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REVIEW ARTICLE

Control of NO_3^- and N_2O emissions in agroecosystems: A review

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Abstract Energized electron flows through biological systems sustain nature's complexity. They drive bacterial, archaeal, and fungal oxidation–reduction processes and enable to introduce CO_2 and N_2 from the atmospheric pool. Electron flux-based food webs convert soil organic matter (SOM) in virgin forest and permafrost soils, over-fertilized agricultural land, grassland systems, compost/wastewater treatment plants, oceans, rain forests, savannahs, and forests of the temperate climate zones, and have their strategy adapted on the system in which they are active. Thus, the electron driving power is responsible in our industrializing world that carbon and nitrogen returns to the atmosphere presently with an annual N_2O-N proportion of 0.5 to 4.2 terragrams (Tg) or an annual atmospheric N_2O-N increase of 0.25 %. N_2O is a 300-times more potent greenhouse gas than CO_2. Nature's water-soluble soil carbon (C_{H2O})/NO_3^- ratio balancing is seen as a model of how N_2O emissions could be kept in a tolerable range. Sub strategies beyond are (a) an annual 400–800 terragrams (Tg) photosynthate-C (90–95 % sugars) release into plant rhizospheres, (b) spot-wise N enriching animal excrement and wide C/N ratio litter fall distributions, (c) viral shunts or life shortcuts to supporting O_2 consuming, N supplying, and denitrifying recycler communities, (d) subterranean organic–inorganic soil components mixing and O_2 diffusion promoting NO_3^- formation, and (e) the release of nitrification inhibiting compounds as neem, karanjin, or specific humic acids which help in controlling nitrate formation and denitrification. Soil microbial transport vehicles are fungal hyphae, plant roots, and subterranean animals. Through their activities, aerobic–anaerobic gradients in the soil crumb mosaics emerge. Plant root intertissue spaces, animal guts, and co-transported soil crumbs where under carbon-dominated C_{H2O}/NO_3^- ratios preferred microbes reside are mobile locations in well-aerated soils. In such reduction-equivalent surplus environments, denitrifying communities are forced to use during anaerobic respiration available nitrate-, nitrite ions, NO, and N_2O economically. Though at carbon-dominated C_{H2O}/NO_3^- ratios more N_2O is reduced to N_2 than in nitrate surplus environments, a complete prevention of N_2O emissions is not a reality and even not desirable from the climate point of view. After describing N_2O formation and emissions from a compost pile, a municipal wastewater treatment plant, a constructed wetland, and mineral N-fertilizer, sewage sludge or nitrification inhibitor-stabilized N-fertilizer amended soils with their aerobic-anaerobic mosaics, this review tries to deduce exercisable CN (C_{H2O}/NO_3) ratio shaping and N_2O emission lowering strategies for ecologists, agriculturists, and waste managers in our industrializing world.

Keywords Ecosystems · C_{H2O}/NO_3^- ratio management · Nitrification · Denitrification · N_2O · CO_2 · CH_4 emissions · Compost · Sewage sludge · Constructed wetlands · Fertilized fields · Organic amendments · Nitrification inhibitors

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1 Introduction

In many developing countries, there is not only a continuing unscientific usage of chemical fertilizers, pesticides, and urban refuse material with adverse consequences on bradyrhizobial populations, VA mycorrhiza, nematodes, earthworms, and growing, aphid-punctured plants (Fig. 1) but also on soil biological quality and agricultural sustainability. From organic farm soils receiving plant uptake adapted organic amendments, it is known that more copiotrophs (+52–119 %), oligotrophs (+25–79 %), actinomycetes (92 to 100 %), more of the arbuscular mycorrhizal protein, glomalin, are present than in conventionally treated fields (Chander and Brookes 1991; Lambers and Weidensaul 1991; Weiss and Larink 1991; Madariaga and Angle 1992; Morgan and Morgan 1992; Vega et al. 1992; Christie and Kilpatrick 1992; Roldan and Alexander 1993; Malhotra et al. 2015). Quantifications of 16S rDNA qPCR abundances showing a 1.8-fold increase in both organic cropping and organic orchard soils and a 22-fold ammoniumoxidaseA gene decrease in organic cropping and orchards allow to conclude that organic amendments improve the biological quality and may thus be appropriately included in the group of *Ecosystem Engineers* that selectively modify the environment and make soil ecosystems more sustainable (Aparna et al. 2014).

