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ORIGINAL ARTICLE

Linking dissolved organic carbon, acetate and denitrification in agricultural soils

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Abstract This study focuses on the factors affecting nitrate removal via microbial denitrification in agricultural soils, and particularly on the quantity and quality of dissolved organic carbon. To assess the relationship among dissolved organic carbon, nitrate and low molecular weight organic acids (acetate and formate), grids of ceramic suction cups were established in the four most representative soil types of the lower Po River floodplain, cropped with maize. Results highlighted a direct relation between acetate and dissolved organic carbon in all sites. The best fit was obtained in soils were the main source of organic carbon was the maize residues. By comparing dissolved organic carbon and acetate versus nitrate concentration revealed that acetate can be used as a better proxy for denitrification in the field with respect to dissolved organic carbon.

Keywords Suction cups · Acetate · Nitrate · Soil type · Maize

Introduction

The recent international concern about the adverse effects of fertilizer-derived nitrate (NO_3^--N) on environmental quality and public health has newly drawn attention to the research on the mechanisms and factors promoting NO_3^--N removal via denitrification in agricultural soils (Galloway et al. 2008; Barnes and Raymond 2010; Yang and Liu 2010).

In agricultural soils and aquifers, the organic matter availability for denitrifying bacteria that use organic carbon as the electron donor has been identified as the most important factor limiting denitrification (Rivett et al. 2008; Jahangir et al. 2012). In most of the studies, the rate of denitrification is related to DOC concentrations rather than the amount of total organic carbon and this has been motivated in reason of the higher biodegradability of the low molecular weight organic acids (LMWOA) which may account for a significant portion of DOC.

In porewater and groundwater DOC concentrations are generally low, indicating a potential DOC limitation of denitrification (Rivett et al. 2008; Zarnetske et al. 2011). Conversely, DOC availability in subsoil expresses a buffering capacity of the excess of NO_3^--N preventing the risk of percolation to groundwater (Böhlke et al. 2002; Green et al. 2008).

In agricultural soils cultivated with maize, the incorporation of crop residues is a traditional farming practice for enhancing soil nutrient availability and productivity (Spedding et al. 2004). In soils receiving synthetic fertilizers, the annual crop residues are often the only input of C_{org}, buried within the top soil and slowly converted into monomers by the soil microbial community (Liu et al. 2006; van der Perk 2006).

Low molecular weight organic acids, like acetate and formate, originate from plant litter breakdown, root exudation and C_{org} decomposition in anaerobic micropores of agricultural soils and can be used further by soil microbes as a carbon (C) source when oxic conditions prevail (van Hees et al. 2003). In fact, they are converted to carbon dioxide (CO_2) or methane (CH_4) after a short residence time, but can also accumulate because of absorption to clay minerals and hydroxides (Strobel 2001).

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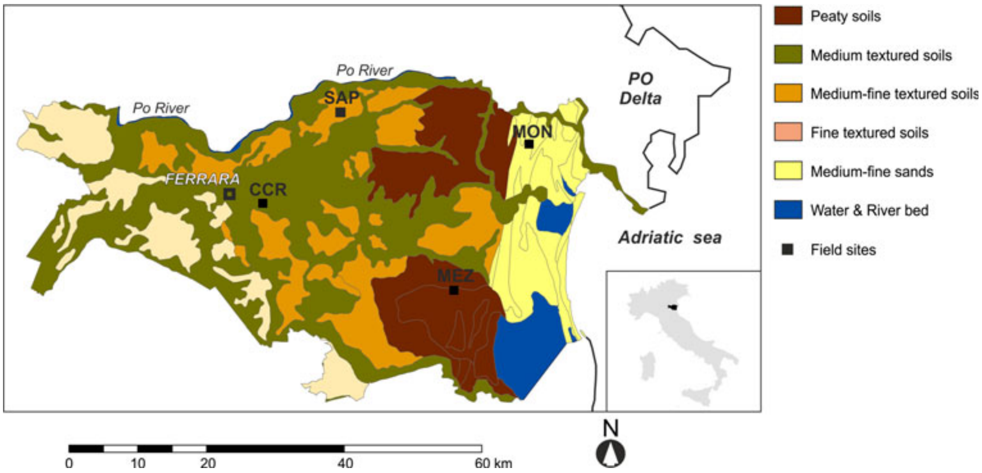

