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<p><b>Project Title:</b></p> <p>Greenhouse gas budget and methane dynamics in a no-tillage sequence</p> <p><b>Key Words:</b> <u>greenhouse gases, methane, nitrous oxide, no-tillage, global warming potential</u></p>	<p><b>For Higher Education Program              Proposals Only:</b></p> <p>Need Area: _____</p> <p>Discipline: _____</p>

## **Abstract**

Nearly a quarter of all cropland in the US is under no-till (NT) - a management practice with well-documented positive effects on soil C sequestration, and soil quality maintenance. Although widespread adoption of no-tillage farming can help mitigate the global warming effect, there is a paucity of field-based data regarding the long-term impact of NT on greenhouse gas (GHG) fluxes. Accumulation of crop residue on soil surface, increased soil moisture and availability of labile organic substrates could stimulate the emission of carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O) and methane (CH<sub>4</sub>) from NT systems. Conversely, long-term adoption of no-tillage could lead to the evolution of a more active population of methanotrophs, increase soil macroporosity and ultimately improve the CH<sub>4</sub> sink strength of croplands. Our primary hypothesis in this study is that ***the longer no-tillage is continuously applied to a soil, the greater becomes the soil's ability and potential to oxidize CH<sub>4</sub>***. We also recognize that expression of the CH<sub>4</sub> oxidation potential of NT soils could be hindered by other soil factors. Thus, there is a need to examine discrepancies between biological potential and actual CH<sub>4</sub> fluxes in NT soils. Based on these considerations, the proposed research will address the following three objectives:

- (1) Assess the impact of long-term no-tillage on GHG fluxes, and examine relationships between gas fluxes and biophysical properties of NT soils.***
- (2) Evaluate the link between soil drainage characteristics and the impact of long-term no-till on GHG fluxes.***
- (3) Characterize the CH<sub>4</sub> oxidation potential of NT soils, and identify factors limiting expression of that potential.***

Although CH<sub>4</sub> is a major focus, the proposed research is a comprehensive assessment encompassing all three major soil-derived GHG (CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>) in order to fully appraise the global warming mitigation potential (GWMP) of no-tillage farming. We will monitor GHG fluxes (2 full years) at research stations and farmers' fields in similar soil type and under no-tillage for up to 46 years. Detailed studies using kinetic approach, stable C isotope and selective inhibition techniques will be conducted in order to characterize the community of CH<sub>4</sub>-oxidizers in NT soils and assess the effect of no-tillage duration and soil drainage characteristics on GHG dynamics. These experiments will be conducted to determine the potential for no-tillage soils to oxidize CH<sub>4</sub> and to provide guidance as to management options that can be used to achieve this potential. Our goal is to provide a basis to enhance and project the contribution of no-till farming to regional GHG inventory under various scenarios of conservation tillage adoption in US agriculture. The proposed research addresses several priorities of the Air Quality program and most specifically priority #2 "increase adoption of best management practices to reduce agricultural emissions".

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## D. PROJECT NARRATIVE

### I. Introduction

Accumulation of the greenhouse gases (GHG) carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) in the atmosphere alters the earth's atmosphere energy balance and is thought to contribute to the accelerated "greenhouse effect". This accumulation also has implication for atmospheric chemistry given the participation of CH<sub>4</sub> and N<sub>2</sub>O in stratospheric ozone depletion (IPCC, 2007). Nitrous oxide and CH<sub>4</sub> are highly potent GHG with global warming potentials (GWP) 310 and 21 times that of CO<sub>2</sub>, respectively (IPCC, 2001). Land use change and agricultural activities (e.g. fertilization, livestock, rice production) contribute an estimated 25 %, 60-65 % and 90 % of the total anthropogenic CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> emissions, respectively (Watson et al., 1992; Duxbury, 1994; Smith 1997; IPCC, 2001). Croplands can be net emitters or biological sinks for GHG depending on land-use and management practices (Smith et al., 2000; Chapuis-Lardy et al., 2007). In recent years, numerous assessments have been made to evaluate the potential of croplands to offset GHG emissions from the energy and industrial sectors. No-till farming figures prominently among the management practices recommended for that purpose. However, because GHG fluxes in NT systems are not well quantified, **the net global warming mitigation potential of no-till agriculture remains debatable.**

Conservation tillage, including no-till (NT), has gained wider acceptance in US agriculture. The total cropland area under no-till (Table 1) has grown steadily during the last 2 decades (Cannell and Hawes, 1994; CTIC, 2005; Fawcett and Towery, 2002; Johnson et al., 2005) and, if this trend is maintained, it has been projected (Subak, 2000) that 46 Mha of US cropland could be under no-till by the year 2012. While the positive effects of NT on soil erosion, water quality, and soil C sequestration are well documented (Kern and Johnson, 1993; Lal, 1997; Paustian et al., 1997; Dick et al., 1998; Frey et al., 1999; Collins et al., 2000; Subak, 2000; Smith et al., 2001), **major gaps remain in our understanding of the impact of no-till farming on soil/atmosphere exchange of GHG.**

Table 1. No-tillage trend in US agriculture.

	Year							
	1990	1992	1994	1996	1998	2000	2002	2004
millions ha	6.8	11.4	15.8	17.4	19.4	21.1	22.4	25.2
% of cropland	6.0	9.9	13.7	14.8	16.3	17.5	19.7	22.6

Sources: CTIC, 2005; Fawcett and Towery, 2002.

Long-term implementation of no-tillage practices is often accompanied with an increase in soil bulk density (Kladivko et al., 1986; Lal et al., 1994; Cannel and Hawes, 1994) that could impede soil-atmosphere exchange of gases. Further, crop residue accumulating on the soil surface in NT agro-ecosystems forms a physical barrier that reduces diurnal soil temperature variation, decreases evaporative soil water loss and, through increased availability of labile organic substrates, could stimulate soil respiration and CO<sub>2</sub> emission. This in turn could lead to a gradual depletion of available O<sub>2</sub> and the development of anaerobic micro-sites where production of CH<sub>4</sub> (methanogenesis) and N<sub>2</sub>O (denitrification) can take place. Application of N fertilizer could further intensify these processes (Ball et al., 1999).

GHG fluxes at the land surface are determined by production, transformation and transport processes within the soil profile. The intensity and duration of tillage practices affect a wide range of soil properties linked to

these processes. However, most field studies relating tillage practices and GHG dynamics do not account for the temporal evolution of the biophysical properties of NT soils. In addition, results of these studies are often compared without consideration of the effect of soil type. These may have contributed to the conflicting reports in the literature regarding the impact of no-till on GHG emission. The diagram below (Fig. 1) is an attempt to illustrate possible effects of long-term no-till on GHG fluxes and to summarize the competing scenarios that emerge from the existing literature (Robertson et al., 2000; Six et al., 2004; Johnson et al., 2005), with some reports concluding that long-term no-till has a positive global warming mitigation potential (GWMP) while others argued just the opposite (Fig. 1). Finally, these investigations quite often focus on a single biogenic trace gas, which makes it difficult to undertake complete analyses of the GHG emission mitigation potential of no-till systems. The need for comprehensive assessments of tillage management on  $N_2O$  and  $CH_4$  fluxes is widely recognized (Chan and Parkin, 2001; Six et al., 2004; Franzluebbers, 2005).

We therefore propose to conduct a chronosequence study involving NT croplands established on similar soil types and in the same eco-region in order to isolate the impact of no-tillage from other soil and environmental factors.

