Soil-atmosphere exchange of nitrous oxide, methane and carbon dioxide in a gradient of elevation in the coastal Brazilian Atlantic forest

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Abstract

Soils of tropical forests are important to the global budgets of greenhouse gases. The Brazilian Atlantic Forest is the second largest tropical moist forest area of South America, after the vast Amazonian domain. This study aimed to investigate the emissions of nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) fluxes along an altitudinal transect and the relation between these fluxes and other climatic, edaphic and biological variables (temperature, fine roots, litterfall, and soil moisture). Annual means of N_2O flux were 3.9 (±0.4), 1.0 (±0.1), and 0.9 (±0.2) ng N cm^{-2} h^{-1} at altitudes 100, 400, and 1000 m, respectively. On an annual basis, soils consumed CH_4 at all altitudes with annual means of -1.0 (±0.2), -1.8 (±0.3), and -1.6 (±0.1) mg m^{-2} d^{-1} at 100 m, 400 m and 1000 m, respectively. Estimated mean annual fluxes of CO_2 were 3.5, 3.6, and 3.4 μmol m^{-2} s^{-1} at altitudes 100, 400 and 1000 m, respectively. N_2O fluxes were significantly influenced by soil moisture and temperature. Soil-atmosphere exchange of CH_4 responded to changes in soil moisture. Carbon dioxide emissions were strongly influenced by soil temperature. While the temperature gradient observed at our sites is only an imperfect proxy for climatic warming, our results suggest that an increase in air and soil temperatures may result in increases in decomposition rates and gross inorganic nitrogen fluxes that could support consequent increases in soil N_2O and CO_2 emissions and soil CH_4 consumption.

1 Introduction

The Brazilian Atlantic Forest is a heterogeneous region that includes a large variety of forest physiognomies and compositions (plant and animal species) and is distributed in different topographic and climatic conditions such as areas of coastal flooded forest (restinga), lowland, submontane and montane forests (Metzger, 2009; Vieira et al., 2008). It originally covered an area of 148 million ha, corresponding approximately to 17.4% of the Brazilian territory, extending for over 3300 km along the eastern Brazilian coast

between the latitudes of 3 and 30°S (Metzger, 2009; Ribeiro et al., 2009). The Atlantic forest represents the second largest tropical moist ecosystem of South America, after the vast Amazonian domain (Oliveira-Filho and Fontes, 2000), and it is also considered a hotspot in terms of biodiversity and endemism (Myers et al., 2000). Nevertheless, the Atlantic Forest is among the most threatened tropical forests in the world because its location coincides largely with the most populated areas of Brazil, where the settlement of European pioneers and African slaves started four centuries ago (Oliveira-Filho and Fontes, 2000). Currently the Atlantic Forest is reduced to only 12% of its original cover (Metzger, 2009), and most remnants are small and disturbed fragments (< 50 ha) or larger areas sheltered on steep mountain slopes (Metzger, 2009; Ribeiro et al., 2009).

Despite the importance of the Atlantic Forest biome there are very few data concerning its function (Maddock et al., 2001). Soils of tropical forests are considered as important contributors to the global gas budgets as source of atmospheric nitrous oxide (Bouwman et al., 1995; Maddock et al., 2001), and carbon dioxide (Keller et al., 1986), and as sink of methane (Reiners et al., 1994; Reiners et al., 1997). Although considerable research has been done for a long time aiming to quantify the main global sources and sinks of the main greenhouse gases (N_2O, CH_4, and CO_2) the uncertainties in the overall budgets of these gases remain large in part because of the limited spatial and temporal extent of the sampling in tropical regions (Maddock et al., 2001; Purbopuspito et al., 2006).

The main objective of this paper is to quantify the soil emission rates of N_2O, CH_4 and CO_2 along a gradient of elevation in the Coastal Brazilian Atlantic Forest located in the northern coast of São Paulo state, southeast region of Brazil. Most studies related to tropical forests soil gas emissions are still strongly biased toward lowland tropical forests (Keller and Reiners, 1994; Davidson et al., 2000; Davidson et al., 2001).We chose to work along a gradient of elevation because of differences in climatic conditions, species composition

and structure (Marrs et al., 1988), nutrient supply (Grubb, 1977) and soil physical and chemical properties (Sollins, 1998; Tanner et al., 1998). Climate and soil properties are well known factors that modulate the emission of trace gases by soils (Davidson, 1993; Steudler et al., 1996; Breuer et al., 2000; Davidson et al., 2000; Kiese and Butterbach-Bahl, 2002; Moreira and Siqueira, 2006). Therefore, we expected soil gas emissions to vary with altitude responding to combinations of the factors described above. Although tropical forest soils are expected to respond to global warming few studies have investigated soils from forests along a gradient of elevation that might provide some insight into controls on future trace gas exchange (Riley and Vitousek, 1995; Purbopuspito et al., 2006).

2 Material and methods

2.1 Study area

This study was conducted in the Coastal Brazilian Atlantic Forest, on the northern coast of the São Paulo State, within the management units (*nucleos*) of Picinguaba (lowland, 23° 31’ to 23° 34’S and 45° 02’ to 45° 05W) and Santa Virginia (montane, 23° 17’ to 23° 24’S and 45° 03’ to 45° 11’W) of the *Serra do Mar* State Park. Three areas (treatments) were selected at the altitudes of 100 m (lowland), 400 m (submontane), and 1000 m (montane) (Alves et al., 2010). Historical monthly average temperatures of the study areas ranges from 19.1 to 25.5 °C (Sentelhas et al., 1999). According to Oliveira-Filho and Fontes (2000) and Talora et al. (2000), the lowland and submontane areas (100 m and 400 m) are characterized as tropical moist forests under a tropical climate (*Af* type in Köppen), whereas the montane area (1000 m) is considered a tropical montane forest (Tabarelli and Mantovani, 2000) under subtropical climate (*Cfa* according to Köppen). For a full description of the forest classification and structure see Alves et al. (2010).

According to the meteorological stations of the Department of Water and Energy of São Paulo State (DAEE-SP) the historical annual mean precipitation (1973-2004) at the municipality of Ubatuba located at 220 m altitude is 3050 mm and in the municipality of Natividade da Serra, near altitude 1000 m, the annual mean precipitation decreases to approximately 2300 mm. During May through August, the total historical precipitation is 200 mm, about half as much as in other months. In this study we considered these four months as *dry season* and the other eight months as *rainy season*.

Soils of the study sites are mostly sandy, but with higher clay contents at 100 m (Table 1). Soils at the three altitudes have low carbon (C) and nitrogen (N) contents and these nutrients are concentrated in the upper soil layer (up to 10 cm depth), decreasing with depth (Martins, 2010). Soil C and N concentrations and stocks progressively increase along the altitudinal gradient (Table 1).