The Earth's atmosphere contains around 20 % O_2 and, consequently, in converting virgin forest and permafrost soils, N over-fertilized monocultures, CN highly enriched compost/wastewater treatment plants or sewage up taking river networks, to mention some productive environments, energy conservation is hierarchically organized (Klüber and Conrad 1998). After O_2-consuming aerobic respiration and nitrification–denitrification set in at first among the anaerobic processes whereby annually N_2O in amounts of 0.5–4.2 Tg N $year^{-1}$ at annual increase of 0.25 % are released into the atmosphere (Fig. 2; Michel and Wozniak 1998; Steffen et al. 2011; Crutzen et al. 2008; Ravishankara et al. 2009; Rockström et al. 2009; Akiyama et al. 2010; Steffen et al. 2011; Gross 2012; Decock and Six 2013; Yu et al. 2013; Benckiser et al. 2015). The emission of N_2O depends in their magnitude on N_2-fixation, a process which is closely conjoined with photosynthesis (Fig. 2). Ammonium, the result of N_2-fixation is oxidized to NO_3^- and this NH_4^+ formation and oxidation to nitrate depends on altitudinal–latitudinal allocation gradients of leaf and root litter, fertilizer-excrement distributions, seasonality, length of dormant periods, microclimate (freeze-thaw cycles, precipitation, solar radiation), O_2 availability, microbial conversion activities, NH_4^+-release, NH_4^+ biomass-humus-clay binding, spatially–temporally occurring vegetation–food web interactions, viral shunts, biomass burning and industry contributions, and denitrifier/nitrate ammonifier community activities returning the NH_4^+ introduced by N_2-fixation as NO, N_2O, N_2, or NH_3 back to the atmosphere from where it came (Figs. 2, 3, and 4; Scherer 1993; Benckiser 1997; Filip et al. 2000; Benckiser and Bamforth 2011; Nacry et al. 2013). A prerequisite for NO_3^- formation is a good O_2 availability, while the NO_3^- returning denitrification process as the

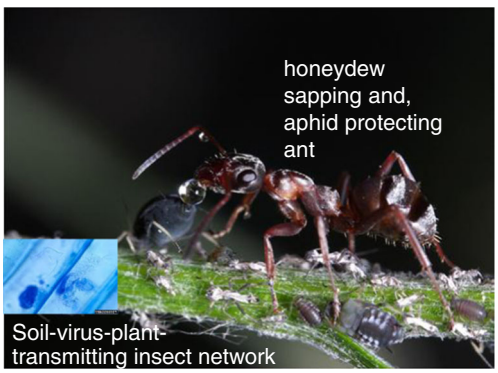

Fig. 1 Soil borne wheat mosaic virus (Furovirus SBWMV (Florida Division of Plant Industry Archive, Florida Department of Agriculture and Consumer Service; http://www.forestryimages.org/browse/detail.cfmimgnum=5266027#sthash.pSQu8HbE.dpuf and a phloem-sucking, honeydew-excreting aphid, protected by honeydew-imbibing ant (photo publication authorized by Thomas Griessler breschniak@gmx.net)

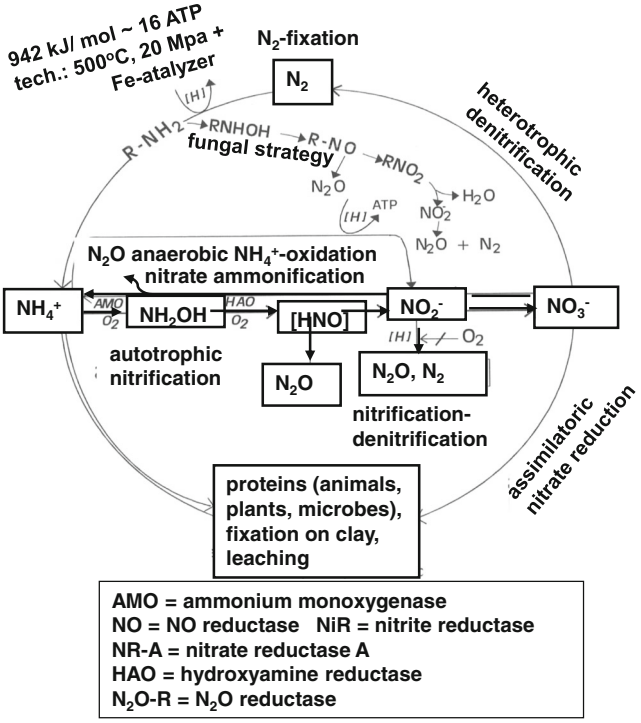

Fig. 2 Nitrogen cycle in terrestrial and aquatic ecosystems (Arcand et al. 2013; Flasconaro et al. 2013; Benckiser et al. 2015)

