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Greenhouse gases (CO₂, CH₄ and N₂O) in lowland springs within an agricultural impacted watershed (Po River Plain, northern Italy)

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In the Po River Plain, nitrogen surplus in permeable soils results in elevated downward nitrogen fluxes, mostly as nitrate. Lowland springs, aligned along interfaces between gravel and sandy soils, recycle part of this nitrogen to the surface and we hypothesised that they may be hot spots of N₂O and other greenhouse gases, due to incomplete denitrification in the suboxic environment. In early and late summer 2009, water flow was measured and water samples were collected at the outlet and ~1 km downstream at 14 springs; physico-chemical parameters [temperature, pH, dissolved inorganic nitrogen (DIN) and dissolved gases (O₂, N₂O, CH₄, CO₂)] were analysed. All springs were characterised by elevated nitrate concentrations (154–1411 μM) and recycled to the surface inorganic nitrogen (~180 kg N NO₃⁻ · day⁻¹ on average). Spring waters were suboxic (40–60% of O₂ saturation) and CO₂, CH₄ and N₂O supersaturated (26.6–2399.0, 0.002–1.02 and 0.02–1.02 μM, respectively). CO₂ and N₂O underwent a significant degassing process from the supersaturated waters to the atmosphere. Calculated N₂O emissions (up to 0.646 g N₂O · m⁻² · d⁻¹, among the highest reported for aquatic environments) highlight the role of lowland springs as hotspots of N₂O. We conclude that lowland springs located in heavily impacted watersheds recycle groundwater nitrate and have an extremely elevated potential as greenhouse gas emitters.

Keywords: CO₂; N₂O; CH₄; gas saturation; spring; N-pollution

1. Introduction

Eutrophication of aquatic environments has been mostly ascribed to nonpoint sources [1]. Agricultural practices affect surface and groundwater quality due to the large use of chemical and organic fertilisers, largely in excess compared with crop uptake [2]. One of the most serious threats connected with fertilisers use is the increasing concentration of biologically available nitrogen (reactive nitrogen) in both the atmosphere and biosphere [3]. Of critical importance is assessment of the nitrogen balance at the watershed scale because, as proposed by Oenema et al. [4], the quantification of the 'soil system N budget' done by comparing input and output N loads

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results very frequently in a missing amount of N. The probable fate of this unaccounted for N is atmospheric loss as N_2 due to denitrification and the progressive accumulation and storage of N in groundwater [5]. Groundwater can exhibit elevated NO_3^- concentrations and, particularly when it is in contact with surface or deep organic horizons, can be the site of intense microbial transformations due to the extremely developed surfaces and multiple oxic and anoxic niches [6]. Also N_2O can be found with elevated concentrations in groundwater, because it is an end- or byproduct of denitrification and nitrification, respectively [7,8]. Owing to the complex nature of substrates and to extremely variable oxygen availability, it is very difficult to measure, model and predict dominant processes and metabolism in aquifers; these processes can vary along the vertical and horizontal sediment profile, with feedbacks for the dissolved chemical forms. Because groundwater can emerge from the soil when the soil porosity changes, chemical analyses of water may allow inferences about chemical and microbial processes from end-products. In the Po River Plain (>70,000 km², northern Italy) lowland springs are common along the transition areas from high- to low-permeability aquifer [9]. They are located at both banks of the Po River and are aligned at the interface between gravel and sandy sediments. Lowland springs are characterised by constant temperature and chemical composition throughout the year; high nitrate concentrations are mainly due to agricultural activities in feeding basins [10,11]. Analysis of the spring water and detailed understanding of pathways of macro-pollutants as nitrogen (i.e. from the surface to groundwater and back again to the surface) are particularly relevant in the Po River Plain, which is the most exploited area of Italy for farming, agriculture and human settlement, with an estimated 5,800,000 pigs and 2,800,000 cows (64 and 44% of national production, respectively) and 16,200,000 inhabitants (27% of total population) concentrated in 23% of national surface [12,13]. Changes in soil use and the intensification of agricultural practices have resulted in widespread pollution of surface and groundwater; a large fraction of the Po River plain is vulnerable to nitrate pollution.

In this study we analysed the water of several springs in order to evaluate their potential role as a hotspot for greenhouse gas emission, in particular N_2O , and as recyclers of an important amount of the nitrate loss by percolation.