2.2 Soil gas flux

At each altitude four plots (replicates) of 1 ha were delimited (Alves et al., 2010). Gas samples were collected once a month from September 2006 through August 2007, in each plot with a day of collection per altitude, generally between 08:00 and 18:00 h local time. Fluxes of nitrous oxide (N_2O), carbon dioxide (CO_2), and methane (CH_4) were measured at random points along 30 m transects that were initiated at randomized seed points in randomized directions each month with eight cylindrical PVC chambers (8 sub-sample chambers per plot) consisting of a pipe that served as a base (0.29 m diameter) and a cap that fit snugly on the base (Keller et al., 2005). For N_2O and CH_4, four samples of 60 mL of the air from the chambers were withdrawn at intervals of 1, 10, 20 and 30 min after closing with 60 mL syringes and then transferred to previously evacuated glass serum vials sealed with gas impermeable, butyl rubber septum stoppers. Samples were analyzed by gas chromatography (SHIMADZU GC-14A Model) within five days of collection. Lab tests

showed that N_2O and CH_4 concentrations were unaffected by storage for up to thirty days. Gas concentrations were calculated by comparing peak areas for samples to those of commercially prepared standards (Scott-Marin) that had been calibrated against standards prepared by the National Oceanic and Atmospheric Administration/Climate Monitoring and Diagnostic Laboratory (NOAA/CMDL). Fluxes were calculated from linear regressions of concentration versus time.

A dynamic flow system was used for measurements of CO_2. Air flowed from the soil enclosure through a Teflon-lined polyethylene sample line 5 m in length and then it entered an infrared gas analyzer (Li-Cor 820). Data was stored in a palmtop computer and fluxes were calculated from the linear increase of concentration versus time adjusted for the ratio of chamber volume to area and the air density within the chamber (Keller et al., 2005). Because of instrument malfunctions, CO_2 fluxes were not available for several months of the year (see Results).

2.3 Litterfall and fine roots

Litterfall data were obtained by thirty 80 cm diameter litterfall traps per plot deployed at randomized points in two plots at each elevation and samples were collected every fifteen days, kept in paper bags, labeled, and dried at 60°C. After drying, samples were weighed. In addition, surface litter layer mass was weighed to assess litterfall stocks simultaneously with litterfall. Thirty surface litter samples were collected from randomly located 0.3 x 0.3 m plots marked by a rigid frame for two plots at each altitude, every thirty days. Samples were kept in paper bags, dried at 60°C and weighed to determine stocks of litter on soil surface. Litterfall and surface litter collections started six months after gas sampling (March 2007) and therefore overlapped the gas collections for only 6 months (March through August 2007). Decomposition rates were calculated according to the model

144 proposed by Olson (1963) and decomposition time was determined according to Shanks
145 and Olson (1961).

146 Five fine root soil cores samples were randomly collected from 0 to 10 cm depth in every
147 plot of each altitude, and treated according to Vogt and Persson (1991). Fine root samples
148 were analyzed for total C and N concentration using a Carlo Erba elemental analyzer at the
149 Laboratory of Isotope Ecology, CENA-USP. For statistical tests, the mean of the five root
150 samples collected at each plot was considered as one of the four replicates per gradient of
151 elevation.

152 **2.4 Soil water filled pore space (WFPS) and N contents**

153 Once a month during one year of collection, and after soil gas collection, the surface litter
154 was removed from each chamber location and a soil core about 5 cm diameter and 10 cm
155 deep was collected. After collection, soil samples were transported on ice in an insulated
156 cooler to the Laboratory of Isotope Ecology at CENA-USP and stored at ~4°C until
157 analysis. Soil samples were sieved (sieve 2 mm mesh) to remove roots and large stones,
158 and a ten grams subsample was oven-dried at 105°C for 24 h to determine water content
159 gravimetrically and N contents (NH_4^+ and NO_3^-), and N-mineralization and N-nitrification
160 processes as the procedures described by Piccolo et al. (1994). Water filled pore space
161 (WFPS) was obtained from soil core samples collected once a month from each chamber
162 location and calculated according to Carmo et al. (2007). Additionally, we recorded air and
163 soil temperatures (2 cm depth) using electronic thermometers.

164 **2.5 Statistical analysis**

165 All data were first tested for normal distribution and for homoscedasticity by the
166 Kolmogorov-Smirnov test. Because of the non-normal distribution of the fluxes for CH_4
167 and N_2O, these data were log-transformed to homogenize variances. We analyzed gas
168 fluxes and other variables in a 2-way ANOVA design using altitude and month as

169 treatments. Four plots served as replicates at each altitude. Months could be considered as
170 treatments because the collection points for chambers were randomized every month.
171 Tukey's *post-hoc* analysis was used to make comparisons among altitudes. Pearson
172 correlation coefficients between N_2O, CO_2, and CH_4 fluxes, soil N contents, soil
173 temperature, and soil moisture also were calculated. Statistical analyses were performed
174 using Minitab version 15 software (Minitab Inc., 2006).

175 Cumulative annual flux of N_2O and CH_4 were calculated by linear interpolation and
176 integration of fluxes among the sampling dates. The difference among cumulative annual
177 fluxes by altitude was also tested by one-way-ANOVA. We estimated the missing CO_2
178 fluxes by fitting an exponential relation between soil temperature and CO_2 flux (Doff Sotta
179 et al., 2004). After filling the missing CO_2 data, the accumulative annual CO_2 flux was also
180 estimated.

181 **3 Results**

182 **3.1 Soil temperature and soil chemical-physical properties**

183 As expected, lower soil temperatures (P<0.05) were found at higher altitude (1000 m) and
184 soil temperature increased downwards (Figure 1).

185 Soil moisture expressed as WFPS was significantly higher (P<0.05) in the plots at 100 m
186 and 400 m than in soils located at 1000 m (Figure 2). The trends in WFPS reflect in part
187 the soil porosity and packing (Beare et al., 2009). Soil bulk densities at 5 cm depth were
188 greater at the lower elevations (0.98 g m^3 at 100 m and 1.06 Mg m^3 at 400 m) compared
189 to the montane site (0.8 g m^3 at 1000 m).

190 There was no difference (P>0.05) in annual net mineralization and net nitrification rates
191 among altitudes. However, ammonium (NH_4^+) and nitrate (NO_3^-) concentrations were
192 significantly higher (P<0.05) at altitude 1000 m (9.7±0.6 and 19.1±1.0 µg g^{-1},

193 respectively). No significant correlations were found between soil nitrate or ammonium
194 concentrations and flux of soil gases during the sampling period nor was soil net N, net
195 mineralization and net nitrification rates significantly correlated to soil gas emissions.

196 **3.2 Fine root and litter production**

197 On average total fine root biomass (0-10 cm depth) was greater (P<0.05) in the dry season
198 than in the rainy season. During the rainy season fine roots had larger live mass (P<0.05)
199 than dead mass and fine root mass (live and dead) was larger (P<0.05) at 1000 m (Table
200 2). In the dry season, there was no significant difference (P>0.05) between live and dead
201 mass along the altitudes but greater root mass (P<0.05) was again found at 1000 m
202 altitude.

203 Carbon to nitrogen (C:N) ratio of fine roots (live and dead) collected during the rainy
204 season was significantly higher (P<0.05) than in the dry season (Table 3). In both seasons,
205 the C:N ratio of live roots was significantly (P<0.05) higher than dead roots. There was no
206 significant difference of C:N ratio of fine roots among altitudes (Table 3).

207 Although a decrease in litterfall was observed at higher altitudes, there was no significant
208 difference among altitudes (Table 4). Litterfall stocks on soil surface were significantly
209 higher (P<0.05) at 1000 m (Table 4). Calculations using Shanks and Olson's model (1961),
210 showed that litter decay rate decreases as altitude increases (P<0.05, Table 4); litter takes
211 18 months for 95% loss at 100 m and about 50% more time at 400 and 1000 m.

