Environ Sci Pollut Res (2014) 21:3783–3791
DOI 10.1007/s11356-013-2355-9

RESEARCH ARTICLE

Compaction stimulates denitrification in an urban park soil using ^{15}N tracing technique

Shun Li · Huan Deng · Christopher Rensing · Yong-Guan Zhu

Received: 2 September 2013 / Accepted: 7 November 2013 / Published online: 28 November 2013

Abstract Soils in urban areas are subjected to compaction with accelerating urbanization. The effects of anthropogenic compaction on urban soil denitrification are largely unknown. We conducted a study on an urban park soil to investigate how compaction impacts denitrification. By using ^{15}N labeling method and acetylene inhibition technique, we performed three coherent incubation experiments to quantify denitrification in compacted soil under both aerobic and anaerobic conditions. Uncompacted soil was set as the control treatment. When monitoring soil incubation without extra substrate, higher nitrous oxide (N_2O) flux and denitrification enzyme activity were observed in the compacted soil than in the uncompacted soil. In aerobic incubation with the addition of $K^{15}NO_3$, N_2O production in the compacted soil reached 10.11 ng N h^{-1} g^{-1} as compared to 0.02 ng N h^{-1} g^{-1} in the uncompacted soil. Denitrification contributed 96 % of the emitted N_2O in the compacted soil and 36 % of the emitted N_2O in the uncompacted soil; total denitrification rate was higher in the compacted soil (up to 79.35 ng N h^{-1} g^{-1}) than in the uncompacted soil (0.11 ng N h^{-1} g^{-1}). Under anaerobic incubation with the addition of $K^{15}NO_3$, no statistical difference in total N losses and ^{15}N-(N_2O+N_2) flux between the uncompacted soil and the compacted soil was detected. Compaction promoted soil denitrification and may impact urban N biogeochemical cycling.

Keywords Compaction · Nitrous oxide · Denitrification · Urban soil

Responsible editor: Zhihong Xu

S. Li · Y.-G. Zhu (✉)
Key Laboratory of Urban Environment and Health, Institute of Urban Environment, Chinese Academy of Sciences, 361021 Xiamen, China
e-mail: ygzhu@rcees.ac.cn

H. Deng
Department of Environmental Sciences and Engineering, Nanjing Normal University, 210023 Nanjing, China

C. Rensing
Department of Plant and Environmental Sciences, University of Copenhagen, Frederiksberg, Denmark

S. Li
University of Chinese Academy of Sciences, 100049 Beijing, China

Y.-G. Zhu
State Key Lab of Urban and Regional Ecology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, 100085 Beijing, China

Introduction

Compaction occurs under rapid urbanization in urban soils, which is principally caused by the use of heavy machinery, the relocation of building materials, and trampling by humans, especially near sidewalks or driveways, at construction sites, and on public green space (Jim 1998; Pouyat et al. 2007). Compaction can affect C and N biogeochemical cycles through directly changing soil physical properties (Lorenz and Lal 2009). Compaction disrupts soil structure, increases bulk density and penetration strength, as well as decreasing aeration porosity and gas diffusivity. These changes lead to a higher probability of anaerobic conditions in soils.

Denitrification is a key process involved in nitrogen cycling and is mainly driven by denitrifiers which reduce nitrate and nitrite to gaseous nitrous oxide (N_2O) and dinitrogen (N_2). The intermediate N_2O is a powerful greenhouse gas that contributes to global warming (Schlesinger 2009) and causes stratospheric ozone destruction (Ravishankara et al. 2009). Soil is a predominant contributor to the atmospheric N_2O, mostly released by the microbial processes of nitrification and denitrification (Thomson et al. 2012). The increased anaerobic micro-sites in soils induced by compaction are probably favorable for denitrification and can increase the emission of N gasses into the atmosphere (Bakken et al. 1987; Ruser et al. 2006). In view of

this, soil compaction may have a pronounced impact on urban N cycling, particularly for denitrification. Since this physical degradation of soils is permanent and difficult to restore, its effects on urban biogeochemistry might be irreversible.

Numerous studies have examined the factors that influence different pathways involved in denitrification, such as nitrate (Magalhães et al. 2005), carbon availability (Henderson et al. 2010), pH (Liu et al. 2010), and copper concentration (Magalhães et al. 2011; Felgate et al. 2012). Nevertheless, compaction, as a physical factor, has not received as much attention as the above chemical factors associated with denitrification. Furthermore, related studies mainly focused on the impact of compaction on soil N_2O emission in agriculture systems and forest systems (Sitaula et al. 2000; Yamulki and Jarvis 2002; Teepe et al. 2004; Bhandral et al. 2007). At present, limited data are available on denitrification (Bakken et al. 1987; Douglas and Crawford 1993), particularly lacking quantitative evidence on denitrification in urban soils. However, urban soil compaction is also widespread and is an increasingly important issue due to urbanization, most probably altering N transformations in urban ecosystems. In addition, pathways of compaction impacting denitrification in urban soils may differ from agricultural soils. To our knowledge, nitrogen input into urban soils is largely nitrate through atmospheric deposition, while in agricultural soils, ammonium predominates the N input via fertilization. Urban soils could enrich nitrate and provide the substrate for denitrification. Bhandral et al. (2007) reported that in compacted soil, the highest N_2O was emitted from the treatment with nitrate application compared to those from other N sources like urine, ammonium, and urea, suggesting that denitrification in soils with high nitrate could probably be promoted under compaction. Therefore, studying the effects of compaction on urban soil denitrification is needed for better understanding on how urbanization affects urban N biogeochemical cycling and urban greenhouse gas emission. Methodologically, most relevant studies examining compaction were conducted in field trails where N_2O emissions may depend on spatiotemporal variation and other variables like climate (Bakken et al. 2012). In contrast, laboratory experiments actually diminish the influence of uncontrollable factors and limit any intrinsic soil heterogeneity. Therefore, in this study, we compressed an urban park soil manually in the laboratory and conducted incubations to understand the mechanisms involved.