2. Materials and methods

2.1. Study area

Selected springs were located within the Po River watershed, along the medium plain portion of Lombardy and Emilia Romagna regions ($n = 12$ and 2 , respectively) (Table 1). Springs 500–1500 m long without any visible water input or output were chosen in order to study gas transformation along the downstream flow. Site selection was carried via technical regional maps 1:10,000 and Google Earth™ (Google Inc., Mountain View, CA, USA) followed by field surveys.

2.2. Water sampling and analyses

Water samples were collected from all springs during two sampling campaigns, in late June and late August, to take into account possible hydrological changes from wet spring to dry summer weather. Two sampling stations were selected for every spring, one near the spring output (H) and a second at 500–1500 m downstream (D). Temperature and pH were measured *in situ* using a multiparametric probe (YSI Instruments, 556 MPS model). Water samples for laboratory analyses were collected just below the water surface using glass bottles, and taking care to avoid gas bubble formation. An unfiltered amount of water was collected into glass vials (internal volume 12 mL, Labco Exetainers®, UK) for dissolved oxygen analysis (iodometric titration) [14]. Filtered amounts of water were transferred to glass vials and analysed for dissolved inorganic carbon (DIC)

Table 1. Coordinates, length of the reach connecting the headwater with the downstream sampling station and water flow of the studied lowland springs.

Spring	Coordinates		Length (m)	Flow (June) (m ³ · s ⁻¹)	Flow (August) (m ³ · s ⁻¹)
	Latitude	Longitude			
1	45°28'37.53"	10°1'16.40"	630	0.34	0.44
2	45°24'31.74"	9°30'20.73"	580	0.13	0.29
3	45°28'2.19"	9°57'46.31"	600	0.06	0.06
4	45°26'18.72"	9°30'37.89"	240	0.06	0.16
5	45°27'35.05"	9°35'34.33"	550	0.23	0.35
6	45°26'1.66"	9°31'32.01"	560	0.45	0.68
7	45°28'38.94"	9°49'59.91"	630	0.04	0.18
8	44°47'32.03"	10°23'34.21"	560	0.01	0.05
9	44°57'0.84"	9°55'20.53"	760	0.01	0.02
10	45°22'44.99"	9°51'6.38"	510	0.12	0.11
11	45°26'12.13"	9°35'41.54"	880	0.21	0.16
12	45°28'15.40"	9°45'14.49"	840	0.09	0.12
13	45°25'52.68"	8°59'16.48"	610	0.02	0.05
14	45°27'20.65"	9°45'40.34"	1330	0.05	0.20

using a six end-point titration method with 0.1 N HCl [15]. Calcium was analysed by titration with EDTA [14]. Subsamples of water for ammonium, nitrite and nitrate analysis were stored in PET tubes and analysed using standard spectrophotometric methods [14]. Gaseous samples for CO₂, CH₄ and N₂O analysis were collected from the headspace of a glass bottle (1150 mL) filled with 500 mL of water, tightly capped and shaken vigorously for 2 min to reach gas equilibrium [16]. Site water was then introduced through the stopper septum and headspace volume (40 mL) was transferred via needles in gastight Exetainers, flushing ~3 × the internal volume. CH₄ was analysed by injecting samples into a gas chromatograph (Series 9000 Fisons, Mainz, Germany) equipped with a flame ionisation detector and a QPLOT column. N₂O concentration was determined using a gas chromatograph (Series 800; Fisons) equipped with an electron capture detector (ECD) provided with a 2 mL loop automated injection port connected to a 10-port valve. Precolumn (1 m) for frontflush and backflush events and a column (2 m) (Porapak 80–100 Q; o.d. 1/8', 0.08' i.d.) were maintained at 60 °C. The ECD was held at 280 °C; pure nitrogen was used as carrier gas at a flow rate of 40 cm³ · min⁻¹. Carbon dioxide concentration was measured with a TraceGC (ThermoFinnigan, San Jose, CA, USA) gas chromatograph equipped with a thermal conductivity detector (TCD). The column (a capillary RTQPlot; 30 m) was maintained at 33 °C; the temperatures of the injector and detector were held at 100 °C, respectively. The flow rate of helium carrier gas was 1.5 · min⁻¹.