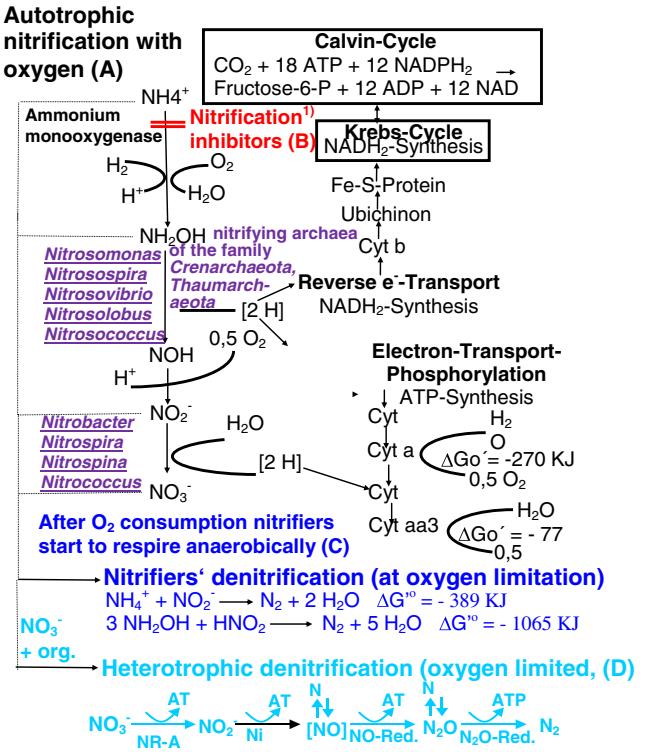

Fig. 3 Nitrite, nitrate, biomass formation, and energy gains during autotrophic nitrification (the nitrification process needs oxygen for conserving ATP and can be inhibited with compounds like N-allylthiourea, dimethylether, acetylene (C_2H_2) nitrapyrin (2-chloro-6-trichloromethyl-pyridine), 3,4 dimethyl pyrazolephosphate (*DMPP*) or dicyandiamide (*DCD*). After O_2 consumption, nitrifiers can gain energy by respiring anaerobically the aerobically formed nitrate with NH_4^+ derived electron and protons. This additional energy conserving possibility differs from heterotrophic denitrification where reduction equivalent are of organic origin) in comparison with anaerobic nitrate respiration by nitrifiers' denitrification and heterotrophic denitrification and the control possibility by nitrification inhibitors (*NI*).

succeeding anaerobic respirations and fermentations are blocked in presence of O_2. In soil crumbs with their aerobic-anaerobic gradients, all oxidizing and reducing activities occur simultaneously and thus the resulting gaseous emissions collected in soil covers are mean values of all aerobic and anaerobic soil processes (Benckiser et al. 1984; Klüber and Conrad 1998; Trost et al. 2013).

Until the end of the nineteenth century, N_2 fixing, nitrifying, and denitrifying bacteria and archaea shaped the world's N demand (Figs. 2 and 3) and N shortage was the norm. The worldwide adapting plant communities counteracted to overcome N-related growth limitations by transporting annually 400–800 Tg photosynthate-C (90–95 % sugars) into their rhizospheres for attracting free-living and symbiotic N_2 fixing bacteria. Besides, the height of N_2O-N emissions was controlled by using N_2O as electron acceptor economically. In addition to nitrification and N_2O+N_2 loss controllers as N_2-fixing microbial activities, a distribution of wide C/N ratio litter fall, soil clay–humus complex and biomass NH_4^+ binding, and release of nitrification-inhibiting compounds such as neem, karanjin, or certain humic acids were and are still employed for C_{H2O}/NO_3^- ratio balancing (Hassett et al. 1987; Scherer 1993; Majumder et al. 2001 and 2004; Kehr 2006; Tadeno and Takeda 2010; Abasi et al. 2011; Carvalhais et al. 2011). Nitrifying bacteria and archaea and the denitrifying communities among the 10^{30} annually newly produced in biofilms around rhizospheres and soil particles consume oxygen and absorb soil nutrients. Bacteria, archaea, fungi, and protists switch after oxygen consumption by using alternatively NO_3^-, NO_2^-, NO, and N_2O as electron acceptors (Figs. 2 and 3). If the availability of NO_3^-, NO_2^-, NO, and N_2O is low but the availability of electron donors high, then an economic thinking sets in and N_2O is preferred reduced to N_2 or even to NH_3 before it can escape from the cell membrane into the surrounding cell atmosphere (Tiedje 1988; Simarmata et al. 1993; Benckiser 1997, 2007a, b, 2010; Ottow 2011; Decock and Six 2013; Cabello et al. 2004; Bardgett and Wardle 2010; Flemming and Wingender 2010; De Long 2012). If N_2O reached the atmosphere, recapturing is limited (Vieten et al. 2008).