To investigate how soil compaction impacts denitrification, we designed three different experiments. In the first experiment, we incubated soils without any additional substrates, such as water or nitrate, to examine how compaction impacts N_2O emissions and denitrification enzyme activity (DEA) under pristine conditions. In the second experiment, we combined ^{15}N labeling with acetylene inhibition to quantify denitrification with a series of indicators in compacted and uncompacted soils under an aerobic incubation. In the third experiment, to check whether compaction regulates denitrification by controlling oxygen (O_2) availability within the soil, we conducted an anaerobic incubation using ^{15}N labeling, and we hypothesized that if initial O_2 were eliminated, the following compaction would have no impact on soil denitrification.

Materials and methods

Soil sampling

In January 2013, upper soil samples (0–15 cm in depth) were collected from Xingdong Park, which is located in Xiamen City, Fujian Province of China (24 °33′N, 118 °02′E). The local climate is subtropical oceanic with an average annual temperature of 21 °C and a mean annual precipitation of 1, 200 mm. Vegetation in the sampling site consists mainly of *Zoysia japonica*. The soil has never received fertilizer. After sampling, the fresh soil samples were passed through a 2-mm mesh, then stored at 4 °C until use and partially air-dried for chemical analysis. The soil was classified as loamy sand (77.82 % sand, 21.03 % silt, and 1.15 % clay) containing 25.7 g kg^{-1} total C, 2.2 g kg^{-1} total N, and 0.5 g kg^{-1} total S with an initial pH value of 7.3.

Experiment 1: Measurement of background soil N_2O emission and DEA

The experiment was conducted to check how compaction influences the background soil N_2O and CO_2 flux as well as observe changes in DEA under laboratory incubation. Twenty grams of freshly sieved soils were transferred to 60 ml serum bottles. For the compacted treatment, the soil was hand compressed to generate a final bulk density of 1.58 g/cm^3. For the uncompacted control treatment, the bulk density of soil without compression was 1.02 g/cm^3. For each treatment, 13 replicates containing the surrounding atmosphere were sealed with butyl rubber stoppers and aluminum caps. Soils were incubated at 25 °C in an incubator under dark conditions for 3 days. Four specific flasks were used to determine N_2O and CO_2 concentrations in the headspace at time zero and subsequently every 12 h. At 12, 36, and 72 h, three randomized replicates of the reaming flasks were selected, and soils in these bottles were sampled to assess the soil DEA by using a robotize incubation system (Molstad et al. 2007). DEA was determined in soil slurries amended with unlimited NO_3^- by anaerobic incubation (Myrold and Tiedje 1985; Well et al. 2005). Six grams of field moist soil

(six replicates) were weighed into a 120-ml serum bottle, and 6 ml KNO_3 solution (100 mg N kg^{-1}) was added as denitrification substrate with a magnetic bar. Immediately, afterwards, all bottles were capped with butyl rubber stoppers and aluminum caps. These samples were made anaerobic by vacuumizing and flushing with pure He for four cycles and finally vented to atmospheric pressure. Half of the flasks were injected with 10 % *v*/*v* C_2H_2 to inhibit nitrification and arrest the reduction process of N_2O to N_2. Consequently, the N_2O accumulated in the headspace was regarded as total denitrification flux, while the other half without C_2H_2 was used to quantify N_2O emission rates. Prior to use, the C_2H_2 was purified by scrubbing through a high concentration of H_2SO_4 and pure water to remove the acetone involved (Gross and Bremner 1992). The moment of acetylene injection was considered the start of incubation (denoted as 0 h). All flasks were instantly incubated in a water bath at 25 °C for 5 h to attain the full diffusion of acetylene into the soil samples, while gas monitoring was only conducted at 0.5 h after the C_2H_2 injection and at 5 h of the incubation.

Experiment 2: Quantification of soil denitrification under atmospheric incubation

The ^{15}N labeled method was combined with the acetylene inhibition technique to quantify the denitrification of both compacted and uncompacted soils under aerobic incubation. Three milliliters of labeled $K^{15}NO_3$ solution (30 mg N kg^{-1}, 25 atom% ^{15}N) was uniformly sprayed onto 300 g fresh soils in a plastic valve bag and homogenized by incessantly kneading and shaking; these samples were then mixed with non-spiked soil at the ratio of 1:3 and homogenized again. The change in soil gravimetric water content was negligible. Afterwards, soils containing label ^{15}N tracer of 20 g were immediately placed in 60 ml serum bottles and were compressed in the same way as the first experiment. For the uncompacted and compacted treatments, in each made six replicates, deionized water was added by pipetting uniformly over the soil surface to attain a residual moisture of 30 % (*w*/*w*) that was favorable for denitrification. The moistening procedure caused minor change in the volume occupied by the soils; hence, the bulk densities for uncompacted and compacted treatments were in fact 1.05 and 1.46 g/cm^3, respectively. The samples were sealed with butyl rubber stoppers and aluminum caps. For each treatment, three replicates were injected with 10 % *v*/*v* C_2H_2 by replacing inner atmosphere in the same volume to measure total denitrification. To attain the inner gasses' equilibrium and accelerate the diffusion of C_2H_2 into the soils, the plunger of the syringe was pumped up and down several times. Another three flasks were added without C_2H_2 to assess the N_2O production flux. Incubation was performed at 25 °C in an incubator under dark conditions throughout a 2-day period. At time zero and after 48 h of incubation, the changes of N_2O and CO_2 concentrations in all flasks atmosphere were determined by robotize sampling system. For time zero, the ^{15}N content of N_2O in flasks without C_2H_2 was identified by testing the background atmosphere, soil NO_3^-, NH_4^+ contents, and ^{15}N-NO_3^- enrichment were measured just after the ^{15}N mixing and before the start of incubation. For 48 h, after N_2O and CO_2 measurement, the flask atmosphere of 12 ml was sampled with a gas-tight syringe from bottles without C_2H_2 and stored in 12 ml pre-evacuated glass vials for subsequent ^{15}N-N_2O analysis. Soils collected from these flasks were used to determine NO_3^-, NH_4^+ concentrations as well as ^{15}N content in nitrate.