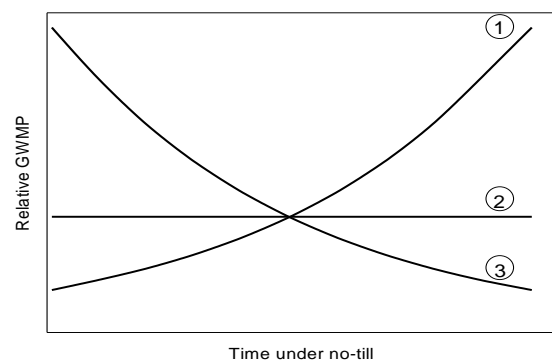


Fig. 1. Relative global warming mitigation potential (GWMP) with long-term adoption of no-till.

*Scenario 1:* Lower  $N_2O$  emission and increased  $CH_4$  uptake with time. This scenario illustrates a positive GWMP of no-till.

*Scenario 2:* Soil C sequestration offset by  $N_2O$  and  $CH_4$  fluxes. No net GWMP of NT farming.

*Scenario 3:* Increased  $N_2O$  and  $CH_4$  emission with longer duration of NT. Under this scenario, NT practices have a negative impact on the GWMP of agro-ecosystems.

## II. Research Objectives

The proposed research focuses on assessing the overall effect of no-tillage farming on GHG fluxes, and elucidating the biological and physical controls of trace gas fluxes in no-till soils. ***Our primary hypothesis is that the longer no-tillage is continuously applied to a soil, the greater becomes the soil's ability and potential to oxidize  $CH_4$ , thus diminishing the greenhouse warming impact of  $CH_4$ .***

The formation and fate of GHG in NT soils are regulated by several soil biophysical properties, but we have a limited understanding of the temporal evolution of these soil properties and their interdependence. It is fair to assume, however, that the physical soil environment (moisture and aeration) and the biological processes regulating the dynamics of biogenic trace gases vary with both soil type and duration of no-till farming. In the proposed research, we will control for soil type. Duration of no-tillage management will thus be the experimental factor. We will accomplish this by conducting our study in a chronosequence of no-till croplands established on similar soil types within the same eco-region. From this investigation, we will gain insights into the temporal evolution of NT-induced soil processes and their relationships with GHG dynamics. The specific objectives of the proposed research are to:

- 1. Assess the impact of long-term no-tillage on GHG fluxes, and examine relationships between gas fluxes and biophysical properties on NT soils.**

**2. Evaluate the link between soil drainage characteristics and the impact of long-term no-till on GHG fluxes.**

**3. Characterize the methane oxidation potential of no-till soils, and identify factors limiting expression of that potential.**

### **III. Rationale and Significance**

The agricultural sector has been identified as important sources of GHG, especially N<sub>2</sub>O and CH<sub>4</sub>. Natural and agricultural soils account for nearly 58 % of the global N<sub>2</sub>O emission (10.2 out of 17.7 Tg N<sub>2</sub>O-N y<sup>-1</sup>; IPCC, 2001). Agriculture also accounts for about 75 % of the anthropogenic CH<sub>4</sub> emission (Kreileman and Bouwman, 1994). Methane (CH<sub>4</sub>) is the second most important greenhouse gas (GHG) after CO<sub>2</sub> and contributes approximately 15-19 % of the global warming (IPCC, 2001). Although a decline in the growth rate of atmospheric CH<sub>4</sub> has been reported (Dlugokensky et al., 2003), current atmospheric CH<sub>4</sub> concentration (1.77 μL L<sup>-1</sup>) corresponds to an increase of 145 % relative to pre-industrial level of 0.7 μL L<sup>-1</sup>) (Watson et al., 1992). Likewise, the concentration of CO<sub>2</sub> has increased from 280 to 365 μL L<sup>-1</sup> and that of N<sub>2</sub>O from 0.28 to 0.31 μL L<sup>-1</sup> during the same period. This accumulation is the product of an imbalance between increased sources and reduced sink strength (Hütsch, 2001; IPCC, 2001).

No-tillage farming is viewed by many as a proven and one of the most promising options to mitigate GHG emission in the agricultural sector. Grant et al. (2004) for example concluded that conversion from conventional to reduced tillage offers the best potential for reducing GHG emission from cropland in Canada. These optimistic views are based largely on the demonstrated capacity NT soils to sequester organic C, but there has been concern that, in the long-term, increased N<sub>2</sub>O and CH<sub>4</sub> emission from NT soils could offset these C gains. Recent surveys indicate that nearly a quarter of all cropland in the US is in no-till. Although the benefits of no-tillage practices on soil quality are well documented, there is a paucity of data regarding GHG fluxes in no-till systems, and more specifically regarding the temporal evolution of GHG dynamics in NT soils. Through laboratory and field studies in a chronosequence of no-till cropland, we propose to **examine the variation in soil biological and physical properties with duration of no-tillage farming and to link these soil parameters with GHG fluxes in no-till croplands**. The information gained will improve our knowledge regarding the soil quality benefits of no-till and will further our ability to develop robust predictions of the contribution of croplands to GHG emission mitigation. Another unique aspect of the proposed study is that our field investigations will be conducted in farmers' fields and thus will provide a "real world" representation of soil attributes in response to tillage practices as typically implemented in farmlands across Ohio and the US Midwest.

Finally, this project could have methodological implications. During the last 15 years, the stable carbon isotope and the selective inhibition techniques have evolved into useful tools for the study of CH<sub>4</sub> oxidation and production, but their applications have been largely confined to wetlands and landfill systems (Abichou et al., 2006). There have been several recent successful applications of the stable C isotope approach to study CH<sub>4</sub> oxidation in forest and grassland ecosystems (Snover and Quay, 2000; Teh et al., 2005). However, we are not aware of any previous attempt to apply these techniques to agricultural soils. The proposed research could be the first study in which these two techniques will be applied in tandem in a natural ecosystem, allowing for cross validation (Obj. 3).

### **IV. Literature Background**

### *Tillage and GHG dynamics*

Increased soil C storage with implementation of NT is well documented. However, there is also a general consensus that the C storage capacity of soils is finite and that C sequestration rate in NT soils generally decreases with time (Akala and Lal, 2001; West and Six, 2007). Therefore, the viability of no-tillage as a land management tool to mitigate climate warming will, in the long-term, depends largely on the magnitude and direction of GHG exchange between the atmosphere and NT soil surface. Therefore, in order for the scientific community to continue to inform land management and related policy decisions, it is critical that we generate accurate GHG emission factors for long-term NT systems. Robertson et al. (2000) concluded that, among tillage practices, NT was the closest to mitigating all other sources of GWP. Grandy et al. (2006) came to similar conclusions from their study of no-till and till systems in southern Michigan and stated that adoption of no-till increased soil C stocks without N<sub>2</sub>O emission tradeoffs. Johnson et al. (2005) developed a series of scenarios to determine rates of GHG emission that could offset SOC sequestered in NT soils. Although CH<sub>4</sub> fluxes were not included in their analysis, their results suggested that N<sub>2</sub>O emission must increase by 32-97 % to offset the average current rate of C sequestration (0.3 Mg C ha<sup>-1</sup> y<sup>-1</sup>) in US Midwest NT soils. An analysis of available GHG emission data (Six et al., 2004) showed higher N<sub>2</sub>O in NT than MP during the first 10 years of NT adoption, but lower N<sub>2</sub>O fluxes in NT afterwards. Since this evaluation was based on a rather limited data set and large uncertainties were associated with the results, additional field measurements are needed to validate the pattern observed.

