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Short communication

Small-scale heterogeneity in carbon dioxide, nitrous oxide and methane production from aggregates of a cultivated sandy-loam soil

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ABSTRACT

Spatial variability in carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) emissions from soil is related to the distribution of microsites where these gases are produced. Porous soil aggregates may possess aerobic and anaerobic microsites, depending on the water content of pores. The purpose of this study was to determine how production of CO_2, N_2O and CH_4 was affected by aggregate size and soil water content. An air-dry sandy loam soil was sieved to generate three aggregate fractions (<0.25 mm, 0.25–2 mm and 2–6 mm) and bulk soil (<2 mm). Aggregate fractions and bulk soil were moistened (60% water-filled pore space, WFPS) and pre-incubated to restore microbial activity, then gradually dried or moistened to 20%, 40%, 60% or 80% WFPS and incubated at 25 °C for 48 h. Soil respiration peaked at 40% WFPS, presumably because this was the optimum level for heterotrophic microorganisms, and at 80% WFPS, which corresponded to the peak N_2O production. More CO_2 was produced by microaggregates (<0.25 mm) than macroaggregate (>0.25 mm) fractions. Incubation of aggregate fractions and soil at 80% WFPS with acetylene (10 Pa and 10 kPa) and without acetylene showed that denitrification was responsible for 95% of N_2O production from microaggregates, while nitrification accounted for 97–99% of the N_2O produced by macroaggregates and bulk soil. This suggests that oxygen (O_2) diffusion into and around microaggregates was constrained, whereas macroaggregates remained aerobic at 80% WFPS. Methane consumption and production were measured in aggregates, reaching 1.1–6.4 ng CH_4–C kg^{-1} soil h^{-1} as aggregate fractions and soil became wetter. For the sandy-loam soil studied, we conclude that nitrification in aerobic microsites contributed importantly to total N_2O production, even when the soil water content permitted denitrification and CH_4 production in anaerobic microsites. The relevance of these findings to microbial processes controlling N_2O production at the field scale remains to be confirmed.

Soil aggregates are a dynamic component of the soil fabric, formed when soil minerals bind to organic matter and disrupted by erosive forces (wind and water) as well as human activities (e.g., tillage). Macroaggregates (>0.25 mm diameter) form around plant roots, fungal hyphae and coarse organic fragments. Macroaggregates are less structurally stable than microaggregates (<0.25 mm diameter), which result from clay and silt particles binding to extracellular polysaccharides of microbial origin (Tisdall and Oades, 1982). Water and gases circulate through macroaggregates via macropores (>10 μm diameter), while micropores with diameters less than 10 μm permit the transport of aqueous substances and diffusion of gases in microaggregates (Jocteur-Monrozier et al., 1991). Although macroaggregates and microaggregates may be located side by side in soils, they represent distinct microhabitats and thus contribute to small-scale heterogeneity in microbially mediated processes.

Information about the distribution and magnitude of microbial activity within soil aggregates could lead to the identification of "hotspots" of CO_2, N_2O and CH_4 production in agricultural fields (Parkin, 1987). However, there is contradictory evidence about the effect of aggregate size on the production of these greenhouse gases. Gupta and Germida (1988) reported less CO_2 production from microaggregates than macroaggregates, but the opposite relation was observed by several authors (Seech and Beauchamp, 1988; Beauchamp and Seech, 1990; Drury et al., 2004). Schutter and Dick (2002) did not find a relationship between CO_2 production, nitrogen mineralization and aggregate size, probably due to temporal variation in substrate availability and microbial biomass

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within aggregates. Dry-sieved macroaggregates produced less N_2O from denitrification than microaggregates (Seech and Beauchamp, 1988; Manucharova et al., 2001), but the denitrification rate increased as the wet-sieved aggregate size increased (Beauchamp and Seech, 1990). In contrast, Drury et al. (2004) reported more N_2O lost through denitrification in dry-sieved macroaggregates than microaggregates, and the trend was reversed when aggregates were ground. In rice paddy and marshland soils, macroaggregates (0.2–2 mm diameter) produced more CH_4 than microaggregates (Ramakrishnan et al., 2000; Manucharova et al., 2001). We are not aware of literature that examines how aggregation affects CH_4 production in non-flooded agricultural soils.