212 **3.3 Soil-atmosphere emissions of trace gases**

213 Annual means of soil N_2O flux decreased (P<0.05) with the increase of altitude (Table 5).
214 At all altitudes, we observed consumption of soil CH_4 with the smallest consumption
215 (P<0.05) observed at 100 m (Table 5). CO_2 fluxes do not correspond to a full year and
216 valid data correspond to the months from March, 2007 through August, 2007. For these

months, soil CO_2 fluxes averaged 3.1 (±0.3) μmol m^{-2} s^{-1} at 1000 m and were significantly lower (P<0.05) than at 400 m and 100 m (3.3 (±0.3) and 3.6 (±0.2) μmol m^{-2} s^{-1} respectively), which were not distinguishable from one another.

The cumulative annual fluxes of N_2O and CH_4 for the three altitudes were calculated and the ANOVA results for N_2O were similar to the simple averages (Table 5). In contrast, for the cumulative fluxes of CH_4 we found no significant difference among altitudes. We note that the simple data provide a more powerful test than the cumulative data because they include more degrees of freedom.

Higher fluxes of CO_2 were observed in all altitudes between February and April, 2007, during the rainy season, and lower fluxes were measured between May and August, 2007, during dry season (Figure 3c). Carbon dioxide emissions increased with soil temperature (r^2 = 0.7 at 100 m, r^2 = 0.9 at 400 m and 1000 m, respectively, P<0.05), but no correlation was observed with WFPS.

Using an exponential model for CO_2 flux with temperature (Doff Sotta et al., 2004), we estimated the missing CO_2 data (October, 2006 through April, 2007) and then interpolated the data as we did for N_2O and CH_4 to estimate annual fluxes. The cumulative annual fluxes of CO_2 were also estimated and values were 3.5, 3.6 and 3.4 μmol m^{-2} s^{-1} at altitudes 100 m, 400 m, and 1000 m altitudes respectively. Based on the exponential model we also calculated Q_{10} values of 1.6, 2.3, and 2.1 at altitudes 100 m, 400 m and 1000 m, respectively.

3.4 Altitudinal and monthly variations of soil gas fluxes and their dependency on changes in soil temperature and WFPS

At 100 m there was a significant (P<0.05) variation in N_2O fluxes during sampling period, with the highest fluxes measured in the rainy months of December, 2006 and January, 2007 (Figure 3a). A significant positive correlation (r^2=0.86, P<0.05) between soil moisture (WFPS) and N_2O flux was observed exclusively at 100 m while there was no correlation between soil temperature and N_2O flux at the same altitude.

Fluxes measured at 400 m showed significant differences along the sampling period, with the largest N_2O emissions (P<0.05) measured during the rainy season, between August 2006 and January 2007 (Figure 3a). At 1000 m there was a weak but significant (P<0.05) monthly variation of N_2O fluxes, and the largest emissions were observed between the rainy months of November, 2006 and January, 2007 (Figure 3a) whereas significantly (P<0.05) lower fluxes were found in the dry months of July and August, 2007. A weak but significant correlation (r^2 = 0.52, P<0.05) between soil temperature and N_2O fluxes was observed at altitude 1000 m.

At 100 m soil-atmosphere exchange of CH_4 showed only negative fluxes (soil consumption of atmospheric methane) and consumption varied significantly (P<0.05) among months. The largest consumption occurred in August, 2006 (transition between rainy and dry seasons) and in the hot and wet period between February and March 2007 (rainy season). Smaller consumption was measured during the cool and dry months of June, July and August 2007 (Figure 3b).

Methane consumption varied significantly (P<0.05) among months at 400 m altitude. More consumption (P<0.05) occurred in the rainy months of September, 2006 and March, 2007 and less consumption was measured during November, 2006 and December, 2006 (rainy season) and in the dry month of June, 2007 (Figure 3b). At 1000 m consumption of CH_4

also varied among months (P<0.05). The pattern was similar to the pattern at 400 m with less consumption (P<0.05) in the rainy months of November and December, 2006 and more (P<0.05) consumption in September 2006, and August 2007 (Figure 3b).

In general, there was no significant correlation between CH_4 fluxes and soil temperature at any altitude. In contrast, CH_4 correlated weakly (r^2=0.40, P<0.05) with WFPS at 100 m.

4 Discussion

4.1 Soil-atmosphere emissions of N_2O

In order to understand the decrease in soil N_2O emissions with altitude we evaluate our data in relation to the hole-in-the-pipe (HIP) model (Firestone and Davidson, 1989; Davidson et al., 2000). According to this model, at a broad scale, N_2O emissions increase with the nitrogen availability (gross inorganic nitrogen fluxes) in the system. Comparing different tropical regions, Davidson et al. (2000) found specifically that N_2O emissions were correlated with soil nitrate concentrations, N-mineralization and nitrification, and were inversely correlated with the soil ammonium concentrations or the ratio of ammonium to nitrate.

Our data contain some anomalies compared to the findings of Davidson et al. (2000) and other studies. At the 1000 m forest site, soil concentrations of ammonium and nitrate were higher than at other sites and average nitrate concentrations were 30% higher than average ammonium concentrations. Soil pools of ammonium and nitrate reflect a balance in production and consumption processes and do not necessarily correlate with gas fluxes. Nonetheless, the low N_2O fluxes at the montane site are at odds with the trends for higher N_2O emissions where soil nitrate pools exceeded soil ammonium pools (Davidson et al., 2000). Despite the high nitrate to ammonium ratio, N_2O fluxes were significantly lower at the montane site than they were in the lowlands. In part, we speculate that the low N_2O

fluxes resulted from the limitation of denitrification by easy drainage in the sandy soil and the consequent good aeration and perhaps from low gross fluxes of inorganic nitrogen owing to the lower temperatures. WFPS was significantly lower at 1000 m than at l00 and 400 m.

The pace of decomposition is also important. High rates of decomposition consume oxygen promoting low-oxygen conditions that promote greater N_2O emissions in tropical forest soils (Keller and Reiners 1994). The data on litter stocks (Table 4) show that the rate of decomposition (promoted by higher temperatures) is nearly twice as great in the lowlands as in the montane sites. Thus, low N_2O emissions at montane sites could be related to low decomposition rates through the limitation in gross nitrogen transformations and through the limitation on oxygen consumption.

No single factor promoted the greatest N_2O fluxes found in months of December 2006 and January 2007 at elevation 100 m. We speculate that the high fluxes result from a combination of high temperature, elevated soil WFPS, and high rates of decomposition what could result low-oxygen conditions. In addition, we note that methane consumption is diminished at the same time. In this case, the association of low oxygen conditions with high N_2O fluxes is corroborated by the correlation between N_2O and WFPS (r^2=0.86; P = 0.05) at 100 m (McSwiney et al., 2001). The influence of soil temperature over gas emissions is corroborated by the significant positive relation between N_2O and soil temperature at 1000 m (r^2 = 0.5, P<0.05).