Comparisons of GHG emission from moldboard plowed (MP) and NT soils have yielded mixed results. Curtin et al. (2000) reported significantly higher rate of CO<sub>2</sub> emission from NT than MP plots. In a winter wheat (*Triticum aestivum* L.)-fallow rotation system, Kessavalou et al. (1998) measured greater rates of CH<sub>4</sub> uptake with NT than with MP practices. In contrast, other studies have reported no effect (Mosier et al., 2006) or a net emission (Chan and Parkin, 2001) with CH<sub>4</sub> under NT, but it should be noted that, in these later two studies, tillage practice was in place for only three years. Likewise, several studies (Goodroad et al., 1984; Lin and Doran, 1984; Robertson et al., 2000; Baggs et al., 2003) have reported higher rate of N<sub>2</sub>O emission under NT while in other studies lower rates of N<sub>2</sub>O emission under NT were found (Jacinthe and Dick, 1997; Kesavalou et al., 1998). Grandy et al. (2006) reported similar rates of N<sub>2</sub>O emission in till and no-till croplands in Michigan and detected no temporal trend in N<sub>2</sub>O emission with no-till duration that was ascribed to the coarse soil texture (sandy loam) at their study site. Adding to this complexity are the results of Venterea et al. (2005) who measured higher N<sub>2</sub>O emission rates under NT when urea was the N source and lower N<sub>2</sub>O emission when anhydrous ammonia was the N fertilizer applied. Results of modeling exercises are no less uncertain. Using the NGAS model, Mummey et al. (1996) predicted higher N<sub>2</sub>O emission in NT soils and attributed their prediction to greater soil moisture. Grant et al. (2004) similarly evoked soil moisture to explain model prediction of increased N<sub>2</sub>O production with adoption of no-till. In contrast, Li et al.'s (1996) DNDC model predicted the opposite trend. Li et al. (1996) argued that moderate soil temperature, less N mineralization and less frequent wet-dry cycles are the main factors explaining lesser N<sub>2</sub>O emission from NT soils. This interpretation is supported by field studies that have indicated that wet-dry cycles are important drivers of N<sub>2</sub>O fluxes in terrestrial ecosystems (Mummey et al., 1994; Jacinthe and Lal, 2004; Jarecki and Lal, 2006).

Reported changes in soil properties with adoption in no-tillage include soil organic C accretion, increased soil bulk density, greater stability of soil structure and development of soil macro-porosity. Given the positive impact of no-tillage on aggregation and the relationship between aggregation and porosity (Lal et al., 1994; Ball et al., 1997b), it seems reasonable to expect a more efficient soil-atmosphere exchange of gases with no-till. The dependency of CH<sub>4</sub> oxidation on diffusivity and permeability of soils is well demonstrated (Kruse et

al., 1996; Ball et al., 1997b,c). Jacinthe and Lal (2006) reported a negative relationship between macropore volume and CH<sub>4</sub> fluxes in a meadow indicating increased CH<sub>4</sub> uptake with greater availability of large soil pores. Likewise, Bender and Conrad (1994) proposed that “wide pores” in soil provide a favorable environment for methanotrophy. Borken and Brumme (1997) reported a significant increase in CH<sub>4</sub> oxidation (26 to 580 %) following liming of forest soils and attributed these results to soil structure improvement.

The accumulation of crop residue on soil surface in NT systems is an additional factor that could affect GHG dynamics. It has been suggested that surface residue and litter can act a diffusion barrier to gaseous exchange, and this interpretation is supported by reports of CH<sub>4</sub> consumption enhancement following removal of these materials from soil surface (Borken and Brumme, 1997; Burke et al., 1997; Steinkamp et al., 2001a). In addition, as a source of labile organic substrates, the presence of surface residue could stimulate soil biological activity leading to the development of O<sub>2</sub>-deficient anoxic pockets in NT soils (at least in the large soil aggregates) where N<sub>2</sub>O and CH<sub>4</sub> production can take place. Therefore, **even though the potential for biological CH<sub>4</sub> oxidation may exist in no-tillage soils, this potential may not be expressed or could be offset by increased N<sub>2</sub>O and CH<sub>4</sub> production.** This enhancement in N<sub>2</sub>O and CH<sub>4</sub> emission in long-term NT soils is illustrated in Fig. 1 (scenario 3) and is supported by several field studies (Goodroad et al., 1984; Lin and Doran, 1984; Robertson et al., 2000; Baggs et al, 2003).

#### *Methanotrophy and tillage management*

Methane uptake in soils is the only known biological sink for CH<sub>4</sub>. Globally, this sink is estimated at 20-60 Tg CH<sub>4</sub> y<sup>-1</sup> (Dorr et al., 1993; Potter et al., 1996; Smith et al., 2000). It is an oxidative process carried out by methanotrophs and mediated by the methane monooxygenase (MMO), an enzyme system structurally and functionally related to the ammonia monooxygenase (AMO) involved in the conversion NH<sub>4</sub><sup>+</sup> to NO<sub>3</sub><sup>-</sup> (Bender and Conrad, 1994; Hanson and Hanson, 1996; Reay and Nedwell, 2004). These similarities are often invoked to explain the negative impact of NH<sub>4</sub>-generating fertilizers on CH<sub>4</sub> uptake (Bronson and Mosier et al., 1994; Hütsch et al., 1994; Priemé et al., 1997). Consumption of atmospheric CH<sub>4</sub> in upland soils drastically declines with conversion of natural ecosystems to intensive agriculture. It has been estimated that conversion of various temperate ecosystems to agriculture has resulted in 30 to 65 % decline of the terrestrial CH<sub>4</sub> sink strength (Powlson et al., 1997; Smith et al., 2000). Known contributors to this decline include fertilization with ammonia-generating fertilizer (Hütsch et al., 1993; Bronson and Mosier et al., 1994; Suwanwaree and Robertson, 2005), and frequent tillage disturbance resulting in soil structure deterioration and loss in soil macro-porosity (Ball et al., 1997a,b,c; Hütsch, 1998). Conversely, studies have also shown that CH<sub>4</sub> oxidation in soils generally improves when croplands are either abandoned or reverted to woodland and grassland (Ojima et al., 1993; Dobbie and Smith, 1996; Mosier et al., 1997; Hütsch, 1998; Smith et al., 2000). These findings also suggest that a reduction in tillage disturbance could have a similar outcome. Given the scarcity of arable land in the world, it seems unlikely to expect abandonment of cultivated lands to occur on a scale large enough to significantly affect the global terrestrial CH<sub>4</sub> sink. However, widespread adoption of no-tillage in agro-ecosystems is a realistic goal.

The restoration of CH<sub>4</sub> oxidation capacity of soils is thought to be a slow process likely involving soil biophysical factors that change slowly over time (Dobbie and Smith, 1996; Priemé et al., 1997; Hütsch, 1998). This line of reasoning led Hütsch (1998) to propose that “**long-term reduced tillage should be considered as a strategy to minimizing the adverse effects of cultivation on soil CH<sub>4</sub> sink strength**”. Due to a lack of field data, the proposition that long-term implementation of no-tillage farming could help restore the CH<sub>4</sub> sink strength of croplands remains a working hypothesis (Dobbie and Smith, 1996; Dobbie et al., 1996; Priemé et al., 1997; Hütsch, 1998; Smith et al., 2000). However, it is supported by improvement in soil

structural stability and macropore development observed with implementation of NT (Lal et al., 1994; Ball et al., 1997a; Kay and VandenBygaart, 2002; Buczko et al., 2006), and reported strong relationships between these soil properties and CH<sub>4</sub> uptake (Ball et al, 1997a,b,c; Jacinthe and Lal, 2006a). Improved soil structure facilitates gas transport, creates favorable conditions for the development of an active population of methanotrophs, and ultimately could result in increased CH<sub>4</sub> consumption in NT soils. This view is supported by studies that have documented a gradual restoration of CH<sub>4</sub> oxidation capacity when cultivated lands are reverted to forest, grassland or abandoned (Ojima et al., 1993; Dobbie and Smith, 1996; Dobbie et al., 1996; Mosier et al., 1997; Priemé et al., 1997; Hütsch, 1998; Smith et al., 2000). Recent work at our long-term no-tillage plots (i.e. 44 continuous years) located near Wooster, OH have indicated the soil profiles are reverting to that more like the original forest soil than of a conventionally tilled soil profile (Mestelan et al., 2006). This suggests that the soil physical, chemical and biological environment in continuous, long-term no-tillage cropland may be similar to the soil environment that methanotrophs encounter in restored grasslands and forests.