Experiment 3: Assessment of soil denitrification under anaerobic incubation

We investigated whether compaction influences denitrification when the original O_2 within soil is removed under anaerobic condition. Soils after ^{15}N mixing in experiment 2 (20 g) were used for the uncompacted and compacted treatments, each with three replicates. The corresponding bulk densities were achieved as in experiment 1. The flasks were then transferred into an anaerobic operating system filled with pure He (Shel Lab Bactron IV, USA) while compaction was processed in an anaerobic box without the addition of extra deionized water. Compacting procedure performed under the anaerobic condition can avoid differentiation in inner soil O_2 which may occur if compacting was conducted under the aerobic condition. Bottles were immediately transferred out and made anaerobic by vacuumizing and flushing with pure He for four cycles before finally being vented

to atmospheric pressure. Incubation was performed in an incubator at 25 °C for 4 h. After 4 h, gas samples of 0.5 ml were collected using a 1-ml gas-tight syringe and then injected into 12 ml He-filled glass vials for analysis of ^{15}N content in N_2. These steps were processed in the same anaerobic operating system as above in order to prevent the contamination of atmospheric N_2. Immediately, afterwards, the N_2O and N_2 concentrations in the headspace were measured by gas chromatography, followed by collection of a 12-ml gas sample before being injected into a 12-ml pre-evacuated glass vial for ^{15}N-N_2O analysis. Soils in these bottles were also collected for determination of NO_3^- and NH_4^+ concentrations.

Soil and gas measurements

Soil pH was determined with a Dual channel pH/Ion/Conductivity/Do meter (X60, Fisher Scientific) as a soil–water suspension (1:2.5 *w*/*v*). Particle size distribution was analyzed by Laser particle size analyzer (Malvern Mastersizer 2000, UK). Total carbon, nitrogen, and sulfur were determined by an element analyzer (Vario MAX CNS, Germany). Soil NO_3^- and NH_4^+ were extracted in deionized water (Fang et al. 2012) and assessed by ion chromatography (Dionex ICS-3000, USA).

The enrichment of ^{15}N-NO_3^- was determined using the chemical reduction method reported by Cao et al. (2013) which was initially developed by Stevens and Laughlin (1994). Briefly, soil extracts were mixed with sulfamic acid solution to remove any NO_2^- and then transferred to He-filled bottles which contained sodium acetate–acetic acid buffer and copperized cadmium for reaction with shaking. Subsequently, gas samples were withdrawn from the headspace to measure the ^{15}N-N_2O content.

The ^{15}N enrichment in N_2O was determined by direct measurement of ion currents at *m*/*z* 44, 45, and 46 using an isotope-ratio mass spectrometer (Thermo Finnigan Delta V Advantage, Bremen, Germany) which was coupled with an automated pre-GC concentration unit. The ^{15}N content in N_2 was determined by direct measurement of ion currents at *m*/*z* 28, 29, and 30 using the mass spectrometer coupled with a GasBench II. The N_2O, CO_2, and N_2 concentrations were analyzed with an Agilent 7890 gas chromatography (Santa Clara, CA, USA) equipped with a PLOT column and a Molsieve column, as well as three detectors: a flame ionization detector, a thermal conductivity detector, and an electron capture detector. The incubation system (Molstad et al. 2007) was coupled to this gas chromatography through an autosampler, therefore, allowing direct and sequential measurement of headspace gasses.

Calculations and statistics

The percentage of water-filled pore space (WFPS) in soils was calculated using the following equation:

$$\mathrm{WFPS}\ \% = \ (\mathrm{GWC} \times \mathrm{BD})/(1-\mathrm{BD}/\mathrm{PD}) \times 100 \qquad (1)$$

Where GWC is the gravimetric water content of the soil, BD is the soil bulk density, and PD is the soil particle density with an assumed value of 2.65 g cm^{-3}.

In experiment 2, the contribution of nitrification and denitrification on N_2O production was calculated based on ^{15}N data following a method described by Stevens et al. (1997). The ^{15}N abundance of NH_4^+ was not measured in this study since the high NO_3^- content in our soil and no extra organic carbon was added; the process of dissimilatory NO_3^- reduction to NH_4^+ could be neglected (Zhu et al. 2013). Moreover, NH_4^+ concentrations in the soil extracts were too low to make a dependable determination of the ^{15}N enrichment. Thus, the ^{15}N-NH_4^+ was presumed to have retained natural abundance over the entire incubation. The gross nitrification rates were estimated by the ^{15}N isotope pool dilution model initially established by Kirkham and Bartholomew (1954) and improved by Barraclough and Puri (1995).

In all experiments, data were shown on an oven-dry basis. Independent sample *t* test was performed to determine the statistical significance of all data between treatments at the $p<0.05$ level and by using SPSS software 18.0 for Windows.

Results

Soil bulk density and WFPS

Compaction caused an increase in soil bulk density, thereby increasing soil water-filled pore space. In the uncompacted treatment of experiments 1 and 3, soil bulk density was 1.02 g/cm^3 and soil WFPS was 25 %, while in the compacted soil, the corresponding values reached 1.58 g/cm^3 and 59 %, respectively. In experiment 2, values of soil bulk density and WFPS in the uncompacted treatment were 1.05 g/cm^3 and 52 %, respectively, but in the compacted soil, the corresponding values reached 1.46 g/cm^3 and 97 %, respectively. According to the formula shown previously, under the same gravimetric water content, the variation in WFPS was owed to the modification of soil bulk density by compression.