We compare our N_2O emissions with the survey made by Breuer et al. (2000) adding recent emissions measurements made in tropical forests, mainly in the Amazon region (Garcia-Montiel et al., 2001; Garcia-Montiel et al., 2002; Keller et al., 2005). The median value of all these measurements was approximately 2.0 kg N ha^{-1} yr^{-1}. Emissions measured at 400 m and 1000 m forest sites were lower than these values, and near the lower end of

312 the spectrum of emissions. On the other hand, N_2O emissions at the 100 m forest sites were
313 larger (2.2 kg N ha^{-1} yr^{-1}) than the median value, but approximately half as great as the
314 highest observed emissions from tropical forests (6 to 7 kg N ha^{-1} yr^{-1}). N_2O emissions
315 measured at 100 m were similar to the mean fluxes found in the only other study that
316 measured annual N_2O emissions in the coastal Atlantic Forest of Brazil (Tianguá
317 Biological Reserve, Rio de Janeiro, 170 to 300 m *asl*) (Maddock et al., 2001).

318 **4.2 Soil-atmosphere exchange of CH_4**

319 Tropical rain forests can function as a significant sink for atmospheric CH_4 and most
320 studies have reported negative fluxes (Verchot et al., 1999; Breuer et al., 2000; Gut et al.,
321 2002, Kiese et al., 2003). In the Atlantic forest, we observed, on average, only negative
322 fluxes (consumption) of CH_4 at all altitudes, and the annual mean fluxes of CH_4 found in
323 this study are similar to fluxes reported by other studies conducted in tropical forests
324 (Keller et al., 2005). Well-drained soils generally consume CH_4 from the atmosphere and
325 soil water content regulates the flux through its control on the diffusion of CH_4 in the soil
326 (Crill, 1991; Born et al., 1990). Butterbach-Bahl et al. (2004) in a study in an Australian
327 tropical rainforests have shown that CH_4 uptake was correlated with WFPS. Although
328 weak, there was a significant (P<0.05) positive correlation between WFPS and CH_4 flux at
329 100 m forest site (r^2 = 0.4, P<0.05) there was no correlation between WFPS and CH_4 flux
330 at the higher altitudes. We note that temperature and moisture correlate in these systems
331 and that when soil moisture conditions are optimal for CH_4 consumption in the cooler sites
332 (400 m and 1000 m), low soil temperatures probably limit the microbial activity
333 responsible for CH_4 consumption.

334 **4.3 Soil-atmosphere emissions of CO_2**

335 Because of equipment malfunctions, the temporal CO_2 extent of emissions measured in our
336 study was limited to only about one-half year. Using the exponential model of flux by

altitude, the integrated carbon dioxide emissions were similar at all altitudes despite the higher temperatures (Figure 3) and the greater rates of decomposition (Table 4) in the lowlands. We may have failed to capture the true dynamics of soil CO_2 flux because we did not sample in the early part of the Austral summer (Figure 3c) when the combination of hot and wet conditions coincided with an abundant forest floor litter stock. As noted in most studies, soil CO_2 emissions are tightly related to temperature and labile substrate (Joergensen et al., 1990; Kiese and Butterbach-Bahl, 2002; Davidson and Janssens, 2006; Moreira and Siqueira, 2006). In our limited observations, the largest soil CO_2 emissions were observed between February and April, 2007 (Figure 3c) when observed soil and air temperatures were highest (Figure 1), reinforcing the evidence for a strong temperature effect.

5 Conclusion

Overall we found that the emissions of N_2O and the uptake of CH_4 by soils of the coastal Atlantic Forest of Brazil are within the range of other tropical forests of the world. We observed that N_2O and CO_2 emissions were lower at higher altitudes, although the nitrogen and carbon stocks were greater at higher altitudes. According to our results, we speculate this contrast cannot be explained by an isolated factor but by an association of factors including air and soil temperatures, species composition (van Haren et al., 2010), soil physical and chemical properties, decomposition rates and nutrient supply. Amongst all those factors, the temperature gradient was most obvious. An apparently non-linear response of both decomposition and nitrogen cycling to elevated temperature leads to strong seasonal N_2O emissions in the lowlands whereas emissions are relatively low at submontane and montane sites throughout the year. Climate change associated with increasing temperatures may result in increased in microbial activity with a consequent increase in soil N_2O and CO_2 emissions and soil CH_4 consumption. While the responses

362 along the elevation gradient associated with temperature are provocative, we recognize that
363 no single factor in this complex system can adequately predict the response of greenhouse
364 gas fluxes to climate change.

365

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References

Alves, LF, Vieira, SA, Scaranello, MA, Camargo, PB, Santos, FAM, Joly, CA and Martinelli, LA.: Forest structure and live aboveground biomass along an elevational gradient of tropical moist forest (Brazil). Forest Ecology and Management, 260, 679-691, 2010.

Beare, M. H., Gregorich, E. G. and St-Georges, P.: Compaction effects on CO_2 and N_2O production during drying and rewetting of soil. Soil Biol. Biochem. 41, 611-621, 2009.

Born, M., Door, H. and Levin, I.: Methane consumption in aerated soils of the temperature zone. Tellus, 42A, 2-8, 1990.

Bouwman, A. F., Vanderhoek, K. W. and Olivier, J. G. J.: Uncertainties in the global source distribution of nitrous oxide. J. Geophys. Res.-Atmos. 100, 2785-2800, 1995.

Breuer, L., Papen, H. and Butterbach-Bahl, K.: N_2O emissions from tropical forest soils of Australia. J. Geophys. Res. 105, 26353-26367, 2000.

Butterbach-Bahl, K., M. Kock, G. Willibald, B. Hewett, S. Buhagiar, H. Papen, and R. Kiese.: Temporal variations of fluxes of NO, NO_2, N_2O, CO_2 and CH_4 in a tropical rain forest ecosystem. Global Biogeochem. Cycles 18(3), GB3012, 2004.

Carmo, J. B., Piccolo, M. C., Andrade, C. A., Cerri, C. E. P., Feigl, B. J., Sousa Neto, E. and Cerri, C. C.: Short –term changes in nitrogen availability, gas fluxes (CO_2, NO, N_2O) and microbial biomass after tillage during pasture re-establishment in Rondônia, Brazil. Soil Till. Res. 96, 250-259, 2007.

Crill, P. M.: Seasonal patterns of methane uptake and carbon dioxide release by a temperature woodland soil. Global Biogeochem. Cycles 5, 319-334, 1991.

Davidson, E. A. and Janssens, I. A.: Temperature Sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165-173, 2006.

