by protecting SOM in soil aggregates. The additions of SOM under organic farming systems increased soil organic carbon (SOC), total nitrogen (TN), and soil microbial biomass. Increased microbial biomass supported aggregate formation, aggregate stability, and the formation of organo-mineral bonding of microbial products, resulting in more stable SOC and TN under organic farming systems than under conventional farming systems. Therefore, organic farming management could be a strategy for sequestering CO₂ by increasing stabilized SOM.

In chapter three, the study focused on the simulation of GHG emissions using the DAYCENT model. The field data from chapter one were used to test the DAYCENT model. The results showed that the DAYCENT model has a reasonable potential to simulate the effect of single application organic fertilizer treatments (feather meal and blood meal) on N₂O and CO₂ emissions. However, the model failed to simulate the peak of N₂O emissions; therefore, extra mineral N was added to the model to simulate the peak of N₂O emissions. The simulation of soil water content and soil temperature in this study is considered to have low potential and require some improvements.

Overall, regulating GHG emissions from agriculture while maintaining soil fertility and crop yields is challenging. Proper fertilizer management could help to achieve this aim. Multiple applications of organic fertilizers with low C/N ratios, such as cyano-fertilizer, can result in the reduction of N and C substrates for N₂O and CO₂ production, thereby decreasing N₂O and CO₂ emissions, while maintaining high yields. In addition, adoption of organic farming systems can result in higher C inputs, increased microbial biomass and aggregate stability, and greater amounts of protected SOM. Soil organic matter that is protected from microbial activity helps to slow the decomposition rate, resulting in reduced CO₂ emissions.

RECOMMENDATIONS

Based on our results, we recommend that cyano-fertilizer with multiple applications could be an option for mitigating GHG emissions from organic farms. Besides reducing GHG emissions relative to other commercial organic fertilizers (feather meal, blood meal, and fish emulsion), cyano-fertilizer could be a potential N source for organic farms because lettuce yields from cyano-fertilizer treatment did not differ from feather meal, blood meal, or fish emulsion treatments (data not shown). Cyano-fertilizer can be produced on-farm and fertigated to the field through drip irrigation. In addition, cyano-fertilizer did not cause clogging of drip emitters in our study.

In our study, the fertilizers were applied based on general farming practices. Therefore, soil fertilizers (feather meal and blood meal) were applied at full amount prior to growing lettuce, and liquid fertilizers (fish emulsion and cyano-fertilizer) were applied in several small doses to avoid flooding the field. The results showed that fertilization with multiple applications could reduce N_2O and CO_2 emissions compared to fertilization with a single application. Therefore, further study should focus on fertilization with multiple applications of fertilizers. Further study should be performed to understand the effect of organic fertilizers with multiple applications on GHG emissions, N use efficiency and yields in other crops. In addition, long-term sampling should be performed to determine how GHG emissions change over time, and these data will benefit researchers who want to build or improve GHG emission models.

Our study also supports the conclusion that organic farming management has potential to mitigate GHG emissions from vegetable farms through the increase of SOC and N sequestration through protection in soil aggregates, especially in free microaggregates, microaggregates within

macroaggregates, free silt and clay fractions, and silt and clay fractions within macroaggregates. Based on soil aggregate protection, we recommend that organic fertilizer application, cover crop management, and reduced tillage be used to increase stabilization and persistence of SOM. We found that the increase in SOC and N was related to soil microbes. Therefore, microbial products within each aggregate including fractions within free microaggregates should be included in future work in order to understand the mechanisms of protection of SOC and N in response to microbes. The turnover rate of protected SOM within each aggregate following commonly-used agricultural practices and the limitation of soil to protect SOM should be determined to understand the capacity of soil to protect SOM.

In the simulations, DAYCENT reasonably simulated N₂O and CO₂ emissions from irrigated organic lettuce field applied solid fertilizers with single applications, although this was the first time DAYCENT was used for lettuce. However, DAYCENT simulation requires more testing and improvement to simulate N₂O and CO₂ emissions from irrigated organic lettuce field applied liquid fertilizers with multiple applications, soil water content and soil temperature.