During the 2003 growing season, measurement of GHG fluxes was initiated at the Western Branch Experimental station of Ohio State (South Charleston, OH) in an effort to assess the effect of tillage intensity of trace gas emission. Results (Fig. 2) showed that N<sub>2</sub>O emission was lowest in the NT plots compared to the CT and MP plots. Results further showed that the NT plots acted as net CH<sub>4</sub> sink while net CH<sub>4</sub> emission was recorded in the other treatments (Fig. 2). These results are in line with Scenario 1 (Fig 1), and are also encouraging given the soil drainage characteristic (SPD) at the site and that 2003 was a wet year. **Clearly, additional measurements are needed at this and other sites to confirm these observations.**

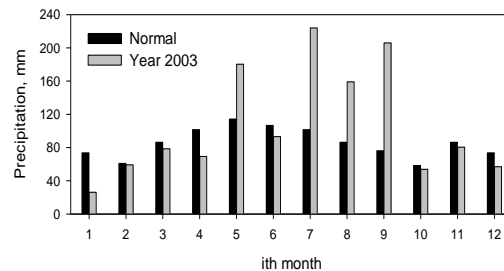
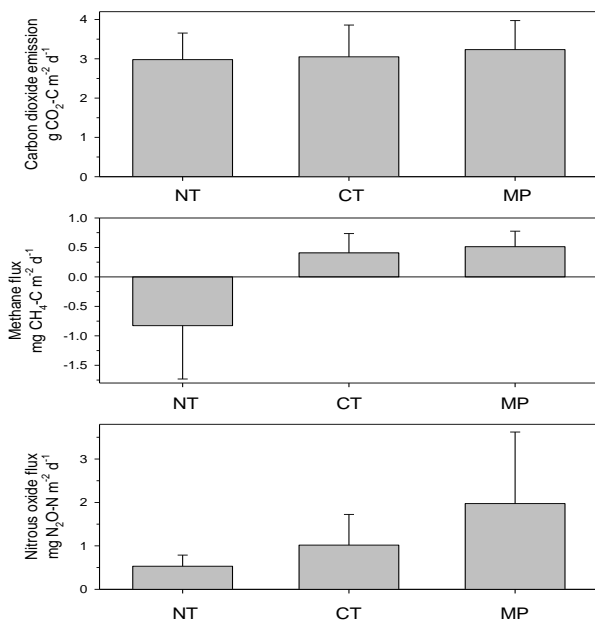


Fig. 2. Greenhouse gas fluxes (*left*) from western Ohio soils under continuous corn no-till (NT), chisel-till (CT) and conventional moldboard plow tillage (MP). GHG fluxes were monitored monthly during the period June – Dec 2003. In 2003 (*above*), precipitation during the period May-Oct (916 mm) was 68% above normal (543 mm).

## V. Experimental Plan

### Description of Study Sites

The proposed research will be conducted at six locations to form a no-tillage chronosequence (Fig. 3) across central Ohio. Long-term precipitation and temperature average 910 mm and 9.3 °C at the northeast end (Wooster) of the transect, and 1043 mm and 10.8 °C at the southwest end. Sites for this no-tillage chronosequence are selected to represent 2 major land-resources areas: MLRA 110 and 111 that extend from



central Ohio into Indiana and eastern Michigan.

The chronosequence includes four farmers' fields and experimental plots at two research stations under no-tillage for periods ranging from 0 to 46 years. In order to obtain a full range of soil disturbance, continuously-plowed soils (zero year no-till) and adjacent secondary growth hardwood forests (reference sites) will be included. The proposed study sites are located near Centerburg (Knox County), Mount Gilead (Morrow County), Bucyrus (Crawford County), Wooster (Wayne County) and South Charleston (Clark County). They form a no-tillage chronosequence (Table 2, Fig. 3) consisting of croplands in corn-soybean rotation under no-tillage for 7 (Mount Gilead), 11 (Bucyrus), 14 (Mount Gilead), 34 (Centerburg), 46 (Wooster and South Charleston) years. Authorization has already been obtained from the landowners to conduct our proposed study at these sites (see letters of support). Every year since 2005, these landowners have consistently pledged their support for our research and express their desire to cooperate.

Table 2. Location and dominant soil series at the study sites.

Sites	Duration of NT, years	Dominant soil series <sup>†</sup> and soil drainage
<b>Farmers' Fields</b>		
Mount Gilead	7	Cardington (MWD) <sup>‡</sup> Centerburg (MWD) Bennington (SPD)
Bucyrus	11	Cardington (MWD) Centerburg (MWD) Bennington (SPD)
Mount Gilead	14	Cardington (MWD) Centerburg (MWD) Bennington (SPD)
Centerburg	34	Cardington (MWD) Centerburg (MWD) Bennington (SPD)
<b>Research Plots</b>		
Wooster	46	Wooster (WD), Canfield (MWD)
South Charleston	45	Crosby (SPD)

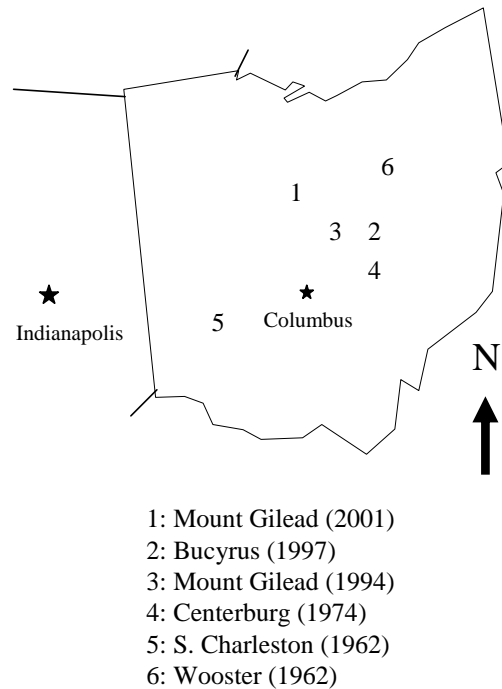


Fig. 3. Location of the proposed study sites in Ohio.

<sup>†</sup> Bennington (Fine, illitic, mesic Aeric Epiaqualfs), Canfield (Fine-loamy, mixed, mesic Aquic Fragiudalfs), Cardington (Fine, illitic, mesic Aquic Hapludalfs), Centerburg (Fine-loamy, mixed, mesic Aquic Hapludalfs), Crosby (Fine, mixed, mesic Aeric Epiaqualfs), Wooster (Fine-loamy, mixed, mesic Oxyaquic Fragiudalfs).

<sup>‡</sup>MWD: moderately well drained; SPD: somewhat poorly drained.

Soils at all the study sites are Alfisols developed from Wisconsinan age glacial till. Across all sites, soil

texture is silt loam in the surface layers, with silty-clay-loam and clay-loam inter-mixed with gravel in the subsurface depending on the site. The Centerburg, Mount Gilead and Bucyrus farmer's fields are in Ohio soil region 5 and the Wooster site is in soil region 1 ([www.dnr.state.oh.us/soilandwater/soils/soilreg3.htm](http://www.dnr.state.oh.us/soilandwater/soils/soilreg3.htm)). By establishing the study sites on the same or very similar soil types, we will be able, in our analysis of the results, to control for soil type as a contributing factor.