Subterranean digging animals like earthworms ingest and digest inorganic, organic components, and microbes, deposit their excrements in ducts, and promote oxygen diffusion into soils similarly as aerenchyma tissues (Benckiser et al. 1984; Horn et al. 2006). In rhizospheres diffusing oxygen finds an electron surplus condition and are comparable to soil crumb environment where virus genomes integrating microbes are active in water-filled pores, which are closely neighbored by air-filled pores (Benckiser 1997; Benckiser and Bamforth 2011). ATP-conserving microbes in soil crumbs respire with O_2, alternatively with electron acceptors as NO_3^-, NO_2^-, NO, N_2O, Fe(III), Mn(IV), SO_4^{2-}, CO_2 or ferment by using internal organic electron acceptors. Fast multiplying microbes at good growth conditions are subjected to virus-mediated life short cuts (viral shunts; Engelhardt et al. 2013). Occurring cell bursts provide the surviving microbial community with additional nutrients, which improve the growth of the survivors (Fig. 4). From oligotrophic lakes and oceans, it is known that virus-mediated cell lysis is a major C, N, and trace element contributor (viral and cell DNA/RNA CN ratio=2; bacterial CN in the exponential growth phase around 3–4; Säwström et al. 2007). The role of viral shunts in soil ecosystems is still a black box (Sime-Ngando 2014). Animals and root-fungal hyphae systems are locations and transport vehicles for viral genomes carrying microbes in well and less well-aerated soils. If reduction equivalent luxuriously available and the electron acceptors nitrate, nitrite NO, and N_2O low concentrated as in rhizospheres or animal guts then the denitrifying communities are forced to think economically and less N_2O will be emitted (Figs. 2 and 3; Benckiser et al. 1984; Tiedje 1988; Benckiser 1997; Klüber and Conrad 1998; Chapman et al. 2006;

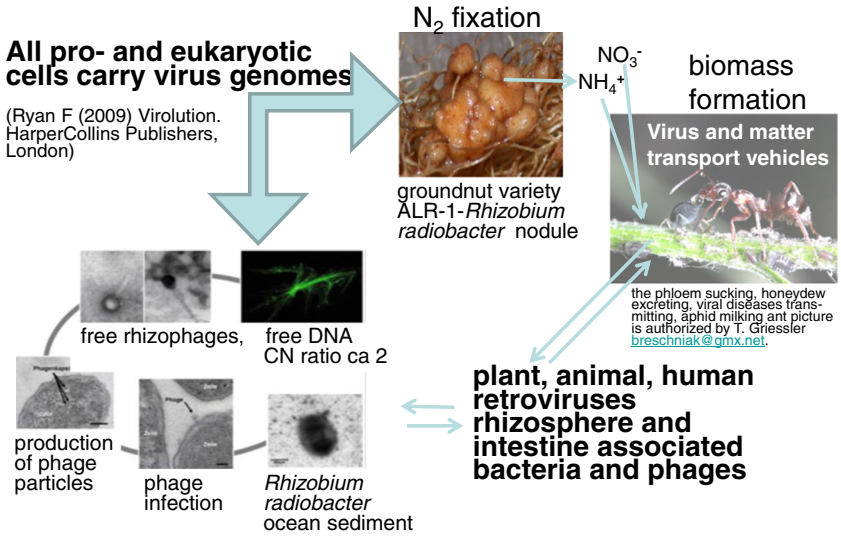

Fig. 4 N and virus cycling through terrestrial ecosystems whereby virus mediated cell burstings, viral life shortcuts, viral shunts contribute to the nutrient supply (adapted from Benckiser 2007b and 2010; Babikova et al. 2013; Engelhardt et al. 2013; Sime-Ngando 2014)

Benckiser 2010; Depkat-Jakob et al. 2010). Plants growing in submerged soils as reed or rice try to keep root respiration going by transporting oxygen in aerenchyma tissue from leaves down to the roots. Thereby, the magnitude of anaerobic energy conservation is concomitantly controlled (Benckiser et al. 1984). Plants also try to avoid too high oxygen consumption by swaying nitrification-inhibiting compounds (Hassett et al. 1987; Majumder et al. 2001; Abasi et al. 2011).