Background N_2O and CO_2 fluxes

Fluxes of N_2O and CO_2 increased with the incubation time for both the uncompacted and compacted treatments. N_2O and CO_2 fluxes were the greatest at the initial 12 h and smaller in the subsequent incubation period. The N_2O flux was significantly higher ($p<0.05$) in the compacted treatment than in the uncompacted treatment throughout the incubation (Fig. 1a). After 72 h, the total cumulative emission of N_2O averaged at 2.17 ng N g^{-1} in the compacted soil, 60 % larger than that in the uncompacted soil (1.35 ng N g^{-1}). Similarly, the CO_2 flux was significantly higher ($p<0.05$) from the compacted treatment than from the uncompacted treatment during the entire observation period (Fig. 1b). At the end of the incubation, total cumulative flux of CO_2 was 30.02 μg C g^{-1} in the compacted soil, 35 % greater than that in the uncompacted soil (22.22 μg C g^{-1}).

Soil DEA

Instantaneous DEA for soils collected at the incubation time of 12, 36, and 72 h illustrated the different denitrification capacities between the uncompacted and compacted treatments. As shown in Table 1, a significant difference ($p<0.05$) was observed between the uncompacted and compacted soils in N_2O emission rate for DEA and N_2 production rate for DEA, as well as total DEA. For the compacted soils collected at 12, 36, and 72 h under aerobic incubation, their total DEA were 550, 484, and 478 ng N h^{-1} g^{-1}, respectively, the corresponding values for the uncompacted soils were significantly lower ($p<0.05$) than the compacted treatment, amounting to 333, 301, and 264 ng N h^{-1} g^{-1}, respectively. Respiration (CO_2 production rate) was quantified from soils incubated without acetylene since acetylene might be used as a carbon source by soil microorganisms. The anaerobic respiration rate of the compacted soil collected at the time-point of 12 h averaged 672 ng C h^{-1} g^{-1}, which was significantly higher ($p<0.05$) than that of the uncompacted soil. For soils collected at 36 and 72 h, the respiration rates showed no statistical significance between treatments. The molar ratios of N_2O to ($N_2O + N_2$) were generally larger ($p<0.05$) in the compacted treatment than the uncompacted treatment.

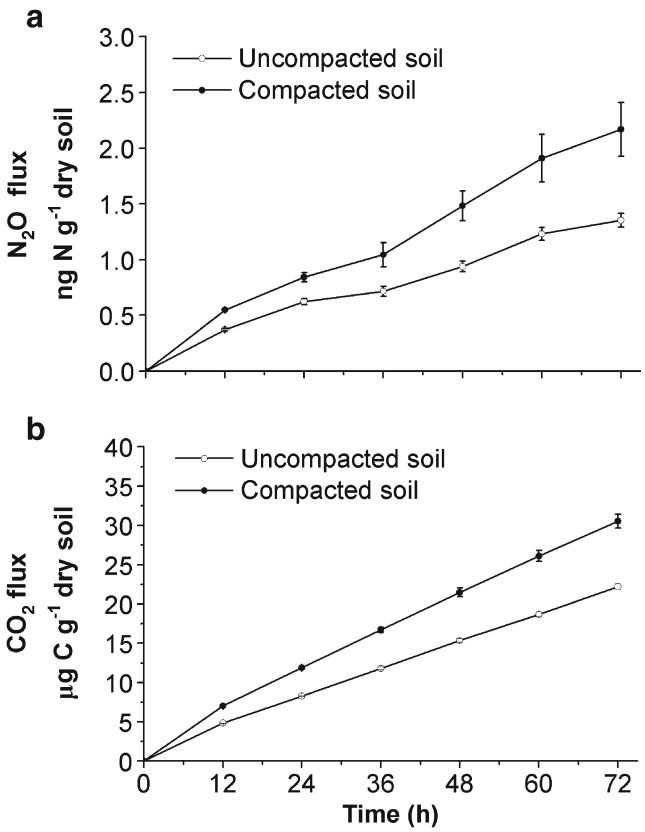

Fig. 1 Mean (±SE, $n=4$) N_2O flux (**a**) and CO_2 flux (**b**) from the uncompacted and compacted soils during the 72-h incubation

Denitrification under aerobic incubation

The compacted soil had significantly greater ($p<0.05$) N_2O production rate and total denitrification rate than the uncompacted soil (Fig. 2). For the C_2H_2-free treatment, the mean production rate of N_2O in the compacted soil (10.11 ng N h^{-1} g^{-1}) was approximately 500 times larger ($p<0.05$) than in the uncompacted soil (0.02 ng N h^{-1} g^{-1}). For the treatment with C_2H_2, the N_2O emission represented total denitrification due to the inhibitory properties of C_2H_2 on nitrification and N_2O reduction. The average total denitrification rate in the compacted soil (79.35 ng N h^{-1} g^{-1}) was approximately 700 times higher ($p<0.05$) than the uncompacted soil (0.11 ng N h^{-1} g^{-1}). Source partitioning of N_2O was estimated by comparing enrichment of ^{15}N-N_2O with ^{15}N-NO_3^- in the treatments without C_2H_2. During the 48-h observation, about 36 % of the emitted N_2O in uncompacted soil derived from denitrification, reaching 0.0075 ng N h^{-1} g^{-1},whereas in the compacted soil, up to 96 % of the released N_2O were produced during denitrification, reaching 9.68 ng N h^{-1} g^{-1} (Table 2). On average, N_2O emission from denitrification was approximately three orders of magnitude larger ($p<0.05$) in the compacted treatment than the uncompacted treatment.