2.3. Gas flux calculations

Fluxes at the water–atmosphere interface were calculated as:

$$J_i = Kd(C_{iSAT} - C_i), \quad (1)$$

where J_i is the gas flux (mol · m⁻² · h⁻¹), d the water depth (m), C_{iSAT} the gas concentration in water in equilibrium with atmosphere (mol · m⁻³) and C_i the gas concentration in water (mol · m⁻³). Reaeration coefficient K (h⁻¹) was calculated using the general formula:

$$K = a \frac{u^b}{d^c}, \quad (2)$$

Table 2. Reported values for coefficients a , b and c of Equation (2).

Ref.	a	b	c
O'Connor and Dobbins [17]	3.74	0.5	1.5
Churchill et al. [18]	5.01	0.969	1.67
Isaacs and Gaudy [19]	4.75	1	1.5
Langbein and Durum [20]	5.14	1	1.33
Owens et al. [21]	5.35	0.67	1.85
Bennett and Rathbun [22]	5.58	0.607	169

where u is the water velocity ($\text{m} \cdot \text{s}^{-1}$) and a , b and c are empirical coefficients estimated by several authors (Table 2). Reaeration coefficients were corrected using Equation (3):

$$\frac{K_1}{K_2} = \left(\frac{Sc_1}{Sc_2} \right)^n, \quad (3)$$

Where K is the reaeration coefficient for gas 1 and 2, Sc is the Schmidt number of gas 1 and 2 and n is a coefficient set to a value of 1, suggested for the stagnant boundary layer model [23]. Schmidt numbers were calculated using the ratio of gas diffusivities and kinematic viscosity of water. Reaeration coefficients were also corrected for temperature effect [24]:

$$K_T = K_{20^\circ\text{C}} 1.0241^{(T-20^\circ\text{C})}, \quad (4)$$

Where K_T and $K_{20^\circ\text{C}}$ are the reaeration coefficients at the T temperature of the water and at 20°C , respectively. Carbonate equilibrium correction for calculation of CO_2 fluxes was not taken into account as water pH was < 8 and DIC > 1 mM [25]. Theoretical fluxes were calculated using K values obtained with coefficients reported in Table 2 and then averaged for both sampling periods.

Expected concentrations of N_2O , CO_2 and CH_4 at D stations (C_{exp} , $\text{mol} \cdot \text{m}^{-3}$) were calculated, in order to estimate gas dynamics during downstream flow, according to the equation:

$$C_{\text{exp}} = (C_i - C_{\text{sat}}) e^{-\frac{Kl}{u}} + C_{\text{sat}}, \quad (5)$$

where l (m) is the reach length. The assumptions of the Equation (5) are: (i) constant temperature (T), velocity (u) and depth (d) during the stream flow; (ii) no interactions among soil-groundwater and water interface in the channel; and (iii) negligible instream production of gases from biological processes. Gas diffusion from water to atmosphere can be modeled considering a static volume of water, with constant parameters (u , d , T , K), in which gas concentration is subjected to an exponential decaying process over time.

2.4. Statistical analyses

Because data were not normally distributed, differences between stations and sampling periods were tested with Wilcoxon's test for paired samples. Statistical analyses were performed with statistical package R (© 2009, R Foundation for Statistical Computing).

3. Results

3.1. Physical and chemical parameters

Average flows of the water measured in the two sampling periods at stations D are reported in Table 1, whereas mean, maximum and minimum values of chemico-physical parameters measured

Table 3. Mean, maximum and minimum values of the physical and chemical parameters analysed at headstream (H) and downstream (D) stations.

	June						August						
	Mean		Max		Min		Mean		Max		Min		
	H	D	H	D	H	D	H	D	H	D	H	D	
pH	7.33	7.66	7.67	8.37	7.08	7.30	7.49	7.68	8.31	8.64	7.17	6.63	
T	°C	16.7	17.3	19.0	22.0	15.0	15.0	17.4	18.4	22.0	22.0	14.0	16.0
O ₂	mg · L ⁻¹	5.63	7.73	8.63	14.04	0.91	4.21	4.67	7.61	8.07	11.76	1.71	4.78
NH ₄ ⁺	μM	0.02	0.28	0.20	2.72	0.00	0.00	0.09	0.77	0.80	8.87	0.00	0.00
NO ₂ ⁻	μM	0.16	1.10	1.19	9.23	0.00	0.01	0.13	1.15	0.95	10.74	0.00	0.00
NO ₃ ⁻	μM	628.6	644.3	1031.2	1159.4	361.6	349.6	532.7	590.5	1410.7	1302.9	154.2	166.4
CO ₂	μM	733.5	456.9	1155.2	870.8	358.8	26.6	912.4	573.9	2161.6	2398.9	128.4	179.5
CH ₄	nM	96.1	181.9	146.5	694.2	17.3	36.8	116.5	152.3	1023.5	688.1	2.4	10.0
N ₂ O	nM	487.7	335.1	974.6	1023.7	205.1	16.6	381.8	260.6	874.0	951.3	66.6	92.6

Table 4. Statistical analyses performed with Wilcoxon test for paired samples.