Fig. 1 Soil map of the Ferrara Province; *square dots* represent the field sites

Laboratory experiments have demonstrated that 70 % of glucose is degraded in soluble metabolites, where acetate is the most important early LMWOA (Kusel and Drake 1994, 1995). Acetate, undergoes a rapid turnover, which can also be linked to denitrification when electron acceptors like NO_3^--N are present and sub-oxic conditions prevail (Chidthaisong and Conrad 2000; Blume et al. 2002). Thus, the flux of dissolved organic carbon (DOC) is usually considered a limiting factor for denitrification (Brye et al. 2001; Taylor and Townsend 2010).

Compared with nitrogen (N), less research has focused on quantifying soluble C leaching under field conditions and various management practices, such as tillage and fertilization (Hadas et al. 2004; Kolář et al. 2011).

To assess the role of acetate and formate in NO_3^--N removal, a grid of suction cups was established in four typical soil types intensively fertilized for decades with synthetic urea [$CO(NH_2)_2$], in the lower Po River floodplain, a premier agricultural districts in northern Italy.

Materials and methods

Study area

The study has been carried out in the lower Po River floodplain (Ferrara Province), a recently reclaimed and intensively cultivated land, characterized by a favourable flat topography and water availability for irrigation. The whole Ferrara Province has been recently declared vulnerable to NO_3^--N from agricultural sources (Water Protection Plan of Emilia-Romagna Region 2006). The predominant soil textures in the Ferrara Province are silty loam and silty clay (68 % of the territory), while peaty (23 %) and sandy (9 %) soils are less common. The surface slope is less than 0.5 % (and mostly less than 0.05 %), allowing to assume surface runoff as minimal and water movement in the unsaturated zone dominantly vertical (Mastrocicco et al. 2010a, b). Four sites, named CCR, SAP, MEZ and MON have been selected (Fig. 1) to monitor the fate of DOC, acetate, formate and NO_3^--N dissolved species in the unsaturated zone. Meteorological stations recording rainfall, wind speed, solar radiation, temperature and humidity are located from 0.5 to 5 km far from the field sites. Meteorological data are available online from the meteorological regional service (http://www.arpa.emr.it/sim/?osservazioni_e_dati/dexter) and from local web service (http://www.meteoveneto.com). The four sites are characterized by a sub-coastal temperate climate by cold winters and warm summers, with moderate precipitations, elevated humidity, low wind speed, moderate daily and seasonal temperature excursions. In each site of the four sites, a rain gauge was installed to record daily rainfall. The cumulated rainfall during 2008 and 2009 was 1,350 mm in CCR; 1,280 mm in SAP; 1,145 mm in MEZ; and 1,092 mm in MON. The lower cumulated rainfall in MEZ and MON was due to their proximity to the coast respect to CCR and SAP (Mollema et al. 2012; Pavan et al. 2008). Two above-canopy sprinkler irrigation events of 30 mm each were applied in June and July 2009 at MEZ and MON sites, while in 2008 during this period rain events were sufficient to cover the crop water demand in every site, thus irrigation was not applied. The relative less precipitation in two of the selected sites was supplemented by sprinkler irrigation, thus the total amount of water was almost the same for all the sites.