The inconsistent relationships between soil aggregate sizes and greenhouse gas production in the literature may be due to (1) differences in soil microbial communities among aggregate fractions (Mummey et al., 2006); (2) unequal concentrations of mineralizable C and other substrates among aggregate fractions, as discussed by Beauchamp and Seech (1990); and (3) soil preparation, aggregate separation methods and the effect of repacking aggregates with similar sizes (Ashman et al., 2003; Uchida et al., 2008). A further challenge is to select an upper size limit for macroaggregates, which varied in diameter from 5 mm (Schutter and Dick, 2002) to 8 mm (Gupta and Germida, 1988; Drury et al., 2004) and 20 mm (Seech and Beauchamp, 1988; Beauchamp and Seech, 1990) in various studies. Artificial and natural macroaggregates with $\geq$8 mm diameter possess anaerobic cores, surrounded by an aerobic outer layer with higher potential nitrification rates (Greenwood and Goodman, 1967; Sexstone et al., 1985; Hoffmann et al., 2007). Such macroaggregates may simultaneously provide a microhabitat for heterotrophic microorganisms and facultative or obligate anaerobes like denitrifiers and methanogens, depending on substrate availability and O_2 diffusion to microbial cells.

The objective of this work was to examine how soil water content affected small-scale variation in CO_2, N_2O and CH_4 production associated with macroaggregates (0.25–6 mm) and microaggregates (<0.25 mm). We hypothesized that microbially mediated processes within these aggregates are controlled principally by O_2 diffusion through pores when other factors (microbial biomass, substrate availability and aggregate separation method) were similar.

The soil used in this study was a sandy loam Typic Endoaquent containing 700 g sand kg^{-1} and 160 g clay kg^{-1} with 15.4 g organic C kg^{-1}, 1.24 g total N kg^{-1} and pH 6.1, from the Macdonald Research Farm of McGill University, Ste-Anne-de-Bellevue, Quebec, Canada (45°28' N, 73°45' W). Soil (0–10 cm depth) was collected with a shovel from a cultivated, uncropped buffer (12 m wide) between corn and soybean research plots described by Sey et al. (2008). The buffer was harrowed (10 cm depth) with an offset disk about six weeks before soil collection in early September 2004. When collected, the soil contained 150 g H_2O kg^{-1}. After sieving (<6 mm mesh) in the field, soil was air-dried (20 °C) in the laboratory for one week, then spread evenly on top of sieves with 2 mm and 0.25 mm openings, placed in a mechanical rotary sieve and shaken for 30 s to generate three aggregate fractions: 2–6 mm, 0.25–2 mm and <0.25 mm. The bulk soil used in this experiment was also sieved (<2 mm mesh).

The experiment used a factorial design to determine how gas production by soil microbiota was affected by two factors: aggregate size (2–6 mm, 0.25–2 mm, <0.25 mm and bulk soil (<2 mm)) and water content (20%, 40%, 60% and 80% WFPS). Each factorial combination (4 aggregate sizes × 4 water contents) was replicated 12 times, for a total of 192 experimental units. The experimental unit was a 160-mL serum bottle containing 40 g of air-dry soil or aggregates, moistened to 60% WFPS by misting with distilled water and gently tapped to achieve a bulk density of 1.1 g cm^{-3}. Five empty bottles were also prepared to serve as blanks for the experiment. Bottles were sealed with a septa and metal cap and pre-incubated at 25 °C for 48 h to re-establish soil microbial activity. Headspace gas was sampled from half of the bottles ($n = 96$) after 48 h to measure the pulse in gas production that occurs when dry soils are rewetted (Godde and Conrad, 1999).

After the pre-incubation period, septa and metal caps were removed from all bottles, including blanks. Three replicates from each of the 16 factorial treatments (48 bottles) were removed for soil analysis (Table 1). In the remaining 144 bottles, the water content was adjusted gradually (during 1 week) to 20%, 40%, 60% or 80% WFPS by air-drying or adding distilled water, based on the relationship:

$$M_w = \Delta\text{WFPS} \times M_s \times \left(\frac{\rho_s - \rho_b}{\rho_s \rho_b}\right) \quad (1)$$

where M_w is the mass of water added or lost through air-drying (g), ΔWFPS is the difference between the treatment WFPS and the actual WFPS (60%), M_s is the air-dry soil mass (40 g), ρ_b is the soil bulk density (about 1.1 g cm^{-3}, checked by measuring the soil height and calculating the soil volume within each bottle) and ρ_s is the soil particle density (2.65 g cm^{-3}). All bottles were then sealed