The net nitrification rates were measured from the increase in NO_3^- occurring in the soils throughout the 48-h period of incubation. As shown in Table 2, soil NO_3^- in uncompacted treatment accumulated and the net increase averaged 46.15 ng N h^{-1} g^{-1}. In contrast, NO_3^- consumption was observed in compacted soil, and the net decrease averaged 104.18 ng N h^{-1} g^{-1}. Soil NH_4^+ contents of all extracts were under the detection limit, probably due to the fact that N mineralization and nitrification occurred simultaneously, and the NH_4^+ mineralized from organic nitrogen could be rapidly nitrified to NO_3^-. Under the compacted condition, the mean

Table 1 Mean (±SE, $n=3$) microbial respiration and DEA for the uncompacted and compacted soils at three specific sampling times

	Time-point (h)	Respiration (ng CO_2-C g^{-1} h^{-1})	DEA N_2O (ng N_2O-N g^{-1} h^{-1})	DEA N_2 (ng N_2O-N g^{-1} h^{-1})	DEA total (ng N_2O-N g^{-1} h^{-1})	Molar ratio N_2O/(N_2O+N_2)
Uncompacted soil	12	604.5±5.5	98.8±1.1	234.2±18.0	333.0±20.8	0.2989±0.0155
	36	641.1±35.3	95.6±4.2	205.6±5.2	301.2±4.3	0.3176±0.0126
	72	610.5±20.8	82.8±7.2	181.2±6.4	264.1±1.9	0.3137±0.0236
Compacted soil	12	672.0±12.4*	181.7±10.6*	368.5±32.1*	550.2±35.6*	0.3331±0.0257
	36	756.7±35.3	180.2±13.9*	304.0±13.1*	484.2±6.1*	0.3722±0.0251*
	72	645.3±25.8	170.4±9.1*	307.5±9.3*	478.0±5.6*	0.3567±0.0170*

*$p<0.05$ significant difference between the uncompacted and compacted soils at the same sampling time

rate of gross nitrification reached 36.45 ng N h^{-1} g^{-1}, significantly lower ($p<0.05$) than 48.25 ng N h^{-1} g^{-1} of the uncompacted treatment (Table 2).

During the 48-h incubation, the production rate of CO_2 was significantly higher ($p<0.05$) in the compacted soil than in the uncompacted soil. As shown in Table 2, mean CO_2 emission rates were 0.68 μg C h^{-1} g^{-1} and 0.51 μg C h^{-1} g^{-1} in the compacted and uncompacted soils, respectively.

Denitrification under anaerobic incubation

Denitrification rates under anaerobic incubation were expressed as the total N gas production ($N_2O + N_2$) per hour during the 4-h period. Of the denitrification rates analyzed in experiment 3, the value of 1,094.65 ng N h^{-1} g^{-1} was obtained from the compacted soil, which was not significantly different from that found in the uncompacted soil (858.98 ng N h^{-1} g^{-1}) (Table 3). With respect to ^{15}N-($N_2O + N_2$) flux, the average value in the compacted soil and the uncompacted soil also showed no significant difference, reaching 90.86 and 82.12 ng N h^{-1} g^{-1}, respectively (Table 3). Moreover, there was no significant difference in soil NO_3^- uptake rates (data not shown) for both the compacted and uncompacted treatments. As shown in Table 3, anaerobic microbial respiration in compacted soil (1.73 μg C h^{-1} g^{-1}) was significantly higher ($p<0.05$) than in uncompacted soil (1.46 μg C h^{-1} g^{-1}).

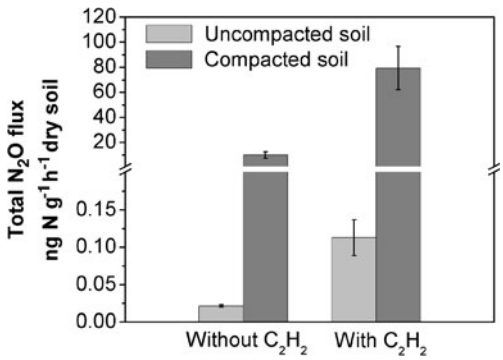

Fig. 2 Mean (±SE, $n=3$) N_2O fluxes from treatments without and with C_2H_2 in the uncompacted and compacted soils during the 48-h aerobic incubation

Discussion

Background N_2O and CO_2 emissions and DEA

Larger N_2O flux was obtained from the compacted soil in experiment 1 because compaction reduced soil pore volume and increased WFPS which in turn impeded the supply of O_2 through diffusion within soil. Consequently, this led to increased anaerobiosis which is favorable for N_2O production by denitrification (Renault and Sierra 1994; van Groenigen et al. 2005; Bhandral et al. 2007). Moreover, our results showed that the DEA increased after compression. It was primarily induced by the increased anoxic micro-sites within soils. The observed higher DEA in the compacted treatment indicated that compaction has the potential to promote soil N_2O emissions through denitrification process.

In experiment 1, the CO_2 emission measured after compaction was found to be higher than from the uncompacted treatment during the 72-h incubation. Interestingly, this finding was contradictory to most previous studies. On one hand, compaction led to a higher bulk density in the compacted soil compared to the uncompacted soil. Noteworthily, increased

Table 2 Mean (±SE, $n=3$) CO_2 flux, N_2O from denitrification, net nitrification, and gross nitrification in the uncompacted and compacted soils from C_2H_2-free treatment during the 48-h aerobic incubation

	Uncompacted soil mean ± SE	Compacted soil mean ± SE
CO_2 flux (μg C h^{-1} g^{-1})	0.508±0.009	0.684±0.018*
N_2O from denitrification (ng N h^{-1} g^{-1})	0.00752±0.0014	9.678±2.545*
Net nitrification (ng N h^{-1} g^{-1})	46.15±7.41	−104.18±19.11*
Gross nitrification (ng N h^{-1} g^{-1})	48.25±0.95	36.45±0.42*

*$p<0.05$ significant difference between the uncompacted and compacted soils

Table 3 Mean (±SE, $n=3$) respiration, denitrification products (N_2O and N_2), total (N_2O+N_2) and total ^{15}N-(N_2O+N_2) in the uncompacted and compacted soils during the 4-h anaerobic incubation