	Flow	pH	T	NH ₄ ⁺	NO ₂ ⁻	NO ₃ ⁻	CO ₂	O ₂	N ₂ O	CH ₄
H-H (June–August)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*
H-D (June)	–	**	n.s.	n.s.	***	n.s.	**	**	**	n.s.
H-D (August)	–	*	**	n.s.	**	n.s.	*	***	*	n.s.

Notes: n.s. $p > 0.5$, * $p < 0.5$, ** $p < 0.1$, *** $p < 0.01$.

at the headwater (H) and downstream (D) stations are reported in Table 3. Differences between water discharge ($0.21 \pm 0.18 \text{ m}^3 \cdot \text{s}^{-1}$ in June and $0.13 \pm 0.13 \text{ m}^3 \cdot \text{s}^{-1}$ in August, respectively, pooled data from the two sampling periods) and between chemico-physical parameters at the headwater sites were not significant in the two sampling periods ($p > 0.05$; Table 4). Temperature remained constant at H sites in both sampling periods, although it increased slightly downstream, particularly in August. Nitrate was the most important dissolved inorganic nitrogen form, with elevated concentrations at H and D in both sampling periods, up to $1400 \mu\text{M}$; nitrite and ammonium concentrations were often below detection limits (Table 3).

All headwaters were under-saturated in oxygen, although significant reaeration occurred downstream ($p < 0.01$; Table 4). Nitrous oxide, carbon dioxide and methane concentrations exhibited large variability among sites but not between sampling periods and were always far above the theoretical water–atmosphere equilibrium values. N₂O and CO₂ concentrations decreased significantly downstream ($p < 0.05$), although this was not the case for CH₄ ($p > 0.05$) (Figure 1).

In both sampling periods, carbon dioxide was positively correlated with nitrous oxide ($\rho = 0.82$) and nitrate ($\rho = 0.78$), nitrous oxide was also positively correlated with nitrate ($\rho = 0.75$) (Figure 2).

3.2. Calculated N₂O, CO₂ and CH₄ fluxes

As spring waters were N₂O, CO₂ and CH₄ supersaturated, the calculated fluxes were all positive (efflux from water to the atmosphere) and reflected the same variability as *in situ* concentrations. Fluxes of N₂O (0.120 ± 0.142 and $0.075 \pm 0.114 \text{ g N}_2\text{O m}^{-2} \cdot \text{d}^{-1}$, respectively), CO₂ (0.194 ± 0.173 and $0.227 \pm 0.336 \text{ kg CO}_2 \text{ m}^{-2} \cdot \text{d}^{-1}$, respectively) and CH₄ (0.008 ± 0.008 and $0.010 \pm 0.026 \text{ g CH}_4 \text{ m}^{-2} \cdot \text{d}^{-1}$, respectively) (Equation (1), pooled data from all sampling sites and for the two periods) did not differ significantly in the two sampling periods ($p > 0.05$).