400 Davidson, E. A., Erickson, H. E. and Keller, M.: Nitrogen oxide fluxes and nitrogen
401 cycling during post agricultural succession and forest fertilization in the humid tropics.
402 Ecosystems 4, 67-84, 2001.
403 Davidson, E. A., Erickson, H. E., Keller, M., Veldcamp, E. and Verchot, L. V.: Testing a
404 conceptual model of soil emission of nitrous and nitric oxides. BioScience 50, 667,
405 2000.
406 Davidson, E. A.: Soil water content and the ratio of nitrous oxide to nitric oxide emitted
407 from soil, in Oremland, R. S., ed. The Biochemistry of Global Change: Radioactive
408 trace gases. New York: Chapman and Hall, 369-386, 1993.
409 Doff Sotta, E., Meir, P., Malhi, Y., Nobre, A. D., Hodnett, M. and Grace, J. Soil CO_2
410 efflux in a tropical forest in the central Amazon. Global Change Biology 10, 601-617,
411 2004.
412 Firestone, M. K and Davidson, E. A.: Microbiological basis of NO and N_2O production
413 and consumption in soil, in Andreae, M. O. and Shimel, D. S., eds. Exchange of trace
414 gases between terrestrial ecosystems and the atmosphere. New York: John Wiley &
415 Sons, 7-21, 1989.
416 Garcia-Montiel, D. C., Melillo, J. M., Steudler, P. A., Neill, C., Feigl, B. J. and Cerri, C.
417 C.: Relationship between N_2O and CO_2 emissions from the Amazon basin. Geophys.
418 Res. Lett. 2(3), 2002.
419 Garcia-Montiel, D. C., Melillo, J. M., Steudler, P. A., Neill, C. and Cerri, C. C.: Controls
420 on soil nitrogen oxide emissions from forests and pastures in the Brazilian Amazon.
421 Global Biogeochem. Cycles 15, 1021-1030, 2001.
422 Grubb, P. J.: Control of forests growth and distribution on wet tropical mountains: with
423 special reference to mineral nutrition. Annu. Rev. Ecol. Syst. 8, 83-107, 1977.
424 Gut, A., van Dijk, S. M., Scheibe, M., Rummel, U., Welling, M., Ammann, C., Meixner, F.
425 X., Kirkman, G. A., Andreae, M. O., and Lehmann, B. E: NO emission from an

426 Amazon rain forest soil: Continuous measurement of NO flux and soil concentration. J.
427 Geophys. Res.-Atmos. 107(D20), 8057, 2002.
428 Joergensen, R. G., Brookes, P. C. and Jenkinson, D. S.: Survival of the soil microbial
429 biomass at elevated-temperatures. Soil Biol. Biochem 22, 1129-1136, 1990.
430 Keller, M., Asner, G. P., Crill, P., Dias, J. D., de Oliveira, R. C., Silva, H. and Varner, R.:
431 Soil-atmosphere exchange of nitrous oxide, nitric oxide, methane and carbon dioxide in
432 logged and undisturbed forest in Tapajos National Forest, Brazil. Earth Interact. 9, 1-28,
433 2005.
434 Keller, M. and Reiners, W. A.: Soil-atmosphere exchange of nitrous oxide, nitric oxide and
435 methane under secondary succession of pasture to forests in the Atlantic lowlands of
436 Costa Rica. Global Biogeochem. Cycles 8, 399-409, 1994.
437 Keller, M., Kaplan, W. A. and Wofsy, S. C.: Emissions of N_2O, CH_4 and CO_2 from
438 tropical forests soils. J. Geophys. Res.-Atmos. 91, 1791-1802, 1986.
439 Kiese, R., Hewett, B., Graham, A., and Butterbach-Bahl, K.: Seasonal variability of N_2O
440 emissions and CH_4 uptake by tropical rainforest soils of Queensland, Australia, Global
441 Biogeochem. Cycles, 17, 13, 104310.1029/2002gb002014, 2003.
442 Kiese, R. and Butterbach-Bahl, K.: N_2O and CO_2 emissions from three different tropical
443 forest sites in the wet tropics of Queensland, Australia. Soil Biol. Biochem. 34, 975-
444 987, 2002.
445 Maddock, J. E. L., dos Santos, M. B. P. and Prata, K. R.: Nitrous oxide emissions from soil
446 of the Mata Atlântica, Rio de Janeiro State, Brazil. J. Geophys. Res.-Atmos. 106,
447 23055-23060, 2001.
448 Marrs, R. H., Proctor, J., Heaney, A. and Mountford, M. D.: Changes in soil nitrogen-
449 mineralization and nitrification along an altitudinal transect in tropical rain forest in
450 Costa Rica. J. Ecol. 76, 466-482, 1988.

451 Martins, S. C.:Caracterização dos solos e serapilheira ao longo do gradiente altitudinal da
452 Mata Atlântica, estado de São Paulo. Doctorate Thesis. Universidade de São Paulo,
453 Brazil, 2010.
454 McSwiney, C. P., McDowell, W. H. and Keller, M.: Distribution of nitrous oxide and
455 regulators of its production across a tropical rainforest catena in the Luquillo
456 Experimental Forest, Puerto Rico. Biogeochemistry-US 56, 265-286, 2001.
457 Metzger. J. P.: Conservation issues in the Brazilian Atlantic forest. Biol. Conserv. 142,
458 1138-140, 2009.
459 Moreira, F. M. S. and Siqueira, J. O.: Microbiologia e Bioquímica do Solo. Lavras, MG:
460 UFLA, 2006.
461 Myers, N., Mittermeier, R. A., Mittermeier, C. G., da Fonseca, G. A. B. and Kent, J.:
462 Biodiversity hotspots for conservation priorities. Nature 403, 853-858, 2000.
463 Oliveira-Filho, A. T. and Fontes, M. A. L.: Patterns of floristic differentiation among
464 Atlantic forests in southeastern Brazil and the influence of climate. Biotropica 32, 793-
465 810, 2000.
466 Olson, J. S.: Energy storage and the balance of producers and decomposers in ecological
467 systems. Ecology 44, 322-331, 1963.
468 Piccolo, M. C., Neill, C. and Cerri, C. C.: Net nitrogen mineralization and net nitrification
469 along a tropical forest-to-pasture chronosequence. Plant and Soil 162, 61-70, 1994.
470 Purbopuspito, J., Veldkamp, E., Brumme, R. and Murdiyarso, D.: Trace gas fluxes and
471 nitrogen cycling along an elevation sequence of tropical montane forests in Central
472 Sulawesi, Indonesia. Global Biogeochem. Cycles 20, 2006.
473 Reiners, W. A., Gerow, K. G. and Keller, M.: Estimating rainy season nitrous oxide and
474 methane fluxes across forest and pasture landscapes in Costa Rica. 3rd International
475 Symposium on Ecosystem Behavior. Philadelphia, Pennsylvania, 117-130, 1997.

476 Reiners, W. A., Bouwman, A. F., Parsons, W. F. J. and Keller, M.: Tropical rain forest
477 conversion to pasture - changes in vegetation and soil Properties. Ecol. Appl. 4, 363-
478 377, 1994.
479 Ribeiro, M. C., Metzger, J. P., Martensen, A. C., Ponzoni, F. J. and Hirota, M. M.: The
480 Brazilian Atlantic Forest: How much is left and how is the remaining forest distributed?
481 Implications for conservation. Biol. Conserv. 142, 1141-1153, 2009.
482 Riley, R. H., and Vitousek, P. M.: Nutrient dynamics and nitrogen trace gas flux during
483 ecosystem development in montane rain forest. Ecology 76, 292-304, 1995.
484 Sentelhas, P. C., Pereira, A. R., Marin, F. R., Angelocci, L. R., Alfonsi, R. R., Caramori, P.
485 H., Swart, S. Balanços Hídricos Climatológicos do Brasil-500 balanços hídricos de
486 localidades brasileiras. ESALQ Piracicaba. Available at:
487 http://www.bdclima.cnpm.embrapa.br/index.php
488 Shanks, R. E. and Olson, J. S.: First-year breakdown of leaf litter in southern Appalachian
489 forests. Science 134, 194-195, 1961.
490 Sollins, P.: Factors influencing species composition in tropical lowland rain forest: Does
491 soil matter? Ecology 79, 23-30, 1998.
492 Steudler, P., Jones, R., Castro, M., Melillo, J. and Lewis, D.: Microbial controls of
493 methane oxidation in temperate forest and agricultural soils. Nato Asi. Ser. Ser. I 39,
494 69-84, 1996.
495 Tanner, E. V. J., Vitousek, P. M. and Cuevas, E.: Experimental investigation of nutrient
496 limitation of forest growth on tropical mountains. Ecology 79, 10-22, 1998.
497 Tabarelli, M., and Mantovani, W.: Gap-phase regeneration in a tropical montane forest: the
498 effects of gap structure and bamboo species. Plant Ecology 148, 149-155, 2000.
499 Talora, D. C. and Morellato, P. C.: Fenologia de espécies arbóreas em floresta de planície
500 litorânea do sudeste do Brasil. Revista Brasil. Bot., São Paulo, v.23, 13-26, 2000.