Nature's N management functioning within boundaries is meanwhile reasonably understood. Farmers use the new insights and try to approach within the functioning of nature's boundaries high biomass yields with monoculture crop plants and an adapted N management. Until the invention of technical N_2 fixation (TNF) around 100 years ago, monoculture cropped plants suffered under N shortage; and for overcoming the N shortage, farmers selected better-adapted crops on fluctuating N sources and applied animal manure (Sturm et al. 1994; Lambers et al. 2011; Chen et al. 2013; Nacry et al. 2013). From 1908 to 1960, the worldwide N inputs increased nine-fold and since then mobile NO_3^- ions reach increasingly the groundwater and N_2O enriches in the atmosphere. Both, nitrate and N_2O overloads generate enormous costs (Sutton et al. 2011). Ecologists, farmers, waste managers, and industries try meanwhile increasingly, scientifically and governmentally supported by having landscape conservation in the focus, to approach nature's evolutionary developed N overloads avoiding principles. Soil compaction minimizing tillage techniques are continually improved for achieving a better mixing of organic residues and inorganic soil components, a plant requirement-tuned mineral, animal manure and compost fertilization is practiced, intercropping with promising N_2 fixing bacteria legume relationships is increasingly on the screen and a steadily developing petroleum saving, computerized precision farming that factors local differences is employed. Governmental organization platforms such as the Association of German Agricultural Research and Experimental Stations (VDLUFA) analyze periodically the nutrient contents in per hectare recommended animal manure amounts, which contain about 76, 19.2, 64.7, 33.6, 7.8, 0.1 kg N, P, K, Ca, Mg, and Cu, respectively (Wiesler 1998). More reluctantly recommended is meanwhile the field application of sewage sludge, which may contain 3.8, 1.6, 0.4, 0.6, 5.3 % N, P, K, Mg, Ca and 202, 5, 131, 349, 53, 3, and 1446 mg Pb, Cd, Cr, Cu, Ni, Hg, Zn per kg dry matter, respectively (Benckiser and Simarmata 1994; Sturm et al. 1994; Fytili and Zabiotou 2008). Compost spreading rather recommended by the European Union (EU), is in the EU annually produced from about 300×10^6 tons of waste and spread on the 123,391,000 ha agri-, horti- and viniculturally used EU land (Barth et al. 2008; Beylich et al. 2010; PICCMAT 2011). Collected wastes generally consist of biowastes (5 %), green wastes (54 %), pre-digested products (30 %), sewage sludge, street litter, dead leaves, and industrial waste. Green wastes may contain hard or softwood bark, cardboard, corn stalks, grape pumice, hay from grass/lucerne, rough grass, round bales, green grass, olive pumice, paper newsprint, peat\moss, pine needles, pinewood shavings, sawdust, sawdust hardwood, straw from oat or wheat, shrub or tree trimmings, hard, soft woodchips or yard waste mixtures with single CN ratios of 125, 135, 520, 40, 27, 25–95, 70, 400, 64, 110, 350, 365, 996, 120, 55, 120, 30, 430, 500, and 44, respectively, and produced composts out of such a material mix have CN and C_{H2O}/NO_3^- ratios of 15 and around 1000, respectively (Pauli and O'Malley 2008). Soil incorporated plant residues, composts, or biochar, a pyrolysed compost/animal manure mixture, recommended as soil ameliorators, improve the soil texture, enhance soil quality, health, and workability, sequester CO_2, and reduce fossil fuel demand and erosion (Bouma 2014). They enrich soils with surplus reduction equivalents, dilute soil nitrate, and balance the C_{H2O}/NO_3^- ratio in a way that the