Visits and on-site interviews with farmers will be conducted in order to establish study areas at each of the farms. Field under conventional (MP) and no-tillage (NT) practices will be selected and three study areas per tillage practice will be delimited. Soil maps will be used during delimitation with care being taken to locate the study areas within the same or very similar soil series. Since the dominant soil series at the farmers' sites are closely related (Table 2), we expect to find areas that meet these criteria at all four farmers' sites. The study areas will not be truly random, but they could nonetheless be treated as pseudo replicates during statistical analysis of the data.

Experimental plots were established in 1962 by the Ohio Agricultural Research and Development Center (OARDC) near Wooster and in 1963 near South Charleston (OH) to evaluate the effects of tillage and crop rotations on soil properties. Cropping system includes corn-soybean and corn-oat-meadow rotations at the Wooster site, and continuous corn (CC, *Zea mays*) at the South Charleston site. At each site, there are three tillage treatments: no-till (NT), chisel plow (CP) and conventional tillage (CT). Each tillage treatment is replicated three times (Wooster) or four times (South Charleston) in a randomized block design. In the proposed study, we will sample three of the continuous corn NT and MP plots at each site (for a total of 6 plots per research station).

**Objective 1. Assess the impact of long-term no-tillage on GHG fluxes and examine relationships between gas fluxes and soil biophysical properties.**

**Hypothesis** *Compared to conventional-tillage (CT), no-tillage (NT) provides a greater global warming mitigation potential (GWMP) through the combined effect of soil C storage, reduced N<sub>2</sub>O emission and greater CH<sub>4</sub> uptake in the short-, mid- and long-term.*

## **Procedure**

### ***General***

As stated above, at each farmer's field, three study areas under NT and three study areas under MP will be delimited. A study area will also be established at a nearby woodlot.

### ***Soil physical properties***

In each study area, duplicate soil cores (5 cm diam, 5 cm depth) and composite soil samples will be collected in the 0-15 and 15-30 cm soil depth at the beginning of each growing season. Soil cores will be water-saturated and then equilibrated in a pressure plate extractor at a matric potential of -33 kPa (field capacity). Upon cessation of water dripping out of the extractor, the weight of each core will be recorded. The loss of water between saturation and -33 kPa will be taken as a measure of macropore volume (the volume of soil pores that is water-free at field capacity) (Roseberg and McCoy, 1990). Soil cores will then be used for determination of soil bulk density, total porosity and pore size distribution (Marshall, 1958). Composite soil samples will be air-dried, sieved and used to determine soil pH (soil:water ratio of 1 to 2), particle size distribution (hydrometer method), aggregate stability (wet sieving method; Kemper and Rosenau, 1986).

### Mineral N and nutrient cycling

Duplicate soil samples (0-30 cm) will be collected from each study area on a monthly basis during the growing season. Sampling frequency will be bi-weekly following fertilizer application. Samples will be carried on ice to the laboratory, stored in the laboratory at 4 °C and, within 5 days after collection, extracted with KCl (2 M) for determination NO<sub>3</sub> and NH<sub>4</sub> using a Konelab analyzer (EST Analytical, Fairfield, OH).

A portion of the soil samples collected at the peak of each growing season (between June and August) will be used for assessment of mineralizable C and N (Keeney, 1982; Hart et al., 1994). Duplicate (10 g) field-moist soil samples will be placed in 120 mL serum bottles and incubated for 20 days in the laboratory. The production of CO<sub>2</sub> in the serum bottles will be monitored using an infrared gas analyzer (IRGA). The amount of CO<sub>2</sub> produced during the incubation period will be taken as a measure of readily mineralizable C. At the end of the incubation period, soil in the serum bottles will be extracted (2 M KCl) for determination of inorganic N (NH<sub>4</sub> and NO<sub>3</sub>). The rate of net N mineralization will be determined as the difference between initial and final mineral N concentrations.

### Measurement of gas fluxes

GHG fluxes (CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub>) will be monitored during 2 growing seasons using the static chamber technique. The distribution of chambers at the study sites is summarized below (Table 3). Each study area will also be instrumented with soil temperature and moisture probes interfaced with data loggers.

Table 3. Distribution of static chambers at the study sites.

Study site	Number	Tillage/Landuse	Sampling units	Chambers
Farmers' fields	4	MP	3 delimited areas	12 (4 per sampling area)
		NT	3 delimited areas	12 (4 per sampling area)
		Forest <sup>†</sup>	2 delimited areas	6 (3 per sampling area)
<i>Total chambers at farmers' fields</i>				$(24 \times 4) + (6 \times 2) = 108$
Research stations	2	MP	3 plots	12 (4 per plot)
		NT	3 plots	12 (4 per plot)
		Forest	2 delimited areas	6 (3 per sampling area)
<i>Total chambers at research stations</i>				$(24 \times 2) + (6 \times 1) = 54$

<sup>†</sup>Wooded areas will be selected at two of the farmers' fields, and only at the South Charleston station.

Large rectangular chambers as described by Venterea et al. (2005) will be used. Chamber consists of a stainless steel base and a lid lined with rubber gasket at their edge. Chamber bases will be inserted 5 cm into the ground, at least one week prior to conducting measurement and will remain at the same place (except for scheduled farming operations) for the entire growing season. The lid will be fitted with a gas sampling port. At the time of sampling, chamber tops (L: 50 cm; W: 30 cm; H: 10 cm) will be placed over the bases and secured with metal clamps. Air samples (~ 10 mL) will be withdrawn from the chamber headspace at 0, 20 and 40 minutes and stored in pre-evacuated glass vials (7 mL) sealed with gray butyl rubber septa. GHG fluxes will be computed using the equation:

$$F = \left( \frac{\Delta C}{\Delta t} \right) \left( \frac{V}{A} \right) k$$

where,  $\Delta C/\Delta t$  is the change in GHG concentration inside the chamber (mass GHG m<sup>-3</sup> air min<sup>-1</sup>), V is the chamber volume (15x10<sup>-3</sup> m<sup>3</sup>), A is the area circumscribed by the chamber (0.15 m<sup>2</sup>), and k is the time conversion factor (1440 min d<sup>-1</sup>). A positive value of F corresponds to a net emission of gas from soil into the

atmosphere. Conversely, a negative F value corresponds to a net transfer (uptake) of gas from the atmosphere into the soil.

Fluxes will be monitored bi-weekly with more frequent sampling during the winter-to-spring transition, and in the 2-3 weeks following fertilizer application. Less frequent measurements will be made during the dormant season. This sampling frequency is based on previous studies (Jacinthe and Dick, 1997; Jacinthe et al., 2002; Gregorich et al., 2005; Phillips, 2007) that had shown that GHG fluxes (especially N<sub>2</sub>O) tend to be vigorous during these periods and could account for a significant portion of annual trace gas budget.

Given the distance between the study sites, project personnel will be divided into two teams for the purpose of trace gas sampling. One team (under the supervision of W. Dick) will sample the Wooster research plots and, the Bucyrus and Mount Gilead farmer's fields. A second team (under the supervision of R. Lal) will sample the South Charleston research plots and the farmers's fields located near Centerburg. We expect that each sampling occasion will take two days to complete at all sites. For a given site and at each sampling occasion, monitoring of trace gas fluxes will be conducted at about the same local time in order to stabilize the effects of diurnal temperature variation on flux variability.