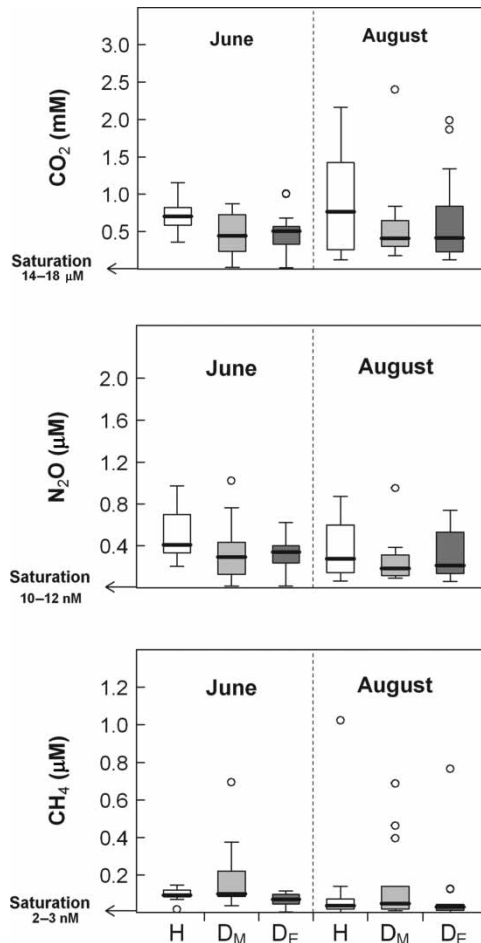


Figure 1. Concentration of CO_2 , N_2O and CH_4 measured at the headwater (H) and downstream (D_M) stations. D_E , the expected concentration downstream, was calculated using Equation (3). Theoretical concentration of the three greenhouse gases at equilibrium is also reported.

Equation (5) was used to calculate the theoretical, expected concentration of the three greenhouse gases at the downstream sites, assuming that the only occurring process was degassing. Expected CO_2 and N_2O concentrations (D_E) were not significantly different from those measured (D_M , $p > 0.05$) while this was not the case for CH_4 , with measured concentrations higher than expected ($p < 0.05$) (Figure 1).

4. Discussion

4.1. Spring waters as sources of greenhouse gases

Spring headwater showed similar physico-chemical parameters in the two investigation periods, probably due to the slow turnover of groundwater. Temperatures such as those at H sites (16–17 °C) or slightly below (14–15 °C) are measured also during winter periods and result in rather constant rates of microbially mediated processes all year round [10]. Most aquatic environments at the same latitude display wide ranges of water temperatures, ranging from slightly above 0 to

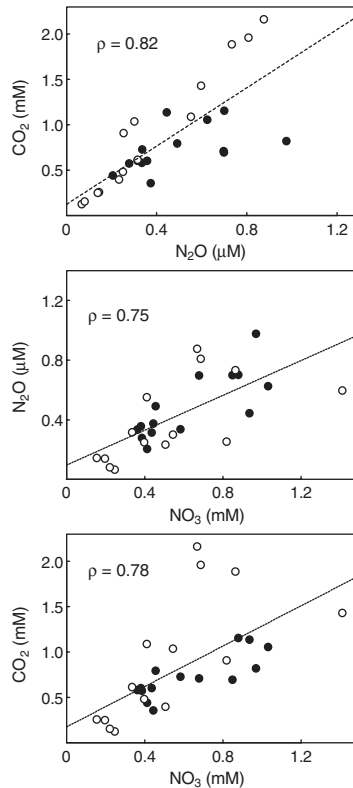


Figure 2. Correlations among nitrate, carbon dioxide and nitrous oxide concentrations measured at headwater (H) of the 14 springs. Black and white dots refer to June and August samplings, respectively. All correlations were highly significant ($p < 0.01$).

30 °C, the extremes of which can affect microbial processes. Significant degassing occurs mostly in summer due to lower gas solubility at higher water temperatures and to increased gas production by microbial communities. Concentrations of greenhouse gases, in particular nitrous oxide, were elevated in nearly all springs. This is particularly relevant, as N_2O has a warming potential which is ~ 298 -fold higher than that of CO_2 [26], moreover N_2O is generally a trace gas in most aquatic environments, where it does not accumulate if not under peculiar environmental conditions (i.e. hypoxic water or under extremely high NO_3^- concentrations) [27]. Average N_2O emission from the springs studied in this study ($\sim 100 \text{ mg } N_2O \text{ m}^{-2} \cdot \text{d}^{-1}$) are ~ 100 -fold higher than average emission reported for a variety of soils [28] which highlights the relevance of lowland springs as hotspots for this gas in the agricultural systems of the Po River Plain. At present, available information does not allow us to assess whether at the watershed level N_2O fluxes from these springs to the atmosphere are relevant and a more spatially extended study would be necessary.