denitrifying communities are forced to use the electron acceptors NO_3^-, NO_2^-, NO, and N_2O economically. The result is a wider N_2/N_2O ratio (Tiedje 1988; Zumft 1997; Barth et al. 2008; Crutzen et al. 2008; Akiyama et al. 2010; Beylich et al. 2010; PICCMAT 2011; Chen et al. 2013; Fungo et al. 2014). A C_{H2O}/NO_3^- ratio balancing is easier achieved than with compost, plant residue or biochar incorporation with nitrification inhibitor-stabilized N fertilizers, which try to control directly nitrate formation especially during the plant's free and early growth period (Sturm et al. 1994; Zerulla et al. 2001). Because of less supplied nitrate, the emission of N_2O is reduced and a wider N_2/N_2O ratio is achievable. Thus, an intensive searching for efficient nitrification inhibitors set in around 50 years ago (Mc Carty 1999) whereby heterocyclic, partly two or three adjacent N atoms and chlorine-containing, unsubstituted, N compounds as etridiazole (5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole, Dwell), 2-chloro-6-trichloromethylpyridine (nitrapyrin), 3-mercapto-1,2,4-triazole, 4-amino-1,2,4-triazole, 3-methylpyrazole-1-carboxamide or 2-ethinyl-pyridine, benzotriazole, 1,2,4-triazole, indazole, pyridazine, 3,4-dimethylpyrazole (DMP) were found to be effective (Kilian et al. 1998; Mc Carty 1999; Zerulla et al. 2001; Tindaon et al. 2012; Benckiser et al. 2013). As efficient nitrification inhibitors, chemicals as *N*-allylthiourea, the N_2O reductase blocking C_2H_2, dimethylether (DME), or dicyandiamide (DCD, $C_2H_4N_4$) were identified (Benckiser et al. 1986; Powell and Prosser 1986; Hallinger et al. 1990; Rajbanshi et al. 1992; Miller et al. 1993; Wagner et al. 1995; Bollmann and Conrad 1997; Philips et al. 2002; Li et al. 2008; de Klein et al. 2011; PICC MAT 2011; Eurostat yearbook, statistical guide to Europe, European Commission 2013). Each of these compounds aims to block the key enzyme of nitrification, the ammonium monooxygenase (AMO), whereby, e.g., dicyandiamide blocks the electron transport in the cytochromes of AMO during the conversion of NH_3 to hydroxylamine or 3,4-dimethylpyrazole phosphate binds indiscriminately to the complex of membrane-bound proteins inclusively the AMO (Fig. 3). Dicyandiamide and 3,4-dimethylpyrazole phosphate have as nitrapyrin an AMO inhibition efficiency of more than 35 %, though their chemical structure is rather naturally than that of nitrapyrin and both seem not to affect non-target soil metabolisms at the recommended field application rates (Akiyama et al. 2010; Tindaon et al. 2012).

Dicyandiamide and 3,4-dimethylpyrazole phosphate are successfully marketed under the trade-names Alzon and Entec, respectively (PICCMAT 2011) and therefore this review tries to deduce from N_2O formation studies in a compost pile, a municipal wastewater treatment plant, a constructed wetland and mineral, NI stabilized N-fertilizer or sewage sludge amended soils possible N_2O emission reducing ways in our industrializing world.

2 N_2O formation in composting biowastes

Compost companies as the Herhof Company, Giessen (http://www.herhof.com/de/geschaeftsbereiche/trockenstabilat.html) receive municipal green biowastes differing in composition from delivery date to delivery date as the material brought on May 06 and May 13 display (May 06/May 13: green cutting 30–35/15 %, DM contents 54.4/41.4 %, total C 400/275, total N 12.8/16.3 g kg^{-1} DM, C/N ratio 31.4/16.9, pH 6.4/6.7, NH_4^+-N, NO_3^--N, NO_2^--N and C_{H2O} contents 723/1293, 26/142, 2.0/4.9, 10.1/12.5 mg kg^{-1} DM; for method details, see Benckiser et al. 1996). The May 06 biowaste, pre-composted in Herhoff Rotteboxes, where it was wetted around 7 to 10 days with recirculating seepage water until a temperature of about 65 °C was reached, was piled after being removed under the shelter of a front side open 15-m high halls to trapezoid, regularly turned around and wetted heaps (ca. 100×4×1.50 m) over 10 weeks for reaching compost stabilization. Samples from the original and pre-composting material and stabilizing compost samples, weekly with an auger taken at ten different heap locations out of the heap depths of 0–5 and 50 cm, were combined, separately per depth, mixed and in situ as 40–60-g proportions filled into airtight closable 250-ml flasks (Schott, Germany; three parallels). For driving out the N_2O retained by the original, pre-composted, and stabilizing biowaste material, the airtight closed flasks were heated in a water bath (80 min at 80 °C) before in the flask headspace N_2O was gas-chromatographically determined and calculated in mg N_2O-N kg^{-1} compost DM (Weiske et al. 1995). The compost material pasteurization during the measuring procedure prevents a de novo N_2O synthesis. In separate materials, ammonium, nitrate, nitrite, and C_{H2O} formation changes were analyzed.