van Haren, J. L. M., Oliveira Jr., R. C., Restrepo-Coupe, N., Hutyra, L., Camargo, P. B., Keller, M., and Saleska, S. R.: Do plant species influence soil CO_2 and N_2O fluxes in a diverse tropical forest? Journal of Geophysical Research 115, G03010, 2010.

Vieira, S. A., Alves, L. F., Aidar, M., Araújo, L. S., Baker, T., Batista, J. L. F., Campos, M. C., Camargo, P. B., Chave, J., Delitti, W. B. C., Higuchi, N., Honorio, E., Joly, C. A., Keller, M., Martinelli, L. A., Mattos, E. A. d., Metzker, T., Phillips, O., Santos, F. A. M. d., Shimabukuro, M. T., Silveira, M., and Trumbore, S. E.: Estimation of biomass and carbon stocks: the case of the Atlantic Forest. Biota Neotropica 8, 0-0, 2008.

Verchot, L. V., Davidson, E. A., Cattanio, J. H., Ackerman, I. L., Erickson, H. E. and Keller, M.: Land use change biogeochemical controls of nitrogen oxide emissions from soils in eastern Amazonia. Global Biochem. Cycles 13, 31-46, 1999.

Vogt, K. A. and Persson, H.: Measuring growth and development of roots. In Lassoie, J. P. and Hinckley, T. M., eds. Techniques and approaches in forest tree ecophysiology, Boca Raton: CRC Press, 477-501, 1991.

Table 1. Physical-Chemical characterization of the soil layers (0.3 m depth) sampled at the studied sites (mean ± standard error; n=32 for each altitude and depth). Data source: Martins, 2010.

Depth (cm)	N	C	Sand	Clay	Bulk Density
	……..(g kg^{-1})……		……(%).....		(g m^{-3})
100 m					
0-5	3.4 ± 1.4	45.9 ± 19.4	60.4 ± 9.7	31.5 ± 8.0	0.9 ± 0.1
5-10	2.4 ± 1.1	31.8 ± 15.3	56.5 ± 9.3	35.1 ± 8.6	1.1 ± 0.1
10-20	1.9 ± 0.7	25.9 ± 10.1	56.8 ± 9.9	35.3 ± 9.7	1.3 ± 0.1
20-30	1.2 ± 0.4	16.5 ± 5.9	55.8 ±9.6	37.4 ± 9.7	1.4 ± 0.1
400 m					
0-5	4.6 ± 1.1	58.9 ± 15.5	66.7 ± 6.6	16.4 ± 3.8	1.0 ± 0.0
5-10	3.6 ± 0.8	45.8 ± 12.7	62.2 ± 3.8	20.5 ± 3.7	1.1 ± 0.1
10-20	2.7 ± 0.5	34.7 ± 8.8	61.4 ± 6.0	22.1 ± 4.5	1.2 ± 0.1
20-30	2.0 ± 0.3	26.0 ± 5.9	59.5 ± 5.9	23.4 ± 4.2	1.3 ± 0.1
1000 m					
0-5	6.8 ± 3.1	91.5 ± 45.3	57.3 ± 12.2	20.3 ± 8.5	0.8 ± 0.2
5-10	4.5 ± 1.5	58.8 ± 21.2	53.9 ± 14.3	22.3 ± 10.8	0.8 ± 0.2
10-20	3.8 ± 1.2	49.6 ± 17.1	54.0 ± 12.2	19.8 ± 10.7	1.0 ± 0.2
20-30	3.1 ± 1.2	44.4 ± 22.5	53.5 ± 12.3	20.6 ± 11.5	1.1 ± 0.2

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Table 2. Fine root biomass (live and dead) at different altitudes in the rainy and in the dry season. Values represent mean and standard error of four replicates per altitude.

Altitude	**Rainy season (g m^{-2})**		**Dry season (g m^{-2})**	
(m)	**Live**	**Dead**	**Live**	**Dead**
100 m	204.2 (±28.1)a	82.1 (±16.0)b	433.8 (±119.1)a	275.4 (±131.9)a
400 m	293.1 (±38.1)a	143.34 (±17.4)b	310.6 (±87.6)a	219.5 (±98.2)a
1000 m	464.0 (±80.2)a	220.7 (±44.5)b	1098.3 (±89.8)a	896.2 (±82.3)a

Lower case letters indicate difference between columns within seasons.

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Table 3. Concentrations of carbon and nitrogen and C:N ratio of fine roots (<2 mm) at different altitudes in rainy (January,2007) and dry (August, 2007) months. Values represent mean and standard error (in parenthesis) of four replicates per altitude.

Season	Altitude (m)	Category	C (%)	N (%)	C:N
Rainy	100 m	Live	42.8 (±1.2)	1.4 (±0.1)	32.6 (±2.7)a,A
		Dead	37.8 (±1.4)	1.5 (±0.1)	26.5 (0.1)b,A
	400 m	Live	42.9 (±0.3)	1.5 (±0.1)	31.1 (±2.2)a,A
		Dead	38.0 (±2.4)	1.4 (±0.1)	27.1 (±0.5)b,A
	1000 m	Live	45.4 (±1.0)	1.3 (±0.1)	35.7 (±2.1)a,A
		Dead	44.0 (±1.2)	1.5 (±0.1)	29.9 (±0.9)b,A
Dry	100 m	Live	41.4 (±1.2)	1.7 (±0.2)	25.6 (±1.8)a,B
		Dead	37.4 (±0.5)	1.7 (±0.1)	22.1 (±1.5)b,B
	400 m	Live	39.4 (±0.5)	1.6 (±0.1)	26.4 (±1.0)a,B
		Dead	37.2 (±1.0)	1.7 (±0.2)	22.3 (±1.7)b,B
	1000 m	Live	43.6 (±0.8)	1.7 (±0.1)	27.1 (±1.6)a,B
		Dead	39.6 (±0.9)	1.7 (±0.1)	23.3 (±1.4)b,B

Lower case letters indicate difference between altitudes within seasons and upper case letters indicate difference between seasons.

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Table 4. Litterfall inputs and stocks in different altitudes and litter decomposition rates (k) and time (months) for decay of 50% ($t_{0.5}$) and 95% ($t_{0.05}$). Data represents six months of sampling (March through August 2007). Different letters represent statistically significant differences among altitudes.