During the growing season, nearly 850 air samples will be collected monthly (assuming biweekly). Collected gas samples will be forwarded to Jacinthe's laboratory in Indianapolis for analysis. A fully automated gas chromatograph (Varian CP 3800) interfaced with a Combi Pal autosampler is available allowing rapid analysis of air samples and data processing. Two batches of samples are expected to be shipped every month. A delay of several days (~10 days) may occur between collection and analysis of air samples, but we do not expect this to affect the integrity of our samples. Glass vials are widely used for the storage and shipping of air samples. In past studies, we have found that these vials can remain evacuated for 4-5 months (Jacinthe and Dick, 1997). Rochette and Bertrand (2003) have also demonstrated the suitability of glass vials for long-term (136 d) storage of air samples. The slight over-pressurization of the glass vials will further protect against dilution of our samples with air from the open atmosphere.

#### *Soil Carbon Stocks*

A direct assessment of soil C sequestration at the study sites will not be conducted as part of the proposed research. For our farmers' fields, C sequestration rates will be derived using the Century model to integrate soil properties, climatic data and crop yield information. For the experimental plots, SOC sequestration data have been already collected in previous studies (Dick and Durkalski, 1997; Dick et al., 1998; Collins et al., 2000; Jarecki and Lal, 2005).

Gas fluxes will be expressed as CO<sub>2</sub> equivalent (based on the radiative property of each gaseous species) and the net global warming mitigation potential (GWMP) of NT practice will be computed (Robertson et al., 2000; Jacinthe and Lal, 2003; Six et al., 2004; Grandy et al., 2006). The GWP provides an integrated measure of NT impact on atmospheric GHG burden and the "greenhouse effect". To derive GWP, the sum of all emitted GHG will be subtracted from the rate of soil organic C storage.

#### *Data Analysis*

GHG flux data will be analyzed using repeated measures analysis of variance (ANOVA) with years under no-tillage as the class variable and sampling occasion as the repeated-measure factor (Littell, 1989; SAS, 2001). Because of difference in soil drainage characteristics, data from the South Charleston experimental plots will not be included in that analysis. Data from this site will be used to address Objective 2 (below). Annual flux and global warming mitigation potential (GWMP) of no-tillage practice at each study site will be computed

using a time horizon of 100 years (Robertson et al., 2000; Jacinthe and Lal, 2003; Six et al., 2004). Relationships between GHG fluxes and soil properties will be explored using both regression and principal component analysis. Examination of the links between the properties of NT soils and GHG fluxes could perhaps provide clues as to how these properties can be managed to optimize the global warming mitigation potential of no-tillage farming.

**Objective 2. Evaluate the link between soil drainage characteristics and the impact of long-term no-till on GHG fluxes.**

*Hypothesis The capacity of NT soils to biologically oxidize CH<sub>4</sub> increases with the duration of NT and that, in fine-textured soil, this increased capacity is masked by increase in CH<sub>4</sub> production and/or restricted transport of CH<sub>4</sub> to active sites of methanotrophy.*

*Background and Justification*

Hütsch (1998) investigated the CH<sub>4</sub> oxidation capacity of soils with adoption of reduced tillage. After 15 years, results showed significantly higher CH<sub>4</sub> oxidation rates (0.26 mg CH<sub>4</sub>-C m<sup>-2</sup> d<sup>-1</sup>) in direct-drilled (equivalent to NT) than in plowed (0.03 mg CH<sub>4</sub>-C m<sup>-2</sup> d<sup>-1</sup>) plots established on a sandy loam Cambisol. Priemé et al. (1997) also examined the CH<sub>4</sub> oxidation potential of soils supporting aggrading forests (established at sites previously used as cropland) and found a negative relationship between years since conversion to forest and the minimum concentration of CH<sub>4</sub> required to initiate CH<sub>4</sub> oxidation. In other words, the longer the site had been afforested, the greater the ability of soils to consume CH<sub>4</sub> at or even below atmospheric concentration. This trend was interpreted to reflect change in the composition and activity of the CH<sub>4</sub>-oxidizing community with time (Priemé et al., 1997). The above-referenced studies contrasted however with the results of Jacinthe and Lal (2005) who observed no improvement in the CH<sub>4</sub> oxidation capacity after 8 years of no-tillage in a poorly-drained silty clay loam soil (Kokomo series). Chan and Parkin (2001) also reported net CH<sub>4</sub> emissions after 3 years on NT in a clay loam field in Iowa. These results suggest that poor soil drainage may retard the restorative effect of no-tillage on soil CH<sub>4</sub> consumption. This interpretation is consistent with the suggestion that CH<sub>4</sub> oxidation recovery occurs faster in sandy than in fine-textured clay and loamy soils (Dobbie and Smith, 1996). However, this suggestion has not been tested experimentally. *It is thus hypothesized that (i) the capacity of NT soils to biologically oxidize CH<sub>4</sub> increases with the duration of NT and that (ii) in fine-textured soils this increased capacity is not apparent due to concurrent CH<sub>4</sub> production or restricted CH<sub>4</sub> transport to active sites of methanotrophy.* We will test this hypothesis through laboratory and field investigations.

*Field monitoring of net CH<sub>4</sub> fluxes*

The research plots in Wooster and South Charleston have been established at locations with contrasting soil drainage characteristics (well drained and somewhat poorly drained). Therefore, these plots offer a unique opportunity to test this hypothesis, and to determine how four decades of no-tillage, interacting with soil drainage, affects CH<sub>4</sub> oxidation.

GHG fluxes will be monitored at the Wooster and South Charleston plots using the procedures described above (Objective 1). At each site, three NT and three MP plots under continuous corn will be selected. Each plot will be instrumented with four static chambers, as well as soil temperature and moisture probes. GHG fluxes and soil properties will be monitored at the same frequency as described in Objective 1. GHG fluxes data will be analyzed using soil drainage as the class variable, annual CH<sub>4</sub> flux as the response variable and sampling occasion as the repeated measures factor (Littell, 1989).

### *Field-scale measurement of gross CH<sub>4</sub> production and consumption*

In order to get more insights into CH<sub>4</sub> dynamics at these two sites, selective inhibition and stable C isotope techniques will be used on selected sampling occasions (4-5 times during a cropping season) to assess *in-situ* gross CH<sub>4</sub> production and consumption. The latter can be masked if rates of CH<sub>4</sub> production are elevated. The stable C isotope approach will allow us to estimate both oxidation and production, and thus will also serve as an independent confirmation of the reliability of the selective inhibition technique.

Next to each static chamber, soil atmosphere samplers centered at 10, 20 and 40 cm soil depths will be installed. Sampler will consist of cells of silicone membrane (20-cm long; 1.6 cm id, 0.24 cm wall-thickness, Cole-Parmer cat. H-06411-82) closed at one end with a caulked silicone stopper and fitted at the other end with FEP-lined tygon tubing and a stopcock. The soil atmosphere sampler design is based on previous work that has established the permeability of silicone membrane to CO<sub>2</sub>, CH<sub>4</sub> and N<sub>2</sub>O, and the use of this material for sampling the soil atmosphere under saturated and unsaturated conditions (Jacinthe and Dick, 1995; Jacinthe and Groffman, 2001; Jacinthe and Lal, 2004). There will be four sets of soil atmosphere sampler in each plot. Two sets will be sampled for <sup>13</sup>CH<sub>4</sub> analysis and estimation of CH<sub>4</sub> oxidation by the stable isotope approach. The other two sets will be used in connection with the selective inhibition technique.

It has been demonstrated (Oremland and Capone, 1988; Chan and Parkin, 2000) that methyl fluoride (CH<sub>3</sub>F) is a specific inhibitor of the MMO enzyme involved in CH<sub>4</sub> oxidation. Methyl fluoride has also been used in conjunction with closed chamber techniques to assess CH<sub>4</sub> oxidation in a variety of ecosystems including lakebed (Oremland and Culbertson, 1992), wetlands (Schipper and Reddy, 1996; Moosavi and Crill, 1998), but we are not aware of its application in croplands.