N_2O, NH_4^+, NO_2^-, NO_3^-, and C_{H2O} formation in the biowaste and composting material was highest in the 6th week of compost stabilization (Fig. 5). Turning around the piled material assumingly favored NO_3^- and N_2O formation where the upper 5 cm retained about 130.0±114.6 μg N_2O-N kg^{-1} compost DM and the 50 cm heap depth material about 2844.0 ±3988.1 μg N_2O-N kg^{-1} compost DM. N_2O formation was highly significant correlated with nitrite (r=0.97), positive but not significant with nitrate and water-soluble carbon (r=0.61 and 0.55, respectively). The high standard deviations indicate a spot-wise N_2O formation.

3 N_2O-formation in and emission from municipal wastewater treatment plant

N_2O formation in a compost heap or in wastewater depends on temperature, NH_4^+-N, NO_3^--N, NO_2^--N, and organic matter availability (BOD_5, COD_{Mn}). Consequently, in the aerated activated sludge (1700 m^3; retention time 120 min), the aerated nitrification unit, the non-aerated denitrification tank (16,

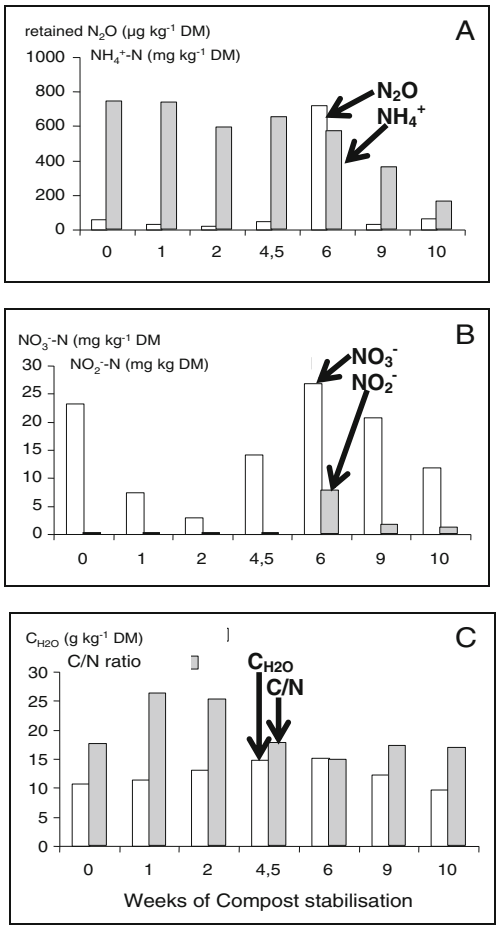

Fig. 5 Daily N_2O-surface fluxes (**a** acetylene inhibition technique) from a minerally fertilized (*a*) or sewage sludge amended (*b*) Parabrown earth (Inceptisol) planted to spring wheat during 1985 and compared with N_2O-production potentials in the soil depths 0–20, 20–40 and 40–60 cm (**b** and **c**)

000 m^3, retention time 140 min) and the effluent of the municipal wastewater purifying plant in Giessen, Germany, dimensioned for about 150,000 inhabitants, N_2O formation and emission in dependency of the above parameters were determined for getting an idea about reduction possibilities. In 40-ml wastewater samples regularly collected in 20 cm depth of each purification unit and the effluent and in situ filled into airtight closable 250-ml flasks (Schott, Germany), which were incubated in a water bath (80 °C, 80 min; Weiske et al. 1995) wastewater-retained N_2O was determined. Emitting N_2O was collected with self-constructed, open PVC covers (60×40×20 cm, four per unit), which had a perforated PVC-plate with 0.8-cm-diameter holes fixed at the bottom for achieving a uniform membrane pump-driven and flow meter (Platon, Germany) controlled air flow of 90 L h^{-1} through each cover. The N_2O separated air flow into a 20 L h^{-1} one that flew 2 h through silica gel and sodium hydroxide traps in order to remove H_2O and CO_2, and a glass column filled with 2 mm pellets of 0.5 nm molecular sieve to absorb the emitting N_2O (Merck, Germany), and a bypassing 70 L h^{-1} one for preventing an uncontrolled cover up-floating in the air-bubbled purification tanks was gas-chromatographically determined after desorption from the molecular sieve (Schwarz et al. 1994) and calculated in grams N_2O-N per day per tank or per effluent after having subtracted the N_2O already present in the sucked through air. The wastewater-retained N_2O was calculated in kilograms N_2O-N per day per tank or per effluent. For method details concerning pH and N-, C-species (N_t, NH_4^+-N, NO_2^--N, NO_3^--N, C_t, C_{H2O}) determination during the measuring periods March to July, 1994, November 1995 to March 1996, see Schwarz et al. (1994), Benckiser et al. (1996).