	Uncompacted soil mean ± SE	Compacted soil mean ± SE
Respiration (μg C h^{-1} g^{-1})	1.464±0.029	1.732±0.047*
N_2O (ng N h^{-1} g^{-1})	569.52±16.69	623.42±16.05
N_2 (ng N h^{-1} g^{-1})	289.46±32.79	471.23±110.95
Total (N_2O+N_2) (ng N h^{-1} g^{-1})	858.98±36.00	1094.65±96.34
Total ^{15}N-(N_2O+N_2) (ng N h^{-1} g^{-1})	82.12±2.77	90.86±2.59

*$p<0.05$ significant difference between the uncompacted and compacted soils

bulk density, in many studies, has been shown to decrease CO_2 production by (a) diminishing soil aeration and subsequently restricting the associated microbial activity (Pengthamkeerati et al. 2005; Silva et al. 2011), (b) improving the proportion of small pores which could physically protect soil organic matter against microbial degradation (Breland and Hansen 1996), and (c) reducing gas diffusivity (Shestak and Busse 2005). However, on the other hand, compaction also caused a higher WFPS in the compacted soil (59 %) than the uncompacted soil (25 %). Previous studies (Linn and Doran 1984; Doran et al. 1990) reported that soil respiration increased linearly between 30 and 60 % WFPS and attained maximum values between 55 and 61 % WFPS and further reduced above this level. In our study, we conclude that at 25 % WFPS, respiration of uncompacted soil was limited by the lower water availability which restricts solutes diffusion and bacterial motility (Sommers et al. 1981; Beare et al. 2009), whereas at 59 % WFPS, the microbial activity was improved in the compacted soil. Consequently, we consider that greater CO_2 release under compaction can mainly be attributed to increased respiration at optimal WFPS offsetting the reduction for higher soil bulk density.

Denitrification under aerobic incubation

Total denitrification flux observed in C_2H_2 treatment showed that compaction stimulated the denitrification process. Bakken et al. (1987) reported that an accumulated denitrification in the soil compacted by tractor traffic was 4 to 5 times greater than the corresponding no compaction treatment throughout a 75-day period. Douglas and Crawford (1993) also observed that the most severe compaction led to the highest total denitrification fluxes in a field trial. In the present laboratory incubation, total denitrification rates of the compacted soil and the uncompacted soil differed by nearly three orders of magnitude. For soils incubated without C_2H_2, emitted N_2O could be attributed to both nitrification and denitrification. In the C_2H_2-free treatment, both the higher proportion and the greater amount of N_2O emission due to denitrification in the compacted soil suggested that compaction exponentially increased denitrification, thus leading to a much larger N_2O flux compared with the uncompacted soil. Additionally, the compacted soil experienced a dramatic decline in NO_3^-, indicating that denitrification was the primary cause of N_2O production under compaction. In contrast, the observed accumulation of NO_3^- in the uncompacted soil supported the view that nitrification was the predominant pathway contributing to N_2O emissions. As discussed earlier, increased anoxic microsites resulting from higher bulk density and greater WFPS are conducive to denitrification. In this experiment, the high N_2O emission from compacted soil at 97 % WFPS supported the assumption of an existing threshold WFPS (65 %) above which N_2O production increased dramatically as reported by Clayton et al. (1997) and was also in agreement with the findings in other studies (Davidson 1991; Dobbie and Smith 2001; Ruser et al. 2006; Beare et al. 2009).

The gross nitrification rate determined by ^{15}N-NO_3^- pool dilution in the compacted soil was relatively lower than the uncompacted soil. It was mainly due to the reduction of soil aeration which restricted aerobic nitrification.

In experiment 2, CO_2 emission from the compacted soil was higher than the uncompacted soil. Although the high soil bulk density and especially the large WFPS (97 %) in the compacted soil could reduce aerobic microbial activity and restrict CO_2 diffusion, they also created extremely anoxic conditions which were favorable for denitrification occurrence. As a result, we believe that the faster CO_2 release in the compacted soil was attributed primarily to the greater anaerobic respiration through denitrification.

Denitrification under anaerobic incubation

As denitrification is an anaerobic process, O_2 availability which controls the activity and synthesis of denitrifying enzymes in the soil is regarded as the most important regulator (Tiedje 1988; Burgin and Groffman 2012). We hypothesized that compaction would influence denitrification predominantly by changing O_2 availability; thus, when the original O_2 within the soil was removed and the soil was incubated under the anaerobic condition, the denitrification rates may not be different between the uncompacted and compacted treatments. As expected, the results obtained from experiment 3 support this hypothesis. The significantly higher respiration in the compacted soil was probably due to its optimal WFPS (59 %) which may enhance the diffusion of solutes (Sommers et al. 1981; Beare et al. 2009). Although compaction caused changes in anaerobic respiration, no statistical difference was found in denitrification rate, ^{15}N-(N_2O + N_2) losses and soil NO_3^- consumption between uncompacted and compacted soils throughout the 4-h anaerobic period. It is suggested that,

under the strictly anaerobic conditions, compaction did not impact on denitrification. Conversely, it may indicate that compaction regulates denitrification mainly by controlling the O_2 availability and that was the reason why we primarily adopt an aerobic incubation rather than an anaerobic incubation in this study. Furthermore, exposure of soils to aerobic surroundings provided more similarity to the realistic environment when compared with anaerobic conditions.

Denitrification of urban soil under compaction

By comparing experiment 1 with experiment 2, we observed that moistening and adding nitrate magnified the distinction of denitrification and N_2O emission between uncompacted soil and compacted soil. In view of this, we notice the fact that in urban areas, growing fossil fuel combustion and industrial production can lead to increasing NO_x emissions. Consequently, urban soils are probably subjected to much greater wet and dry atmospheric N deposition than rural areas in which N input is mainly in the form of nitrate (Lovett et al. 2000; Zhu and Carreiro 2004; Pickett et al. 2011). As urban soil received nitrate which is the most important substrate for denitrification, the compacted soil with limited O_2 may have fast denitrification, especially during rainfall events. In terrestrial ecosystems, soils are the key sites for denitrification, followed by groundwater and riparian zones (Bouwman et al. 2013). Therefore, we assume that compacted urban soils may be recognized as a hot spot for denitrification and N_2O emission.