The SAP soils are deep, moderately alkaline, with silty clay or clay loamy textures; from moderately to highly calcareous in the upper horizons and scarcely calcareous in the lower horizons. The CCR soils are in general moderately alkaline, with the upper horizons characterized by silty clay loamy texture and moderate carbonates content; the lower horizons exhibit silty loamy texture and are highly calcareous. The representative MEZ soil profile shows upper horizons of approximately 40–60 cm thickness with high levels of C_{org}, moderate or low carbonates

Table 1 Soil classification of the four field sites

	Pedological classification	Sedimentological environment	Textural classification
CCR	Haplic Calcisols	Fluvial	Silty loam
SAP	Vertic Cambisols	Interfluvial	Silty clay
MEZ	Thionic Histosols	Marsh lagoon	Peaty
MON	Calcaric Arenosols	Beach dune	Sandy

content and slightly alkaline pH; the lower horizons exhibit low to moderate acid pH and saline conditions (Table 1). SAP, CCR and MEZ have been fertilized using $[CO(NH_2)_2]$ at a rate of 300 kg N/ha/year: 20–25 % in pre-sowing and the rest in the early growing period. A micro-ripper with sub-needles, distributing $[CO(NH_2)_2]$ at 0.05 m below ground level (b.g.l.), has been employed to minimize volatilization. CCR, SAP and MEZ have not received organic fertilizers in the last 30 years.

The MON site is representative of neutral to moderately alkaline soils, with the upper horizons characterized by fine sand texture and low carbonates content; while, the lower horizons exhibit fine to medium sand texture and are alkaline with moderate carbonate content. Due to the intrinsic high permeability and low fertility, these soils are usually amended with organic fertilizers. The MON site in addition to 300 kg N/ha/year of $[CO(NH_2)_2]$, has been amended with 7 t/ha of chicken manure from organic farming, in October 2007 and October 2009. In all the studied soils, maize stalks have been annually incorporated into the soil at ploughing.

Sample collection and analysis

To define the site stratigraphy triplicates core logs have been drilled manually with an Eijelkamp Agrisearch auger equipment down to 1 m b.g.l. In Table 2 the soil parameters for each field site are resembled. From collected core samples at 0.25, 0.50, 0.75 and 1 m b.g.l., particle size curves have been obtained using a settling tube for the sandy fraction and an X-ray Micromeritics Sedigraph 5100 for the finer fraction. C_{org} content has been measured by wet oxidation (Rowell 1994) and the cation exchange capacity (CEC) via the ammonium acetate replacement method (Rowell 1994). Two arrays of soil solution suction samplers have been installed at 0.25, 0.50, 0.75 and 1 m b.g.l., in each field site to analyze soil water quality. Soil water has been collected by applying a vacuum of about 0.065 MPa to the suction cup for at least 1 h, yielding between 10 and 200 ml of water per sample.

Table 2 Grain size distribution, C_{org} and CEC, averaged on three replicates from four different levels 0.0, 0.25, 0.5 and 1.0 m b.g.l

Parameter	CCR	SAP	MEZ	MON
Grain size (%)				
Sand	15.8	10.1	3.10	95.0
Silt	60.7	47.0	78.9	4.10
Clay	23.6	43.0	18.1	0.90
C_{org} (%)	1.06	2.97	13.9	1.23
CEC (mmol/kg)	1.09	2.66	3.41	0.83

In all the four sites the upper soil horizons (suction cups at 0.25 and 0.5 m b.g.l.) have become too dry to produce soil water samples during summer seasons; this has reduced the total number of samples collected and analyzed in a manner inversely proportional to the depth, during dry periods. The number of samples collected during 2008 and 2009 were 180 at CCR, 204 at SAP, 140 at MEZ and 170 at MON.

Oxygen and redox potential were determined in the field with a HANNA Instruments HI 9146N galvanic oxygen sensor with temperature and salinity compensation and a HI 9125N combined AgCl–Pt electrode for redox potential measurements. Water samples have been preserved by adding 0.2 mmol/l of sodium azide, stored at 4 °C in the field and then frozen at −18 °C in the laboratory.