Altitude	**Litterfall**		**Forest Floor**		
(m)	**Inputs (t ha^{-1} y^{-1})**	**Stocks (t ha^{-1})**	**DC[1] (k)**	**$t_{0.5}$**	**$t_{0.05}$**
100	8.4 (±1.5)[a]	4.3 (±0.8)[a]	2[a]	3	18
400	7.4 (±1.8)[a]	4.4 (±0.4)[a]	1.4[b]	5	25
1000	5.5 (±0.9)[a]	4.8 (±0.6)[b]	1.3[b]	5	27

[1]DC= Decomposition coefficient

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Table 5. Simple annual mean (SA) and integrated (Int.) fluxes of N_2O and CH_4 for different altitudes. Different letters represent statistically significant differences among the altitudes. See text for a description of the averaging and integration approaches.

Altitude (m)	**N_2O (ng N cm^{-2} h^{-1})**		**CH_4 (mg CH_4 m^{-2} d^{-1})**	
	SA	Int.	SA	Int.
100	3.9^{a} (±0.4)	4.4^{a} (±0.5)	-1.0^{a} (±0.2)	-1.0^{a} (±0.2)
400	1.0^{b} (±0.1)	1.1^{b} (±0.1)	-1.8^{b} (±0.3)	-1.7^{a} (±0.3)
1000	0.9^{c} (±0.2)	1.1^{b} (±0.3)	-1.6^{b} (±0.1)	-1.4^{a} (±0.1)

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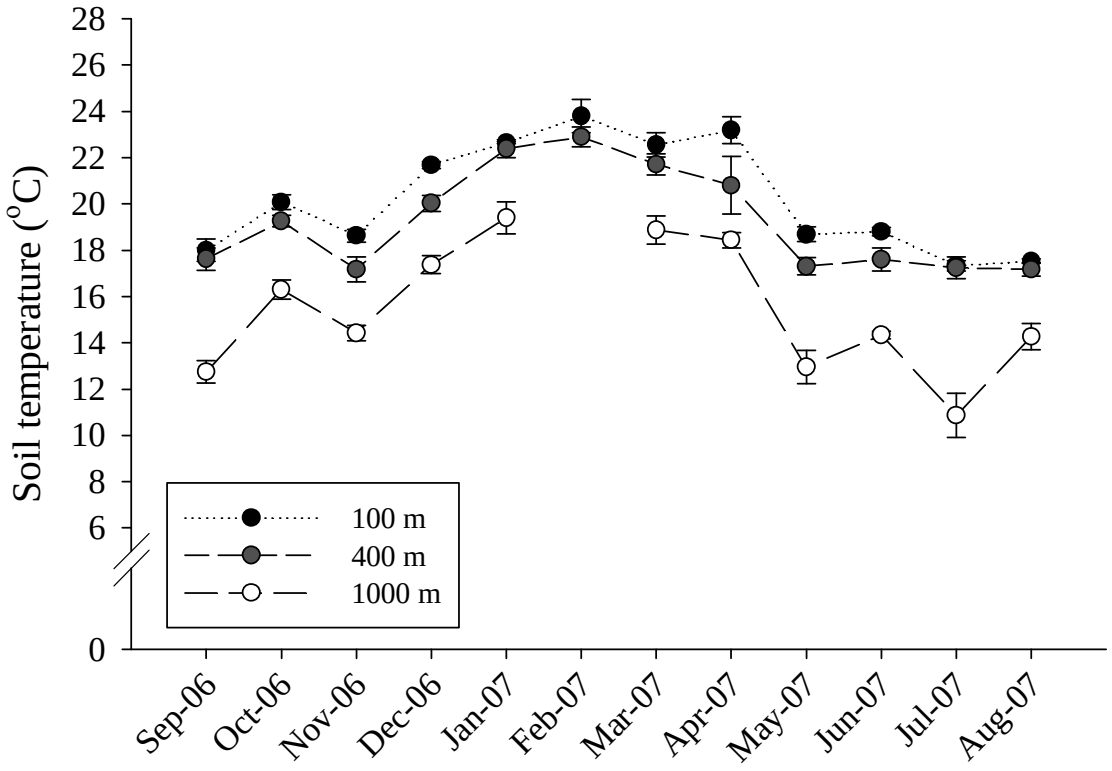

Figure 1. Monthly soil temperatures (2 cm depth) at the three different elevations. Values represent the mean of four replicate plots per elevation, and error bars represent the standard error. Because of weather conditions it was not possible to access the sites at altitude 1000 m in February 2007.

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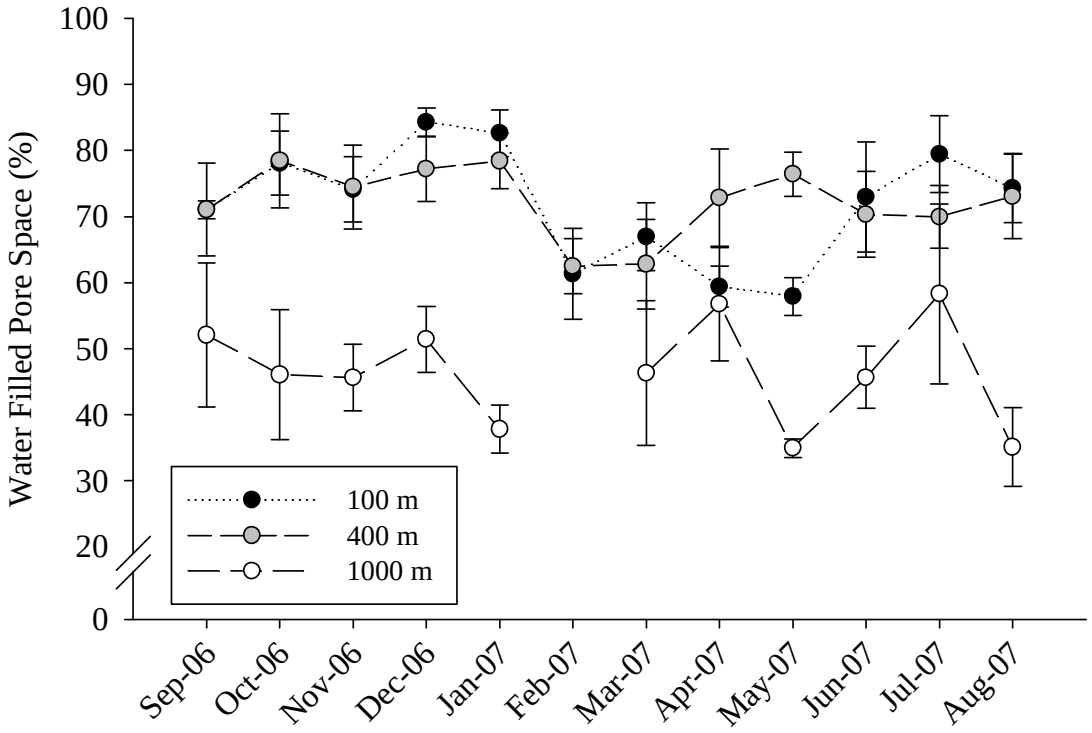

Figure 2. Monthly variation of Water Filled Pore Space (WFPS) at different elevations. Values represent means of four replicates per elevation and bars represent standard errors. Because of weather conditions it was not possible to access the sites at altitude 1000 m in February 2007.