Table 1
Gas production during 48 h pre-incubation ($n = 24$; standard error of the mean in brackets) and characteristics of bulk soil and aggregate fractions following the pre-incubation period ($n = 3$; standard error of the mean in brackets)

	Bulk soil	Aggregate fraction		
		2–6 mm	0.25–2 mm	<0.25 mm
Gas production[a]				
CO_2–C (mg kg^{-1} h^{-1})	0.93 (0.04)a	0.86 (0.09)a	0.64 (0.02)b	0.75 (0.01)a,b
N_2O–N (μg kg^{-1} h^{-1})	0.53 (0.22)b,c	0.96 (0.22)b	0.18 (0.23)c	4.03 (0.74)a
CH_4–C (ng kg^{-1} h^{-1})	4.3 (0.44)b	0.43 (0.38)c	7.6 (0.41)a	7.8 (0.86)a
Soil analyses[a]				
NO_3–N (mg kg^{-1})[b]	39.0 (1.5)b	52.9 (1.5)a	35.1 (1.8)b,c	30.3 (1.5)c
NH_4–N (mg kg^{-1})[b]	3.9 (0.2)b	2.7 (0.2)c	6.2 (0.2)a	6.9 (0.2)a
Mineral N (mg kg^{-1})[b]	42.9 (1.6)b	55.6 (1.6)a	41.3 (2.0)b	37.2 (1.6)b
Dissolved organic N (mg kg^{-1})[c]	9.7 (1.8)a,b	6.8 (1.8)b	12.9 (2.2)a,b	16.4 (1.8)a
Microbial biomass N (mg kg^{-1})[d]	13.9 (1.5)a	8.3 (1.8)a,b	7.2 (1.8)a,b	4.8 (1.8)b
Dissolved organic C (mg kg^{-1})[e]	21.8 (1.8)a	14.7 (1.8)b	26.2 (3.5)a	21.9 (1.8)a

[a] Within a row, values followed by the same letter are not significantly different based on a post-hoc Tukey's HSD test at $a = 0.05$.
[b] 0.5 M K_2SO_4 extracts measured with a flow-injection autoanalyzer (Lachat Instruments, Milwaukee, WI). Mineral N = NO_3–N + NH_4–N.
[c] Alkaline persulfate digest of 0.5 M K_2SO_4 extract (Cabrera and Beare, 1993).
[d] Chloroform fumigation–direct extraction, followed by alkaline persulfate digestion, corrected for extraction efficiency with $K_{EN} = 0.54$ (Brookes et al., 1985).
[e] Wet combustion of 0.5 M K_2SO_4 extract at 680 °C (Shimadzu TOC-V analyzer, Kyoto, Japan).

with a septa and metal cap. Each factorial treatment ($n = 16$) had three replicate bottles without acetylene, three replicate bottles with 10 Pa (0.01% v v^{-1}) of acetylene and three replicate bottles with 10 kPa (10% v v^{-1}) of acetylene. For bottles with acetylene treatment, we first extracted 0.01% or 10% of the air using a gas-tight syringe and injected acetylene to replace the volume of air extracted from the headspace plus soil pore space. All bottles were then incubated at 25 °C for 48 h before headspace gases were sampled.

Gas samples (20 mL) were taken with a gas-tight syringe, injected into evacuated 12 mL exetainers (Labco, Wycombe, UK) and analyzed with a gas chromatograph (Varian Model 3800, Walnut Creek, CA) equipped with automated valve injectors to simultaneously quantify CO_2, N_2O and CH_4 concentrations in ppm v v^{-1} units (Rochette and Hutchinson, 2005). The production of CO_2, N_2O and CH_4 in the headspace was calculated based on Holland et al. (1999), after converting gas concentrations from ppm (equivalent to $cm^3\ m^{-3}$) to mass per volume concentrations (d, g of C or N m^{-3}) with the ideal gas equation and the molecular mass (M, g mol^{-1}) and C or N content (a, g mol^{-1}) of each gas (e.g., $CO_2 = 12$ g C mol^{-1} CO_2):