Acetate, formate and NO_3^--N have been determined with isocratic (1 ml/min and 25 µl of injection loop) dual pump ion chromatography ICS-1000 Dionex, equipped with an AS9-HC 4 × 250 mm high capacity column, a on guard AG9-HC guard column and an ASRS-ULTRA 4 mm self-suppressor for anions (Pfaff 1993; Singh et al. 1996). An AS-40 Dionex auto-sampler has been employed to run the analyses, DOC has been determined with a C analyzer (Carbon Analyzer Shimadzu TOC-V-CSM) after acidification with one drop of 2 mol/l HCl to remove dissolved carbonate (Potter and Wimsatt 2005). The limit of quantification of acetate, formate, NO_3^--N and DOC on the analyzed samples were 0.1, 0.3, 0.05 and 0.08 mg/l, respectively.

Results and discussion

Relationship of acetate and formate with DOC

Figure 2 shows the relationship between acetate and DOC in the four sites. The acetate concentrations found in this study are in accordance with those reported by Brye et al. (2001) and Siemens et al. (2003) for agricultural soils. CCR exhibited the lowest acetate concentrations in correspondence with the lowest soil C_{org} content (Fig. 2; Table 2) among sites. In CCR this agrees with the record of fertilization, in the year of study as well as in the last decades, performed only with synthetic fertilizers and

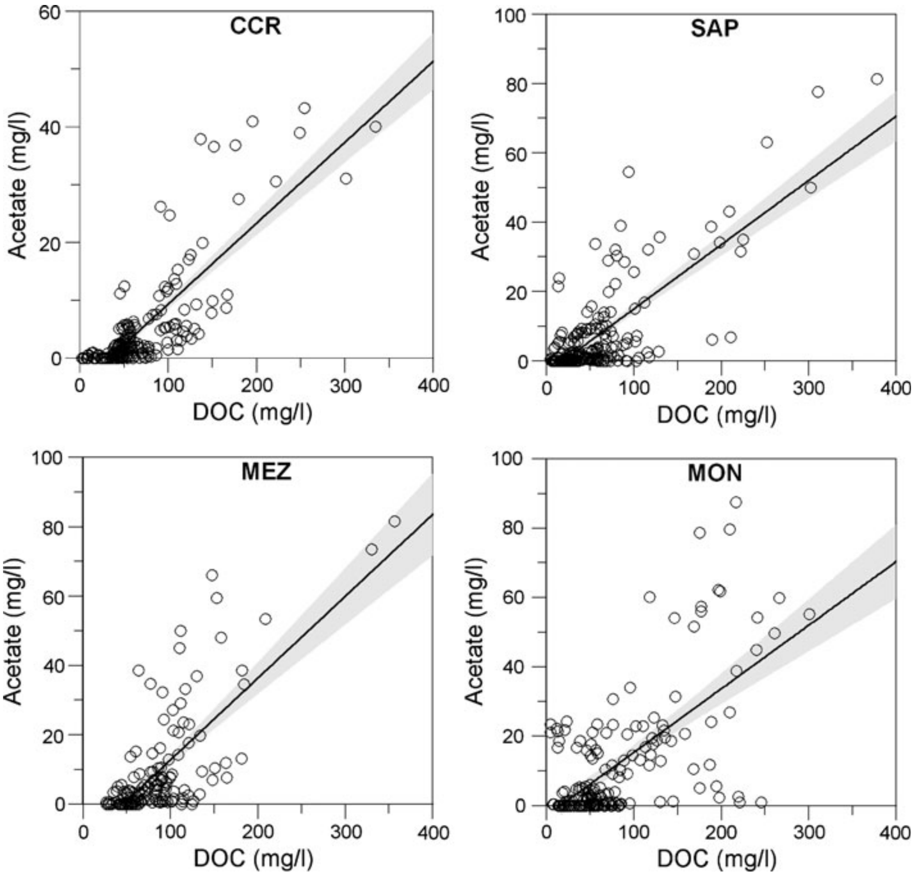

Fig. 2 Linear relationship between acetate (mg/l) and DOC (mg/l) concentration for the four sites, *shaded areas* depict the 95 % confidence intervals. Note differences in *Y* axis scales