The source of nitrous oxide in springs is difficult to infer, since N_2O is a by-product of nitrification and an end product of denitrification and both processes occur in soils and groundwater of the recharge area. A positive correlation between nitrate and nitrous oxide concentration in water, as found in this study, is typical of nitrification process [29]. Acidification of water due to production of protons during nitrification could lead to dissolution of carbonate sediments in the aquifer [30,31], explaining positive correlation between nitrous oxide and carbon dioxide. Oxygen concentrations measured at H sites were always higher than threshold concentrations for denitrification ($1\text{--}2 \text{ mg L}^{-1}$) [32] and were favourable to nitrification. However, denitrification

can occur in anoxic niches within an oxic environment, so oxygen concentration alone is not a predictive parameter [6]. Denitrification in anoxic environments leads to the complete reduction of nitrate to molecular nitrogen and, in much lower amounts, of nitrous oxide. As a consequence, a negative correlation between nitrous oxide and nitrate is expected. However, von der Heide et al. [33] emphasised high concentrations of both nitrate and nitrous oxide in the same aquifer, like those of the current study. Nitrous oxide reductase inhibition by nitrate [27,34] and oxygen [35] might explain the positive correlation between NO_3^- and N_2O . Because denitrification produces carbon dioxide, a positive correlation between N_2O and CO_2 is also expected.

Methane can occur in groundwater in high concentrations, particularly if anoxic conditions and low redox potential are established. The hydrochemistry of groundwater feeding the investigated springs was not optimal for methanogenic bacteria to compete with other anaerobic bacteria, because high nitrate and nitrous oxide concentrations seem to inhibit methanogenesis [36]. Likely due to NO_3^- availability, methane concentrations found in springs were lower than those reported in other aquifers [37]. It is not surprising that methane concentrations did not decrease or tended to increase at the downstream sites because methanogenesis is probably enhanced in vegetated, organic rich and water saturated sediments of the reaches.

The strong link between inorganic carbon and nitrogen, suggested by the positive correlations between CO_2 , NO_3^- and N_2O concentrations, indicates that carbon dioxide supersaturation can be explained by dissolution of carbonate sediments following water acidification (i.e. due to nitrification) or by heterotrophic bacterial processes (i.e. respiration activities). Dissolution of carbonate sediments leads to the production of DIC [38,39] and to the release of Ca^{2+} ions into the water, so positive correlations among calcium, nitrate and DIC are expected (Figure 3). Owing to carbonatic composition of the recharge area of groundwater that feeds lowland springs, decreases in pH are not expected. This is in agreement with pH values found in this study that are, with only one exception, in the 7–8.5 range.

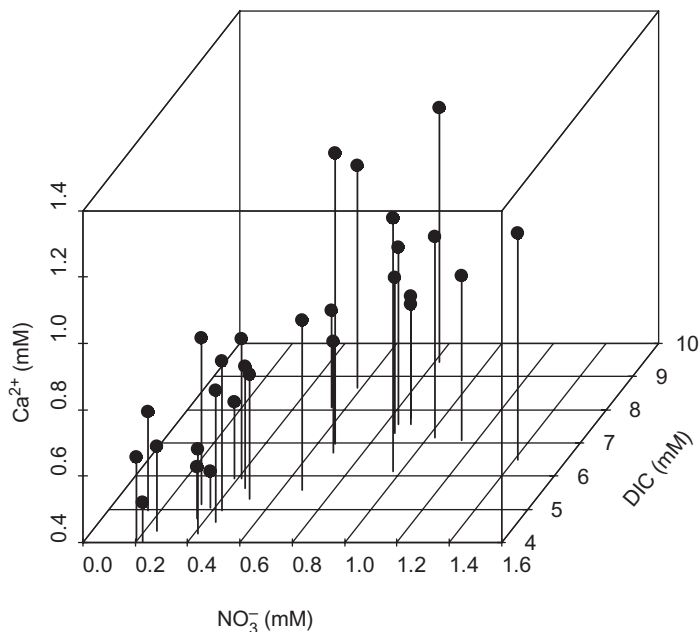


Figure 3. Three-dimensional scatterplot showing positive correlation among calcium, nitrate and DIC. NO_3^- and Ca^{2+} , NO_3^- and DIC and Ca^{2+} and DIC showed positive and significant correlations ($p < 0.01$) with $\rho = 0.67$, $\rho = 0.75$ and $\rho = 0.57$, respectively.