$$d = \frac{MaP}{RT} \tag{2}$$

where P is atmospheric pressure (≈ 1 atm), R is the ideal gas constant (82.06 atm cm^3/mol K) and T is the incubation temperature (298 K). Multiplying d (g of C or N m^{-3}) by the headspace volume ($\approx 1.24 \times 10^{-4}\ m^3$) gave the mass ($C_1$) of CO_2–C, N_2O–N or CH_4–C inside the bottle (mg $bottle^{-1}$) at the end of the incubation. Gas production f (i.e., mg CO_2–C kg^{-1} soil h^{-1}) was then estimated as:

$$f = \frac{(C_1 - C_0)}{(m \times t)} \tag{3}$$

where C_0 is the gas concentration in the blanks, m is the mass of air-dry soil in each bottle (0.04 kg), and t is the incubation period (48 h).

Acetylene added at 10 kPa (10% v v^{-1}) was assumed to inhibit N_2O production from nitrification and prevent N_2O reduction to N_2 (Yoshinari et al., 1977; Davidson et al., 1986; Garrido et al., 2002). The 10 Pa (0.01% v v^{-1}) acetylene treatment was assumed to inhibit nitrification, but not N_2O reduction to N_2 (Davidson et al., 1986).

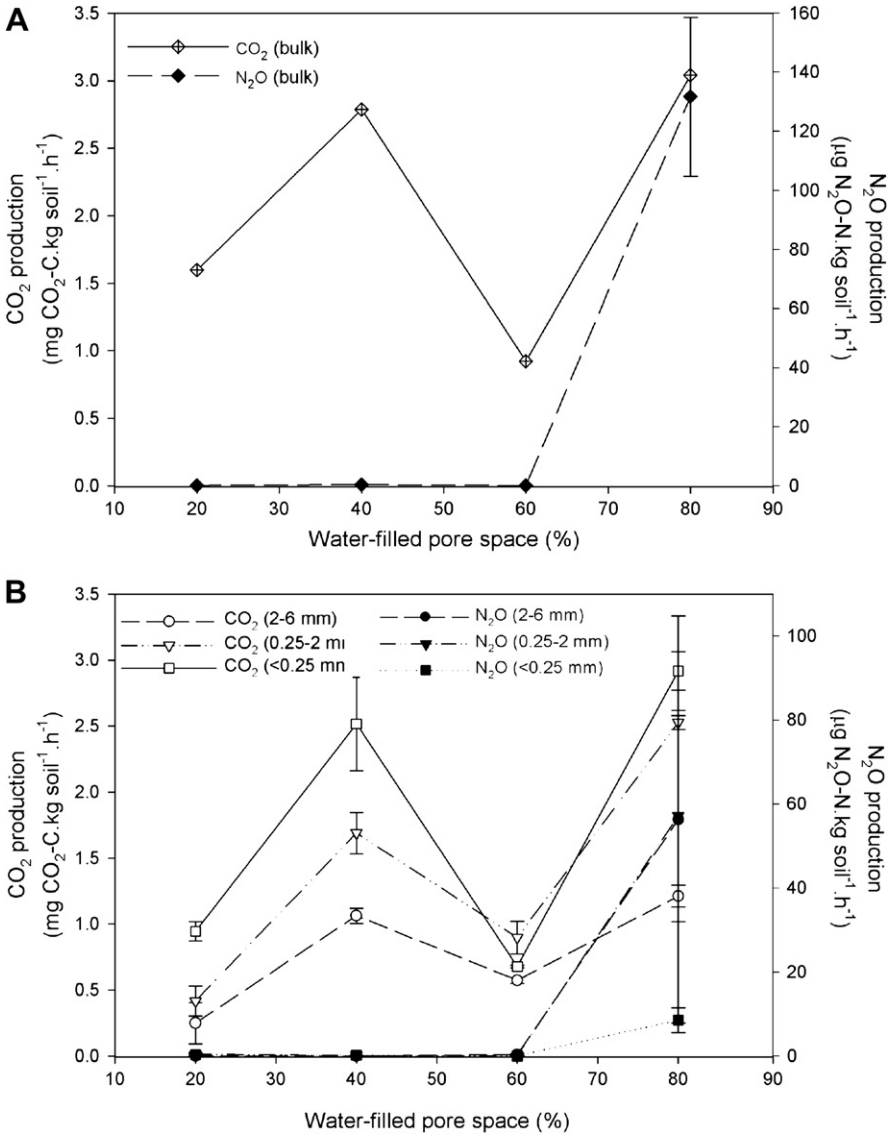

Fig. 1. Carbon dioxide and nitrous oxide production from (A) bulk soil and (B) aggregate fractions, as affected by soil water content (water-filled pore space, WFPS). Values are the mean and standard error ($n = 3$).