To assess gross CH<sub>4</sub> oxidation by the selective inhibition technique, two consecutive CH<sub>4</sub> flux measurements, each of 40 min duration, will be made on a given sampling date. First, CH<sub>4</sub> flux will be measured using the protocol described above (F1). Then, the chamber will be closed and CH<sub>3</sub>F added into the headspace to yield a final CH<sub>3</sub>F of about 1 %. Methyl fluoride will also be added into the soil profile via the silicone cell located at 40 cm soil depth. The goal is to obtain a concentration of CH<sub>3</sub>F of 0.01 - 0.1 % which research has shown is the level needed to inhibit CH<sub>4</sub> oxidation (Oremland and Culbertson, 1992; Chan and Parkin, 2000). Soil air samples will be taken from the 20 cm silicone cell and analyzed for CH<sub>3</sub>F. We expect to reach this concentration within 1 h, but preliminary tests will be conducted at each site to fine tune the timing. Once the required level of CH<sub>3</sub>F is reached, the chamber lid will be removed to vent the excess CH<sub>3</sub>F. The chamber will be closed again, and the concentration of CH<sub>4</sub> in the headspace will be monitored for another 40 min to determine F2. The difference between F2 and F1 will be a measure of the gross CH<sub>4</sub> oxidation.

Biogenic CH<sub>4</sub> is generally depleted in stable C isotope ( $\delta^{13}\text{C} < -50 \text{‰}$ ) compared to the isotopic signature (average  $\delta^{13}\text{C}$ : -25 ‰) of soil organic matter (Garten et al., 2000; Borjesson et al., 2001). During CH<sub>4</sub> oxidation, methanotrophs preferentially consume the light isotope (<sup>12</sup>CH<sub>4</sub>) so that the remaining CH<sub>4</sub> becomes <sup>13</sup>C-enriched. The degree of enrichment is expressed by an isotopic fractionation factor ( $\alpha$ ). If this fractionation factor is known, then the fraction of CH<sub>4</sub> oxidized ( $f_{ox}$ ), gross CH<sub>4</sub> production (Production<sub>gross</sub>) and gross CH<sub>4</sub> oxidation (Oxidation<sub>gross</sub>) can be computed using the following equations (Chanton and Liptay, 2000; Teh et al., 2005; Abichou et al., 2006)

$$f_{ox} = 0.1 \left( \frac{\delta_E - \delta_S}{\alpha_{ox} - \alpha_{trans}} \right)$$

$$\delta_E = \frac{(\delta_f C_f - \delta_i C_i)}{(C_f - C_i)}$$

$$\alpha_{ox} = -4.3 \times 10^{-4} T + 1.042$$

$$\text{Production}_{gross} = \frac{F_N}{1 - f_{ox}}$$

$$\text{Oxidation}_{gross} = \text{Production}_{gross} - F_N$$

where:  $\delta_s$ ,  $\delta_i$  and  $\delta_f$  are isotopic composition of CH<sub>4</sub> at depth in the soil profile (> 40 cm; Teh et al. 2005), in the headspace of the static chamber at the beginning and end of CH<sub>4</sub> flux measurement, respectively;  $\delta_E$  is the time-integrated  $\delta^{13}\text{C}$  of CH<sub>4</sub> in surface flux;  $C_i$  and  $C_f$  are the methane concentrations in the static chamber headspace at the beginning and end of CH<sub>4</sub> flux measurement, respectively;  $\alpha_{ox}$  and  $\alpha_{tarns}$  are isotopic fractionation factors due to microbial oxidation;  $\alpha_{tarns}$  is the isotopic fractionation factor due to transport (1.0013 – 1.0038 depending on CH<sub>4</sub> concentration in the upper soil layer, assumed to be the zone of CH<sub>4</sub> oxidation; Teh et al., 2006); T is the average soil temperature (°C) during the measurement period; and  $F_N$  is the net CH<sub>4</sub> flux as determined by the static chamber method (as described previously).

### **Objective 3. Characterize the methane oxidation potential of no-till soils, and identify factors limiting expression of that potential.**

**Hypothesis:** *No-tillage practices result in the evolution with time of a greater population of methanotrophs and in an increased capacity or potential of soils to oxidize CH<sub>4</sub>.*

#### *Literature Background*

The oxidation of CH<sub>4</sub> in soils is carried out by methanotrophic bacteria. Kinetic approach, phospholipid fatty acid (PLFA) profiles DNA fingerprinting and C assimilation pathways (ribulose monophosphate pathway for type I and serine pathway in type II) have been used to categorize CH<sub>4</sub>-oxidizers into type-I and type II organisms (Hanson and Hanson, 1996; Dunfield et al., 1999; Bull et al., 2000). Based on their affinity for CH<sub>4</sub>, methanotrophs are also categorized as (i) high-affinity organisms adapted to low-CH<sub>4</sub> environments and (ii) low-affinity methanotrophs which dominate in CH<sub>4</sub>-rich systems such as landfills and rice fields (Bender and Conrad, 1992; Hanson and Hanson, 1996). It follows that the values of half saturation constants ( $K_M < 10 \mu\text{L CH}_4 \text{ L}^{-1}$ ) reported for the high-affinity systems are less than for the low-affinity methanotrophs ( $K_M$ : tens to thousands  $\mu\text{L CH}_4 \text{ L}^{-1}$ ; Bender and Conrad, 1992; Benstead and King, 1997; Reay and Nedwell, 2004). Data from one of our recent investigations (Jacinthe and Lal, 2006b) suggest that type II methanotrophy is restored earlier than type I in reclaimed minesoils.

Work to be completed under this Objective includes: (i) laboratory assays to assess the significance of diffusion restriction on CH<sub>4</sub> oxidation in no-till soils, and (ii) establishment of field microplots to evaluate the effectiveness of various organic amendments in improving the CH<sub>4</sub> oxidation capacity of soils.

#### Procedure

##### *Soil sampling procedure*

The biological assays described below will be carried out using soil cores and composite soil samples (0-5, 5-10, 10-20 and 20-30 cm) collected at the peak of each growing season (in July - August). A total of 144 soil samples (6 sites x 2 tillage practices x 3 study areas per site x 4 depths) will be collected. The kinetic assays will be initiated within 5 days of soil sample collection (samples will be stored at 4 °C).

#### *Kinetic of methane oxidation*

Methane oxidation potential will be assessed using intact soil cores and field-moist sieved samples. Soil cores or field-moist sieved (40 g of 2-5 mm size soil aggregate) soil samples will be placed in 900 mL wide-mouth Mason jars. Various amounts of CH<sub>4</sub> will be added to the jar headspace to yield final concentrations ranging between 1.8 and 300 µL L<sup>-1</sup> CH<sub>4</sub>. Methyl chloride (0.1 % CH<sub>3</sub>Cl) will be added to inhibit CH<sub>4</sub> formation (Chan and Parkin, 2000). Rates of CH<sub>4</sub> oxidation will be monitored over a 192 h period. Using non-linear regression procedure (NLIN available in SAS), rates of CH<sub>4</sub> oxidation (v) and initial CH<sub>4</sub> concentration (S) will be fitted to the Michaelis-Menten model [ $v = (V_{\max} S)/(K_M + S)$ ] to derive maximum oxidation rate (V<sub>max</sub>) and half saturation constant (K<sub>M</sub>). The K<sub>M</sub> values - a measure of the affinity of resident bacteria for CH<sub>4</sub> - will provide the first indication whether or not change in the methanotrophic community composition had occurred with duration of tillage practices. Results of assays conducted with sieved soils will be compared to those obtained with intact cores in order to determine the impact of diffusion limitation on CH<sub>4</sub> consumption.