never with manure or organic materials, proving that the sole organic input to the soil was crop residues. Moreover, at this site the high permeability of the thick unsaturated zone guarantees an aerobic environment where acetate is continuously exposed to O_2 and more likely consumed. In CCR, acetate-C approximated 5 % of the DOC while in SAP and MON acetate-C approximated around 7 % and in MEZ up to 9 % of the DOC present at the site. Thus, higher acetate concentrations and a higher acetate to DOC ratio appeared to be directly proportional to the C_{org} content (Table 2) and inversely to saturated condition which in this case have the effect to induce low oxygen availability and therefore slow down acetate mineralization. As a matter of fact, saturation was never present at CCR while it was often present in MON and MEZ, because of the shallow water table and in SAP, due to an ephemeral perched aquifer generated by the very low permeability. This hypothesis was confirmed by the inverse trend evidenced in all sites, between oxygen (O_2) concentrations and redox potential (Eh) and acetate concentration (Table 3). Moreover, in MON, MEZ and SAP, acetate accumulated especially in the bottom layer where anaerobic condition prevailed (Table 3).

Figure 3 shows the comparison between formate and DOC in each field site. Most of the formate concentrations fall between 0 and 4 mg/l in all the four sites, and only few samples recorded in MEZ and MON exceed 4 mg/l. This confirms that formate is a particularly transient species within the LMWOA from C_{org} decomposition.

Table 3 O_2 concentration and Eh averaged on three replicates at 0.25–0.5 m b.g.l., and 0.75–1.0 m b.g.l., in each field site, with *n* indicating the number of samples

Parameter	CCR ($n = 23$)	SAP ($n = 18$)	MEZ ($n = 19$)	MON ($n = 19$)
O_2 0.25–0.5 m (mg/l)	4.6 (2.1)	2.6 (1.7)	2.0 (1.3)	1.6 (1.1)
O_2 0.75–1.0 m (mg/l)	3.9 (3.2)	0.6 (0.5)	>0.1 (0.1)	>0.1 (0.1)
Eh 0.25–0.5 m (mV)	323 (54)	223 (76)	133 (101)	114 (88)
Eh 0.75–1.0 m (mV)	254 (145)	−12 (45)	−75 (23)	−136 (33)

Standard deviation values are given in parenthesis

Table 4 shows that the best fit for DOC-Acetate regression ($p < 0.01$) is achieved in CCR followed by SAP ($p < 0.01$). CCR and SAP have been amended with synthetic fertilizers since decades and the only organic input during the study period was from the maize roots exudates, in the growing season, and the maize residues