Production of N_2O by the denitrification process was estimated from the $N_2O + N_2$ (accumulated as N_2O) in the headspace of bottles treated with 10 kPa acetylene. Production of N_2O by the nitrification process was estimated as the difference in N_2O concentration between bottles treated with 10 Pa acetylene and those without acetylene addition. Bottles containing bulk soil or aggregate fractions moistened to 20%, 40% or 60% WFPS generated less than 0.1 ng N_2O–N g^{-1} h^{-1} when incubated in the presence of 10 kPa or 10 Pa of acetylene, so denitrification and nitrification processes were examined in bottles with bulk soil or aggregate fractions moistened to 80% WFPS.

The CO_2 and N_2O production data were transformed with a $(\ln + 1)$ function, and CH_4 production data was transformed with a square root function. The normal distribution and homogeneity of variance of the transformed data was verified with a Shapiro–Wilks test using the PROC UNIVARIATE function of SAS statistical software (version 9.1, SAS Institute Inc., Cary, NC) before proceeding with analysis of variance (ANOVA). The effect of aggregate size on gas production and soil analyses during the pre-incubation period was evaluated with one-factor ANOVA using the PROC GLM function of SAS. When treatment effects were significant ($P < 0.05$), means were compared with a post-hoc Tukey's Honestly Significantly Different test (Tukey's HSD) at the 95% confidence level. The effect of aggregate size and soil water content on gas production was tested with two-factor analysis of variance using the PROC MIXED function of SAS with the appropriate random error term. Model residuals were normally distributed (PROC UNIVARIATE, Shapiro–Wilks test) and significance tests corrected for heterogeneous variance (Littell et al., 2006).

Gas production during the pre-incubation period indicated a pulse of microbial activity that produced as much as 0.93 mg CO_2–C $kg^{-1}h^{-1}$, between 0.18 and 4.03 μg N_2O–N kg^{-1} h^{-1} and up to 7.81 ng CH_4–C kg^{-1} h^{-1} (Table 1). During the pre-incubation, N_2O production was evident especially from the <0.25 mm fraction (Table 1). The 2–6 mm fraction produced less CH_4 than the bulk soil and aggregate fractions <2 mm (Table 1). At the end of the pre-incubation period, there was more microbial biomass N in bulk soil than the <0.25 mm fraction, with intermediate microbial biomass N concentrations in the 2–6 mm and 0.25–2 mm fractions (Table 1).

The pre-incubated bulk soil and aggregate fractions contained 2.7–6.9 mg NH_4–N kg^{-1} and at least 30 mg NO_3–N kg^{-1} (Table 1). Nitrification is limited when the NH_4–N concentration falls below 0.5 mg N kg^{-1} (Garrido et al., 2002), while NO_3–N concentrations less than 20 mg N kg^{-1} may limit denitrification (Myrold and Tiedje, 1985a,b). Hence, nitrification and denitrification processes in bulk soil and aggregate fractions were probably not limited by N substrates. The DOC pool was similar for bulk soil and aggregate fractions <2 mm (Table 1). The DOC pool is related to CO_2 production (Seto and Yanagiya, 1983) and the availability of C substrates was identified as a limiting factor for nitrification and denitrification processes (Burford and Bremner, 1975; Myrold and Tiedje, 1985a; Seech and Beauchamp, 1988; Stevens et al., 1997). We suggest that the available C pool was similar in bulk soil and aggregate fractions <2 mm after the pre-incubation period.