Hypothesis *Divergence between actual CH<sub>4</sub>-uptake and potential oxidation activity can be reduced and the function of methanotrophic community can be improved through soil amendment.*

In the activities described below, the goal is to identify possible factors limiting expression of the CH<sub>4</sub> oxidation potential of soils and to evaluate various amendments to reduce the gap between potential and actual oxidation rates.

#### *Background*

Agricultural practices such as application of crop residues and bio-solids could have impact on CH<sub>4</sub> dynamics, but available data are limited. The C/N ratio of crop residues appears as a good predictor of their impact on CH<sub>4</sub> oxidation (Boeckx and Van Cleemput, 1996; Hütsch, 2001a). Residues with a high C/N ratio (e.g. wheat straw) were found to have no effect on CH<sub>4</sub> oxidation presumably due to stimulation of N immobilization. However, application of residues with a low C/N ratio (e.g. sugar beet or potato leaves) enhances N mineralization, causing a strong inhibition of CH<sub>4</sub> oxidation, occasionally up to almost 100 %.

Studies have been made of the effects on CH<sub>4</sub> oxidation of farmyard manure (FYM) and bio-solid application to arable soils (Hütsch, 1998a). In the short term, these practices generally result in a net CH<sub>4</sub> emission that has been linked to CH<sub>4</sub> evolved during storage of animal excreta as slurries (Sommer et al., 1996). Work by Chadwick et al (2000) has also shown that the initial burst of emission subsides rapidly with slurries that easily infiltrates into the soils. In these instances, it is proposed that oxidation occurs as CH<sub>4</sub> migrates toward the soil surface.

Application of composted bio-solid to agricultural soils appears to also have short- and long-term effects on CH<sub>4</sub> oxidation. Jarecki and Lal (2006) noted a positive effect of compost application on CH<sub>4</sub> uptake in the months following amendment. Hütsch (2001a) noted a small inhibiting effect two years after application of fresh compost to soils. This effect was ascribed to enhanced N mineralization of fresh compost in the (sandy) soil used. However, the short-term effect of mature compost was an enhancement of CH<sub>4</sub> oxidation (28 %) immediately after application. The increase in CH<sub>4</sub> oxidation probably resulted from an inoculation with



methanotrophic bacteria concomitantly applied with the mature compost. The enhancement of CH<sub>4</sub> oxidation was only temporary (Hütsch, 2001a) probably due to the inability of these additional microorganisms to survive for longer periods of time.

#### *Site selection and amendment*

Based on the results obtained under Objectives 1 and 2, we will identify sites with the widest and sites with the least divergence between actual CH<sub>4</sub> uptake (field) and potential activity as determined during laboratory incubation. Soil property data will be evaluated to test for linkages between the lack of expression and specific soil conditions. As a result of that evaluation, two groups of sites will be selected; sites exhibiting (i) low potential and low actual CH<sub>4</sub> oxidation (LL sites), and (ii) high potential but low actual CH<sub>4</sub> oxidation (HL sites). In addition to these conditions, farmers' willingness to allow future access to the site will be another factor in our selection.

A field experiment will be conducted using plots established at the sites thus identified and selected. Microplots (3 m x 2 m) will be amended with organic materials such as corn residues, chicken manure, gypsum and composted bio-solids. Each treatment (including control) will be applied in triplicate. Methane fluxes and soil properties (physical and biochemical) will be monitored as described in Objective 1. Our goal is to maintain these plots beyond the duration of the present study. Periodic (yearly) evaluation of the oxidation potential and soil physico-chemical properties of the amended-soils will be conducted to determine whether the effects of soil amendments on CH<sub>4</sub> fluxes were transient or can be sustained on a long-term basis.

#### **Analysis of air samples**

Gas samples will be analyzed for CH<sub>4</sub>, CO<sub>2</sub> and O<sub>2</sub> with a Varian (CP-3800) gas chromatograph equipped with a Combipal headspace auto-sampler. All analyses of the gas samples will be conducted in the same laboratory with proper blanks and positive controls. To prevent moisture buildup, analytical columns will be baked for 10 min (120 °C) after every 20 samples. Verification samples (gas standards) will also be included to account for instrument stability. Analysis of samples for CO<sub>2</sub> and CH<sub>4</sub> will be conducted using an Hayesep DB column (300 cm long, 0.3 cm id) connected to a thermal conductivity detector (100 °C, for CO<sub>2</sub> detection) in series with a flame ionization detector (FID at 150 °C, for CH<sub>4</sub> detection). Analytical conditions will be as follows: carrier gas (Helium: 20 mL min<sup>-1</sup>), flame gases (Hydrogen: 25 mL min<sup>-1</sup> and hydrocarbon-free compressed air: 300 mL min<sup>-1</sup>) and oven temperature 90 °C. Carbon dioxide, CH<sub>4</sub> and N<sub>2</sub>O standards for instrument calibration will be purchased from Alltech (Deerfield, IL), and methyl chloride will be obtained from Matheson Tri-Gas (Montgomeryville, PA). Stable isotope composition of CH<sub>4</sub> in soil air samples will be determined using GC-IRMS at the UC Davis Stable Isotope Facility.

#### **VI. Division of Tasks and Schedule of Activities**

A multi-disciplinary research team has been assembled and will bring considerable research experience in soil physics and biogeochemistry - areas of expertise that are needed to successfully address the questions outlined in the proposal. In addition, this team has been involved in several collaborative projects during the last 15 years. Dr. P.A. Jacinthe (PI, Assistant Professor of Soil Biogeochemistry) has a strong record of research and publication in the areas of nitrogen dynamics and soil-atmosphere exchange of trace gases. He will have overall coordination of the project, will handle GHG analysis and will supervise the graduate student working on the field and laboratory assessment of CH<sub>4</sub> oxidation. Dr. R. Lal (co-PI, Professor of Soil Physics) has an extensive research and publication record in the areas of soil management and carbon sequestration. He and the postdoctoral researcher will lead the assessment of soil physical properties and soil

C stocks at the farmer’s fields. Dr. W. A. Dick (Co-PI) is a Professor of Soil Biochemistry with expertise in soil tillage, soil enzymes and nutrient cycling. He, the graduate student and the technician under his supervision will coordinate trace gas sampling activities at the farmers’ fields. He will also handle the installation and maintenance of the micro-plots established under Obj 3. During the last 2 decades, co-PI W. Dick has done extensive work with gypsum by-product to improve soil aeration in no-tillage croplands.

The work described in the proposal is no doubt quite ambitious. However, with careful planning, we believe that we have put together a strong team to accomplish the tasks described. In the budget, we have included funding for one Post-doc and two graduate students. Our field activities will be labor-intensive, and to assist with this work, funding for a Technician (0.2 FTE) during the spring/summer field season also budgeted.

Table 4. Schedule of activities (May 2009 - April 2012).

Task	Year 1	Year 2	Year 3
Recruitment of project personnel	█		
Identification of study areas (Obj. 1 and 2)	█		
Construction/installation of soil air chambers (Obj.1 and 2)	█		
Installation of soil air chambers (Obj. 2)	█		
Determination of chemical and biological properties (Obj. 1, 2)	█	█	
Assessment of soil physical properties (Obj. 2)	█	█	
Methane oxidation in the laboratory (incubation) (Obj. 3)	█	█	█
Field measurement of GHG fluxes (Obj. 1 and 3)	█	█	█
Assessment of gross CH <sub>4</sub> production and oxidation (Obj. 2)	█	█	
Data analysis, and preparation of manuscripts			█