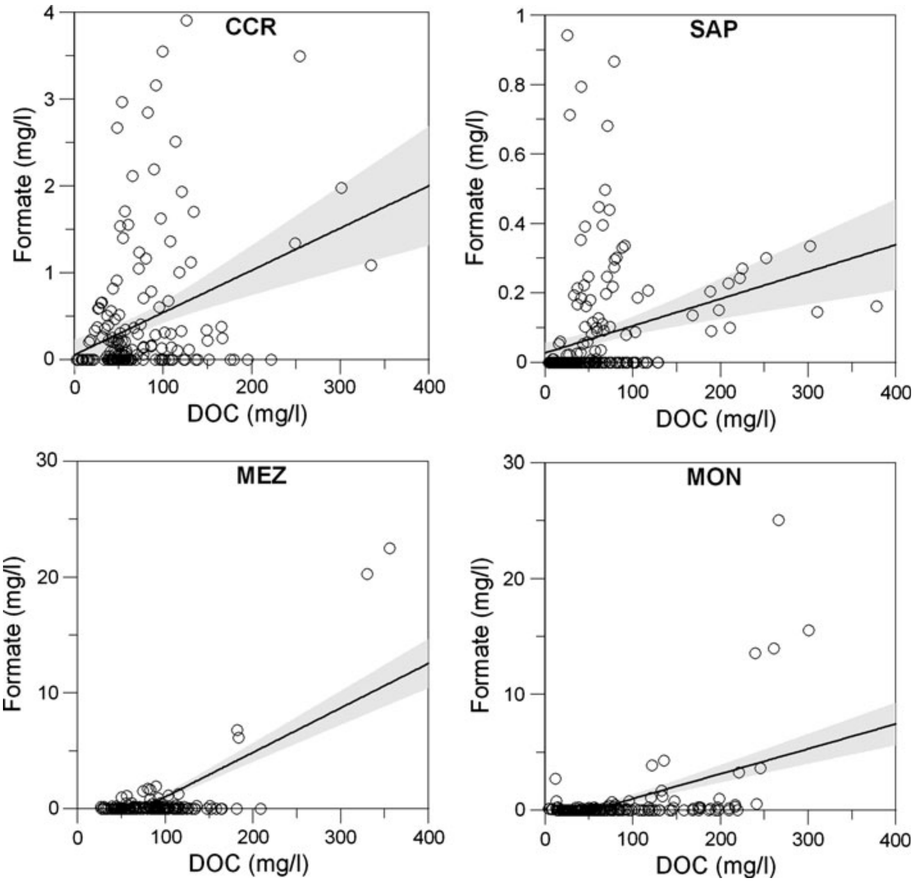

Fig. 3 Linear relationship between formate (mg/l) and DOC (mg/l) concentration in each of the four sites, *shaded areas* depict the 95 % confidence intervals. Note differences in *Y* axis scales

decomposition, after the harvest. Differently, in the peaty soils of MEZ ($p < 0.02$), the mineralization of the abundant C_{org} may have contributed as a secondary source of acetate and in MON ($p < 0.05$) was the addition of chicken manure to act as a secondary source. Table 4 also acknowledges for the poor fit between formate and DOC concentrations. This can be appreciated visually in Fig. 3, where for CCR and SAP the trend of formate against DOC is clearly bimodal while for MEZ and MON only few samples fall along the regression line at elevated concentrations, spoiling the statistical validity of these regressions ($p < 0.5$). Overall, these results indicate that formate is not the most informative tracer of labile DOC fate in agricultural soils because of: (1) the very low concentrations recorded in comparison with acetate and (2) the poor fit with DOC, in all the investigated soils.

Table 4 Regression coefficient (r^2) and residual mean square (RMS) describing the fit to the linear regression models applied to DOC-Acetate in Fig. 2 and to DOC-Formate in Fig. 3, for each field site, with *n* indicating the number of samples

Parameter	CCR ($n = 180$)	SAP ($n = 204$)	MEZ ($n = 140$)	MON ($n = 170$)
r^2 DOC-acetate	0.659	0.601	0.525	0.413
RMS DOC-acetate	26.81	69.63	115.4	190.4
r^2 DOC-formate	0.108	0.075	0.475	0.247
RMS DOC-formate	0.521	0.226	3.73	5.65

Relationship of NO_3^--N with acetate, formate and DOC

The best fitting curve describing the relationship between acetate and NO_3^--N in all four studied soils resulted to be a hyperbolic function [$y = (a/x) + b$] (Fig. 4). The curve fitting procedure confirmed that acetate was the best predictor for denitrification in these soils with a r^2 of 0.83, while formate and DOC were less effective predictors with a r^2 of 0.73 and 0.59, respectively. Following this model, to a significant concentration of acetate corresponds a very low concentration of NO_3^--N and vice versa, supporting the hypothesis that in agricultural soils denitrification capacity and labile C_{org} availability are strictly linked and represent the core regulation of NO_3^--N buffering capacity against ground water NO_3^--N contamination. The DOC instead, accounting for both reactive and non-reactive organic species did not follow a strict hyperbolic pattern ($r^2 = 0.59$), with increasing NO_3^--N concentrations corresponding to DOC concentrations of circa 50 mg/l particularly at CCR. This site presented the highest oxygen