The CO_2 production was affected by aggregate size ($F_{3,47} = 54$, $P < 0.0001$), and was greater from bulk soil than aggregate fractions (Fig. 1a, b). As expected, soil water content affected the CO_2 production ($F_{3,47} = 121$, $P < 0.0001$), with peaks at 40% and 80% WFPS (Fig. 1a, b). The relationship between CO_2 production and soil water content was not consistent with Linn and Doran (1984), who reported that O_2 consumption, CO_2 production and nitrification by aerobic microorganisms reached a plateau between 40% and 60% WFPS and declined as soils became progressively more anaerobic. Our results indicate that aerobic microorganisms were most active within a narrower range of soil water (around 40% WFPS), possibly because O_2 diffusion to sites of microbial activity was constrained as soils became wetter. Skopp et al. (1990) noted that soil respiration is optimal near 60% WFPS for many soils, but could be lower if soil microbial growth was limited due to insufficient substrates, the removal of toxic byproducts or by interactions with other microbiota, e.g., predation or parasitism. The peak in CO_2 production at 80% WFPS corresponded to an exponential increase in N_2O production, suggesting that CO_2 was from the N_2O-producing bacteria.

The greatest N_2O production was measured at 80% WFPS (Fig. 1a, b), which is consistent with other reports (Linn and Doran, 1984; Myrold and Tiedje, 1985a). When no acetylene was added to block N reduction, the <0.25 mm fraction had the lowest N_2O production, probably because complete denitrification ($N_2O \rightarrow N_2$) occurred in this fraction (Fig. 1b). When acetylene was added to block N reduction, total N_2O production (from denitrification plus nitrification) was similar among bulk soil and aggregate fractions ($F_{3,11} = 1.47$, NS), but it was apparent that denitrification generated most of the N_2O produced by the <0.25 mm fraction while nitrification was the dominant process in bulk soil and aggregates >0.25 mm (Fig. 2). One explanation, which is consistent with our hypothesis, is that there was more O_2 diffusion from the headspace into the pores of macroaggregates >0.25 mm than microaggregates. Air contained in the headspace was about 21 kPa O_2 during the 48 h incubation. Horn (1994) reported that an artificial sandy-loam soil had an O_2 partial pressure of about 7 kPa when the soil water potential was −10 kPa. The sandy-loam soil (bulk soil) in this study had a water potential of about −5 kPa at 80% WFPS, so the O_2 partial pressure was likely less than 7 kPa, but probably not lower than 0.35 kPa, the critical level for nitrification (Khalil et al., 2004). We cannot rule out the possibility that ammonium oxidizers, denitrifiers and their substrates were spatially segregated, with ammonium oxiders most abundant in macroaggregates (>0.25 mm) and a dominant denitrifier community in microaggregates (<0.25 mm). In artificial aggregates, anaerobic zones were preferentially colonized by denitrifiers, but the indigenous denitrifier community was similar at the surface and in the centre of a 30 mm macroaggregate (Philippot et al., 1996). Mummey et al. (2006) showed that the Gemmatimonadetes and Actinobacteria subdivision Rubrobacteridae were most abundant within microaggregates, while Acidobacteria were associated with macroaggregates (>0.25 mm), but whether these bacteria function as ammonium oxiders or denitrifiers was not discussed.

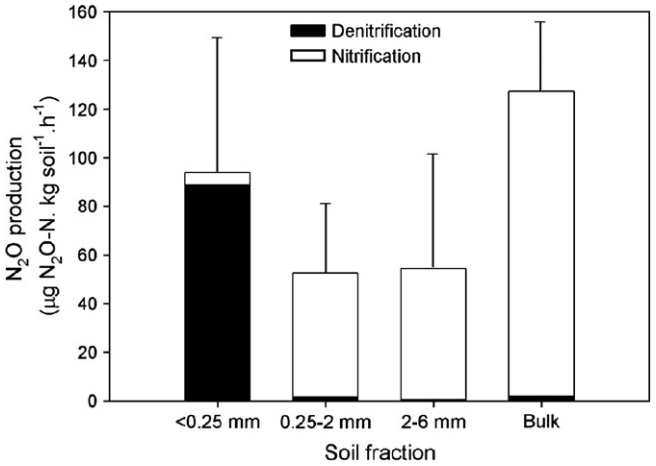

Fig. 2. Nitrous oxide production from denitrification and nitrification processes, determined after bulk soil and aggregate fractions were exposed moistened to 80% WFPS and 10 kPa ($n = 3$) or 10 Pa acetylene ($n = 3$) for 48 h. Standard error bars represent the variation associated with the dominant process that produced N_2O in each soil fraction.