Conclusions

Our study showed that soil compaction, by reducing O_2 availability, promoted denitrification enzyme activity and stimulated N_2O emission mostly contributed by denitrification pathways. Since the compaction effect is perpetual and difficult to reinstate, in a large spatiotemporal scale, it may profoundly alter the urban soil N cycling. As such, we consider that this study provides a valuable perspective to understand how urbanization affects urban N biogeochemical cycling and urban greenhouse gas emission.

Acknowledgments This work was financially supported by the National Science Foundation of China (31270153) and the Ministry of Science and Technology (2011DFB91710). We sincerely thank Yacheng Cao from Institute of Soil Science, Chinese Academy of Sciences for his guidance in determination of ^{15}N enrichment. We thank Lars Molstad from the Norwegian UMB Nitrogen group (http://www.umb.no/nitrogengroup/) for designing and programming our robotized (incubation) system for analyzing gas kinetics. We also thank Valerie Gibson from the Institute of Urban Environment, Chinese Academy of Sciences for checking the grammar of the manuscript.

References

Bakken LR, Børresen T, Njøs A (1987) Effect of soil compaction by tractor traffic on soil structure, denitrification, and yield of wheat (*Triticum aestivum* L.). J Soil Sci 38(3):541–552

Bakken LR, Bergaust L, Liu B, Frostegård Å (2012) Regulation of denitrification at the cellular level: a clue to the understanding of N_2O emissions from soils. Phil Trans R Soc B 367(1593):1226–1234

Barraclough D, Puri G (1995) The use of ^{15}N pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. Soil Biol Biochem 27(1):17–22

Beare MH, Gregorich EG, St-Georges P (2009) Compaction effects on CO_2 and N_2O production during drying and rewetting of soil. Soil Biol Biochem 41(3):611–621

Bhandral R, Saggar S, Bolan NS, Hedley MJ (2007) Transformation of nitrogen and nitrous oxide emission from grassland soils as affected by compaction. Soil Tillage Res 94(2):482–492

Bouwman AF, Beusen AHW, Griffioen J, Van Groenigen JW, Hefting MM, Oenema O, Van Puijenbroek PJTM, Seitzinger S, Slomp CP, Stehfest E (2013) Global trends and uncertainties in terrestrial denitrification and N_2O emissions. Philos T R Soc B 368(1621)

Breland TA, Hansen S (1996) Nitrogen mineralization and microbial biomass as affected by soil compaction. Soil Biol Biochem 28(4):655–663

Burgin AJ, Groffman PM (2012) Soil O_2 controls denitrification rates and N_2O yield in a riparian wetland. J Geophys Res 117, G01010. doi:10.1029/2011JG001799

Cao YC, Zhong M, Gong H, Lu GX (2013) Determining ^{15}N abundance in ammonium, nitrate and nitrite in soil by measuring nitrous oxide produced. Acta Pedologica Sinica 50(1):113–119, in Chinese

Clayton H, McTaggart IP, Parker J, Swan L, Smith KA (1997) Nitrous oxide emissions from fertilised grassland: a 2-year study of the effects of N fertiliser form and environmental conditions. Biol Fertil Soils 25(3):252–260

Davidson EA (1991) Fluxes of nitrous oxide and nitric oxide from terrestrial ecosystems. In: Rogers JE, Whitman WB (eds) Microbial production and consumption of greenhouse gases: methane, nitrogen oxides, and halomethanes. American Society for Microbiology, Washington, pp 219–235

Dobbie KE, Smith KA (2001) The effects of temperature, water-filled pore space and land use on N_2O emissions from an imperfectly drained gleysol. Eur J Soil Sci 52(4):667–673

Doran JW, Mielke LN, Power JF (1990) Microbial activity as regulated by soil water-filled pore space. Transactions 14th International Congress of Soil Science, Kyoto, Japan, August 1990, Volume III, pp 94–99

Douglas JT, Crawford CE (1993) The response of a ryegrass sward to wheel traffic and applied nitrogen. Grass Forage Sci 48(2):91–100

Fang YT, Koba K, Makabe A, Zhu FF, Fan SY, Liu XY, Yoh M (2012) Low $\delta^{18}O$ values of nitrate produced from nitrification in temperate forest soils. Environ Sci Technol 46(16):8723–8730

Felgate H, Giannopoulos G, Sullivan MJ, Gates AJ, Clarke TA, Baggs E, Rowley G, Richardson DJ (2012) The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways. Environ Microbiol 14(7):1788–1800

Gross PJ, Bremner JM (1992) Acetone problem in use of the acetylene blockage method for assessment of denitrifying activity in soil. Commun Soil Sci Plant Anal 23(13–14):1345–1358

Henderson SL, Dandie CE, Patten CL, Zebarth BJ, Burton DL, Trevors JT, Goyer C (2010) Changes in denitrifier abundance, denitrification gene mRNA levels, nitrous oxide emissions, and denitrification in anoxic soil microcosms amended with glucose and plant residues. Appl Environ Microb 76(7):2155–2164

Jim CY (1998) Urban soil characteristics and limitations for landscape planting in Hong Kong. Landsc Urban Plan 40(4):235–249

Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci Soc Am J 18(1): 33–34

Linn DM, Doran JW (1984) Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and nontilled soils. Soil Sci Soc Am J 48(6):1267–1272

Liu B, Mørkved PT, Frostegård Å, Bakken LR (2010) Denitrification gene pools, transcription and kinetics of NO, N_2O and N_2 production as affected by soil pH. FEMS Microbiol Ecol 72(3):407–417