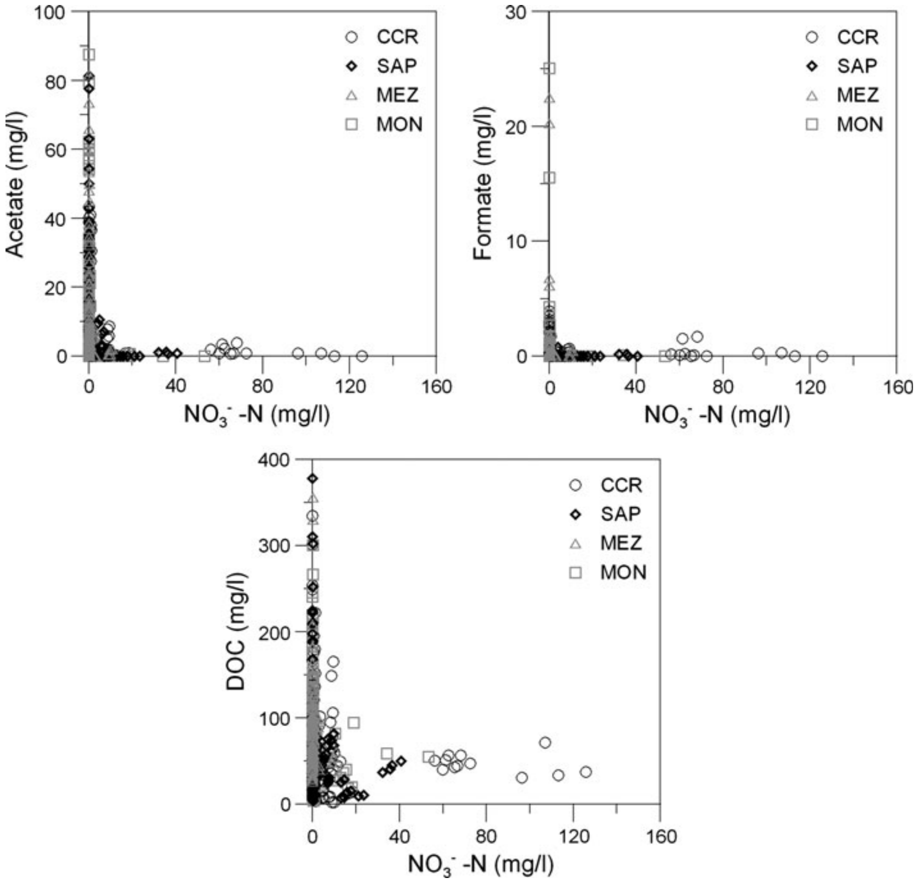

Fig. 4 Plots of NO_3^--N and acetate, NO_3^--N and formate and NO_3^--N and DOC for the four selected sites. Note differences in *Y* axis scales

availability and the lowest C_{org} content, likely characterized by a high refractory nature (Fig. 4). In this view, the concentration of 50 mg/l could be assumed as the reference value of the less reactive fraction in DOC, for the studied soils.

Conclusions

In four typical agricultural soils cultivated with maize in the lower Po River floodplain acetate resulted a good proxy of LMWOA production from labile DOC decomposition, while formate was not suitable as marker species to track the fate of labile DOC in agricultural soils. Acetate correlated well with DOC where the main source of C is the sole maize residues. On the contrary, when the C sources were more than one and different (peat or manure) the linear relation becomes less obvious. Results also indicated the crucial role of acetate in denitrification, in fact acetate versus NO_3^--N concentrations follow a strict hyperbolic pattern. Anyhow, further research on temporal and spatial distribution of acetate and NO_3^--N in agricultural soils is still needed to better characterize their reciprocal trend at different depths and therefore to gain information about the temporal variations of denitrification rate over the year.

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