Bulk soil and macroaggregates of 2–6 mm produced CH_4 at every studied water content and the maximum CH_4 production rates were measured at 80% WFPS for bulk soil and at 40% for 2–6 mm macroaggregates (Fig. 3). In contrast, smaller aggregates (<0.25 mm and 0.25–2 mm) consumed CH_4 (as much as 2.6 ng CH_4–C kg^{-1} h^{-1} at 20% WFPS) and produced CH_4 at higher water contents (Fig. 3). The significant increase in CH_4 production with increasing soil water ($F_{3,45} = 12.7$, $P < 0.0001$) was consistent with the expectation that methane producers are more active when soils are water-logged and aeration reduced (Yavitt et al., 1990). It is notable that CH_4 consumption was numerically largest in the <0.25 mm fraction at 20% WFPS, while CH_4 production was similar in bulk soil and the <0.25 mm fraction at 80% WFPS (Fig. 3). This may indicate that microaggregates <0.25 mm provide a habitat for methane-consuming and methane-producing microorganisms, but this remains to be confirmed in non-flooded agricultural soils. Since the methanogens are strictly anaerobic, they must occupy a different niche than the methane-consuming methanotrophs and other aerobic microorganisms (i.e., nitrifiers). We propose that methanotrophs are active in the boundary between oxic and anoxic zones, which may be found within microaggregates or between the microaggregate particles in repacked soil, but this remains to be confirmed. Chan and Parkin (2001) reported that agricultural soils act as a small sink, consuming as much as 290 ng CH_4–C kg^{-1} h^{-1}, or a small source of CH_4, producing up to 960 ng CH_4–C kg^{-1} h^{-1} in an arable site with periodic flooding. As far as we know, this is the first report showing how soil aggregation and water conditions affect methanogens and methanotrophs.

Water is essential for microbial survival and activity. The effect of soil water on CO_2 production was described by Skopp et al. (1990) as a delicate balance between having sufficient water for substrate transport and microbial requirements, and adequate O_2 for respiration. When O_2 diffusion to microhabitats is constrained and substrate availability is not limited, anaerobic processes like denitrification and methane production are expected to dominate. Since the soil matrix contains aggregates of various sizes, it is likely that the results of this study are relevant to processes occurring in the field, although we cannot overlook the fact that the porosity and substrate availability for microorganisms in an air dried soil, physically separated, repacked and rewetted in the laboratory is quite different than in a field soil. Greater production of N_2O from denitrification is expected from microaggregates (<0.25 mm) when the soil water content exceeds 60% WFPS, however, the importance of N_2O produced by nitrification in macroaggregates (>0.25 mm) cannot be ignored. There are other reports of nitrification as the dominant process leading to N_2O production from arable soils when the O_2 concentration is not limiting (Stevens et al., 1997; Khalil et al., 2004). In coarse-textured soils, it is possible that much of the N_2O production is from nitrification rather than denitrification, but this remains to be confirmed under field conditions.

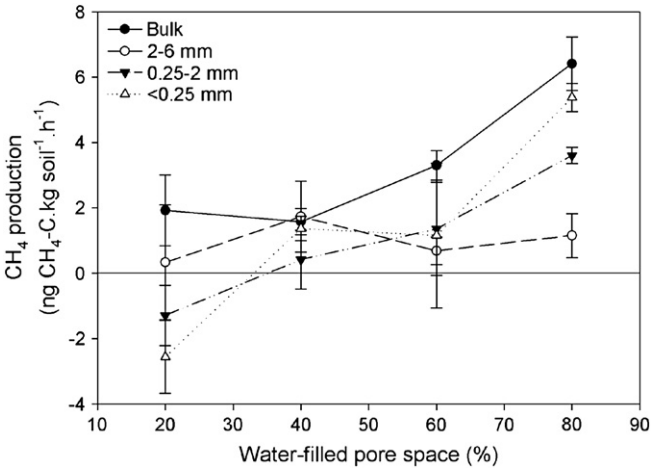

Fig. 3. Methane production from bulk soil and aggregate fractions, as affected by soil water content (water-filled pore space, WFPS). Negative values indicate CH_4 consumption. Values are the mean and standard error ($n = 3$).

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