Lorenz K, Lal R (2009) Biogeochemical C and N cycles in urban soils. Environ Int 35(1):1–8

Lovett GM, Traynor MM, Pouyat RV, Carreiro MM, Zhu WX, Baxter JW (2000) Atmospheric deposition to oak forests along an urban–rural gradient. Environ Sci Technol 34(20):4294–4300

Magalhães CM, Joye SB, Moreira RM, Wiebe WJ, Bordalo AA (2005) Effect of salinity and inorganic nitrogen concentrations on nitrification and denitrification rates in intertidal sediments and rocky biofilms of the Douro River estuary, Portugal. Water Res 39(9):1783–1794

Magalhães CM, Machado A, Matos P, Bordalo AA (2011) Impact of copper on the diversity, abundance and transcription of nitrite and nitrous oxide reductase genes in an urban European estuary. FEMS Microbiol Ecol 77(2):274–284

Molstad L, Dörsch P, Bakken LR (2007) Robotized incubation system for monitoring gases (O_2, NO, N_2O, N_2) in denitrifying cultures. J Microbiol Meth 71:202–211

Myrold DD, Tiedje JM (1985) Establishment of denitrification capacity in soil: effects of carbon, nitrate and moisture. Soil Biol Biochem 17(6):819–822

Pengthamkeerati P, Motavalli PP, Kremer RJ, Anderson SH (2005) Soil carbon dioxide efflux from a claypan soil affected by surface compaction and applications of poultry litter. Agr Ecosyst Environ 109: 75–86

Pickett STA, Cadenasso ML, Grove JM, Boone CG, Groffman PM, Irwin E, Kaushal SS, Marshall V, McGrath BP, Nilon CH, Pouyat RV, Szlavecz K, Troy A, Warren P (2011) Urban ecological systems: scientific foundations and a decade of progress. J Environ Manage 92(3):331–362

Pouyat RV, Yesilonis ID, Russell-Anelli J, Neerchal NK (2007) Soil chemical and physical properties that differentiate urban land-use type and cover types. Soil Sci Soc Am J 71(3):1010–1019

Ravishankara AR, Daniel JS, Portmann RW (2009) Nitrous oxide (N_2O): the dominant ozone-depleting substance emitted in the 21st century. Science 326(5949):123–125

Renault P, Sierra J (1994) Modeling oxygen diffusion in aggregated soils: II. Anaerobiosis in topsoil layers. Soil Sci Soc Am J 58(4):1023–1030

Ruser R, Flessa H, Russow R, Schmidt G, Buegger F, Munch JC (2006) Emission of N_2O, N_2 and CO_2 from soil fertilized with nitrate: effect of compaction, soil moisture and rewetting. Soil Biol Biochem 38(2):263–274

Schlesinger WH (2009) On the fate of anthropogenic nitrogen. Proc Natl Acad Sci U S A 106(1):203–208

Shestak CJ, Busse MD (2005) Compaction alters physical but not biological indices of soil health. Soil Sci Soc Am J 69:236–246

Silva SR, Silva IR, Barros NF, Mendonça E (2011) Effect of compaction on microbial activity and carbon and nitrogen transformations in two oxisols with different mineralogy. Rev Bras Ciênc Solo 35(4):1141–1149

Sitaula BK, Hansen S, Sitaula JIB, Bakken LR (2000) Effects of soil compaction on N_2O emission in agricultural soil. Chemosphere Global Change Sci 2(3):367–371

Sommers LE, Gilmour CM, Wilding RE, Beck SM (1981) The effects of water potential on decomposition processes in soil. In: Parr JF, Gardner WR, Elliott LF (eds) Water Potential Relations in Soil Microbiology. Soil Science Society of America, Madison, pp 97–117

Stevens RJ, Laughlin RJ (1994) Determining nitrogen-15 in nitrite or nitrate by producing nitrous oxide. Soil Sci Soc Am J 58(4):1108–1116

Stevens RJ, Laughlin RJ, Burns LC, Arah JRM, Hood RC (1997) Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. Soil Biol Biochem 29(2):139–151

Teepe R, Brumme R, Beese F, Ludwig B (2004) Nitrous oxide emission and methane consumption following compaction of forest soils. Soil Sci Soc Am J 68(2):605–611

Thomson AJ, Giannopoulos G, Pretty J, Baggs EM, Richardson DJ (2012) Biological sources and sinks of nitrous oxide and strategies to mitigate emissions. Philos T R Soc B 367(1593):1157–1168

Tiedje JM (1988) Ecology of denitrification and dissimilatory nitrate reduction to ammonium. In: Zehnder AJB (ed) Biology of anaerobic microorganisms. Wiley, New York, pp 179–244

Van Groenigen JW, Kuikman PJ, de Groot WJM, Velthof GL (2005) Nitrous oxide emission from urine-treated soil as influenced by urine composition and soil physical conditions. Soil Biol Biochem 37(3): 463–473

Well R, Höper H, Mehranfar O, Meyer K (2005) Denitrification in the saturated zone of hydromorphic soils—laboratory measurement, regulating factors and stochastic modeling. Soil Biol Biochem 37(10):1822–1836

Yamulki S, Jarvis S (2002) Short-term effects of tillage and compaction on nitrous oxide, nitric oxide, nitrogen dioxide, methane and carbon dioxide fluxes from grassland. Biol Fertil Soils 36(3):224–231

Zhu WX, Carreiro MM (2004) Temporal and spatial variations in nitrogen transformations in deciduous forest ecosystems along an urban–rural gradient. Soil Biol Biochem 36(2):267–278

Zhu J, Mulder J, Bakken L, Dörsch P (2013) The importance of denitrification for N_2O emissions from an N-saturated forest in SW China: results from in situ ^{15}N labeling experiments. Biogeochemistry 1–15