4.2. Flux variations during downstream flow

In groundwaters, heterotrophic processes should be dominant and gas supersaturation of microbial products as CO_2 , CH_4 and N_2O is likely to occur [40]. The final fate of greenhouse gases in groundwater is difficult to predict, especially if aquifers are deep. Springs connect aquifers with the surface and feed the secondary drainage network. At the spring headwater, groundwater comes into contact with the atmosphere where the concentrations of greenhouse gases are much lower, resulting in steep gradients and elevated effluxes. Gas release is further enhanced by the morphometry of the segments downstream in the spring, particularly the low depth. As a consequence, calculated fluxes are very high in most springs. The calculated N_2O effluxes from the springs are among the highest reported in the literature for different aquatic and terrestrial environments [41–44] and for this reason lowland springs can be defined as hotspots of greenhouse gas emissions.

Expected CO_2 and N_2O concentrations at D sites, calculated using a simple diffusion model (Equation (5)), were not statistically different from those measured (Wilcoxon for paired data, $p > 0.05$), although this was not the case for CH_4 ($p < 0.01$) (Figure 1). The first outcome can be explained considering that in-stream microbial or plant activities, even if occurring, do not affect significantly the elevated concentrations of the two gas over the relatively short reaches as those considered in this study (500–1500 m), with a water residence time of < 1 h. For these gases, outgassing is likely the main process regulating their concentrations downstream. Nevertheless, trace gas concentrations such as those of methane could be significantly influenced by other factors that are poorly explored, for example, the ingress of supersaturated groundwater from unconfined aquifer or in-stream metabolism. This might be the reason for the discrepancy between theoretical and measured CH_4 concentrations at D sites. The relationship between nitrous oxide and methane concentrations at D stations (Figure 4) allows us to group the studied springs along a gradient of relative abundance from N_2O to CH_4 . The low N_2O to CH_4 ratio might be due to in stream methanogenesis or to a connection with an unconfined aquifer, saturating an organic rich surface soil layer and strictly anoxic. High methane concentrations are, in fact, reported for subsurface water of riparian soils and in the hyporheic zone [45].

4.3. Relevance of groundwater N recycling to the secondary drainage network

Previous research in the same area evidenced high nitrate concentrations in superficial aquifers (up to $1000 \mu\text{M NO}_3^-$) similar to those reported in the present study [46]. Such elevated NO_3^- content is the probable consequence of large N surplus in agricultural soils [47], combined with the traditional irrigation practices, mainly based on flooding over permeable gravel soils. Nitrates

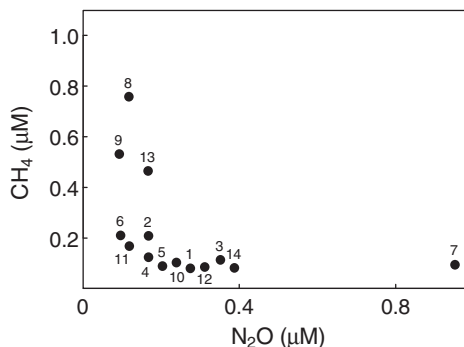


Figure 4. Relationship between nitrous oxide and methane concentrations measured at downstream station (D) in August. The numbers above black dots represent sampled lowland springs.

measured at downstream sites were not significantly different from those measured at H sites, probably due to very low residence time (<1 h) in the considered reach combined with extremely high concentrations. Calculated nitrate loads recycled to the second drainage channel network from each spring are elevated, with a mean value of 177 and a maximum of 456 kg N-NO₃⁻ · d⁻¹. These numbers are relevant if it is taken into account that in the Lombardy Region alone, in the transition area between gravel and sandy soils, >700 springs were censused [48]. Inorganic nitrogen recycled from groundwater to the secondary drainage network by lowland springs has the same order of magnitude of recently estimated nitrogen loads at the closing section of two tributaries of the Po River, the Mincio and Oglio Rivers (average flow 30 and 60 m³ · s⁻¹, respectively) (Pinardi et al. 2007; Racchetti et al. 2008; unpublished works). Nitrate concentrations reported for many aquifers in the Po River Plain and nitrate loads outflowing the springs, emphasise the temporary role of groundwater as N sink and its simultaneous role in recycling nitrogen derived from agricultural activities. We argue that, as groundwater moves, N input to surface waters by springs can occur far from those areas where N contamination of the aquifer occurs. In N-contaminated watersheds, a significant loss of nitrogen from the system could occur via leaching and percolation, but the present study demonstrates that relevant amounts of nitrogen, mostly in the nitrate form, is recycled by lowland springs to the secondary drainage network, where it can undergo assimilative and dissimilative processes. For this reason, such nitrogen amounts should be considered in mass balance calculations.

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