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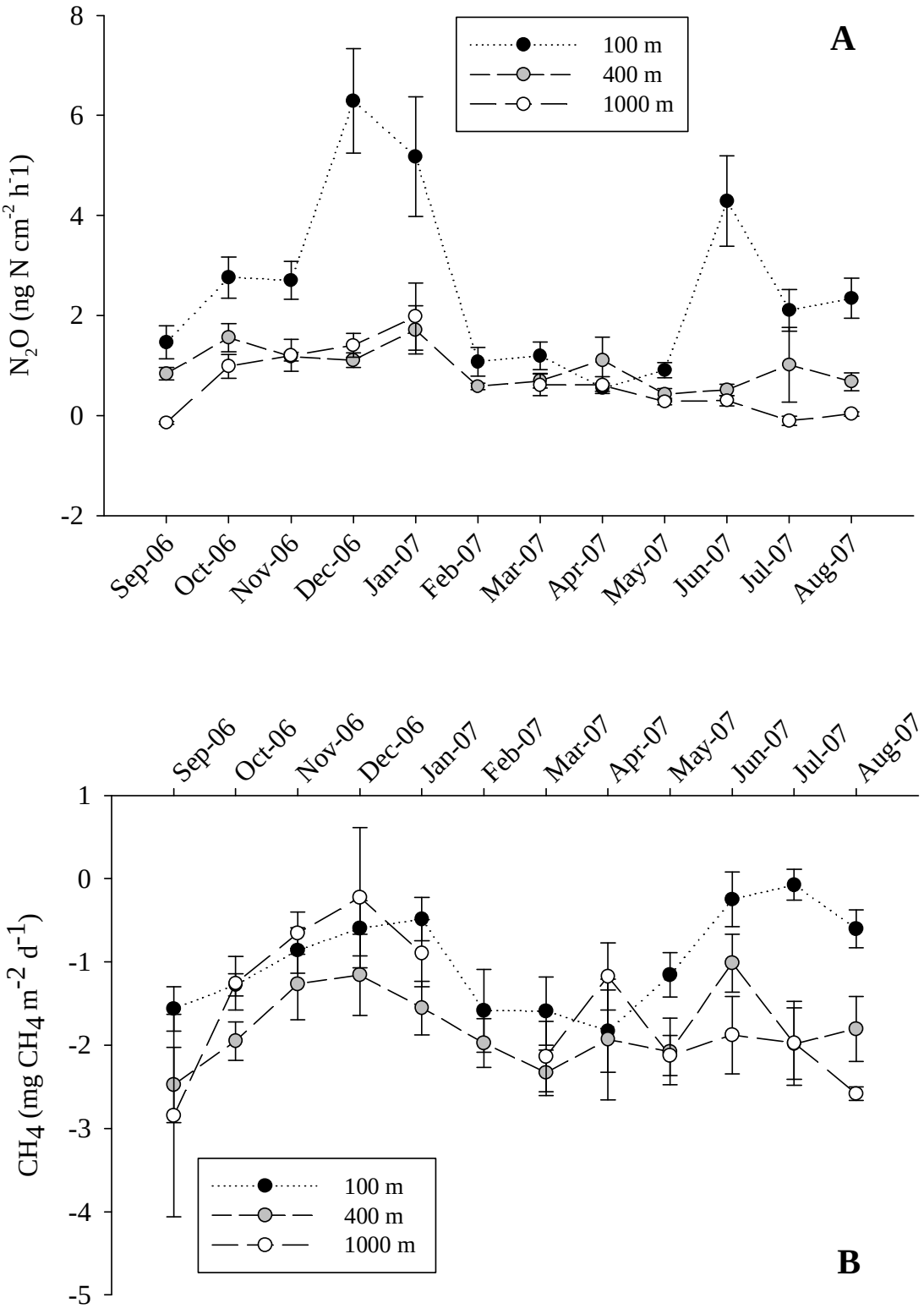
A
100 m
400 m
1000 m
N_2O (ng N cm^{-2} h-1)
8
6
4
2
0
-2
Sep-06
Oct-06
Nov-06
Dec-06
Jan-07
Feb-07
Mar-07
Apr-07
May-07
Jun-07
Jul-07
Aug-07
B
100 m
400 m
1000 m
CH_4 (mg CH_4 m^{-2} d^{-1})
1
0
-1
-2
-3
-4
-5

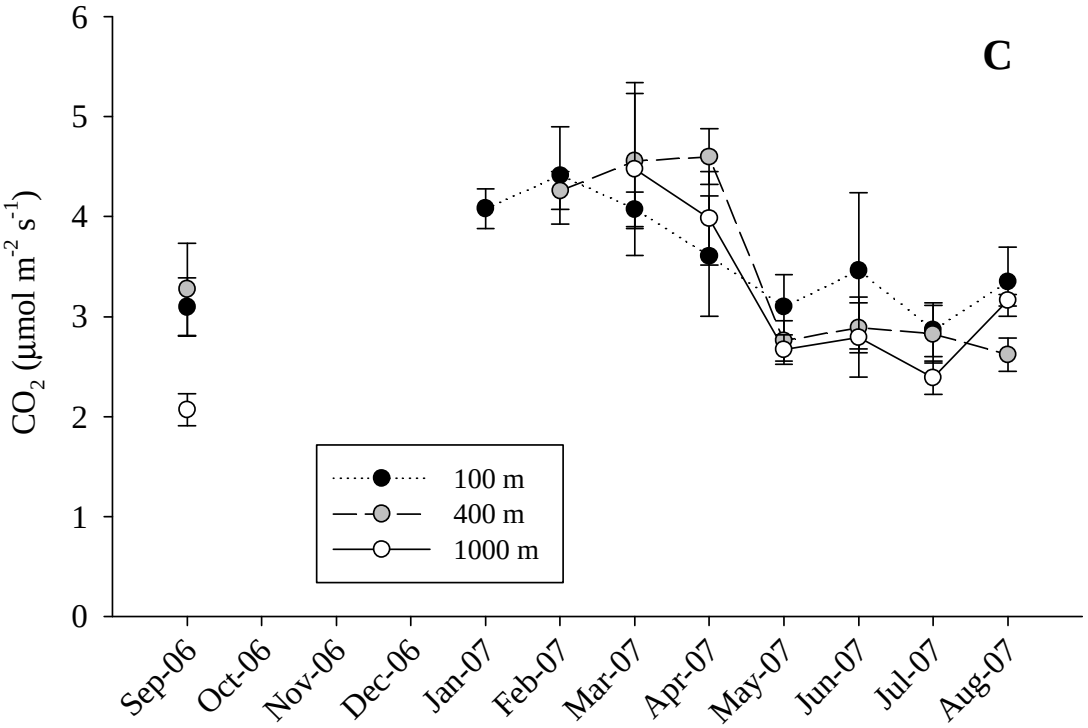

Figure 3. Monthly soil-atmosphere gas flux of (**A**) nitrous oxide (N_2O), (**B**) methane (CH_4), and (**C**) carbon dioxide (CO_2) at different altitudes. Values represent the mean of four replicates per elevation and bars represent standard errors.

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