The mean water temperature, pH and pO_2 of the introduced wastewater were during the March to July, 1994, period 9.6±1 °C, 7.4±0.3, and 6.4±0.6 mg O_2 L^{-1}, respectively, the BOD_5 and COD_{Mn} 170 (10–510) and 196 (32–756) mg O_2 L^{-1}, respectively, and NH_4^+-N, NO_3^--N, and NO_2^--N 28±3.4, 1.9±0.8 and 0.5±0.4 mg L^{-1}, respectively. The water temperature between November, 1995 and March, 1996 was 4.4±3 °C, around 5 °C lower, and dependently the NH_4^+-N, BOD_5, and COD_{Mn} in the aerated activated sludge unit 250 % (NH_4^+-N, 70±40 mg L^{-1}), 170 % (BOD_5, 383±166 mg O_2 L^{-1}), and 268 % (COD_{Mn}, 527±314 O_2 L^{-1}) higher than in the March to July period. Similar during both measuring periods were 1.9±0.8 or 1.9±1.4 mg NO_3^--N L^{-1} and 0.5±0.4 or 0.3±0.23 mg NO_2^--N L^{-1} for the nitrate and NO_2^--N concentration in the aerated activated sludge unit.

NH_4^+-N, NO_3^--N, NO_2^--N, BOD_5, and COD_{Mn} in the aerated nitrification tank (>2 mg O_2 L^{-1}) were in the March to July/November to March periods 3.1±0.9/6.3±8.2, 3.3±1.2/1.8±1.3, 0/0, 0.07±0.05/0.07±0.05 mg N L^{-1} or 1386±483/1987±799, and 4549±2012/4247±1755 O_2 L^{-1}, respectively, and in the unaerated denitrification tank 6.4±5.6/8.7±6.7, 4.4±1.3/1.6±1.2, 0/0.07±0.05 mg N L^{-1} or 1534±297/1532±384 and 4560±1648/2846±863 mg O_2 L^{-1}, respectively. The corresponding March to July/November to March contents in the effluent were 0.4±0.15/5.4±4 (NH_4^+-N), 3.7±1.7/7.4±4 (NO_3^--N), 0.1±0.1/0.18±0.11 mg N L^{-1} (NO_2^--N) or 33.5±22.8/10.9±9 (BOD_5), and 22.5±13/26±36 mg O_2 L^{-1} (COD_{Mn}), respectively.

Due to the prevailing environmental conditions, the N_2O emissions from the aerated activated sludge and nitrification unit of the wastewater treatment plant, Giessen, were about 45 and 72 times higher, respectively, than the N_2O emissions from the non-aerated denitrification tank, indicating that well-aerated wastewaters are a N_2O source par excellence (Table 1). From the introduced NH_4^+-N, roughly 1.56 % left the wastewater treatment system of Giessen as N_2O gas and about 1005 kg N_2O-N in dissolved form with the purified wastewater. Probably because of different designs and operational conditions, nitrous oxide (N_2O) emissions may substantially vary between wastewater treatment plants, ranging from negligible to substantial (a few percent of the total nitrogen load; Law et al. 2012).

Table 1 Mean daily N_2O emissions from different purifying units of the wastewater treatment plant, Giessen, compared with dissolved N_2O amounts in the wastewaters[a]

Purification tank	N_2O emissions g N_2O-N day^{-1} $tank^{-1}$			N_2O dissolved in the wastewater kg N_2O-N day^{-1} $tank^{-1}$ or $effluent^{-1}$		
	March 1993–March 1994	March 1994–July 1994	November 1995–March 1996	March 1993–March 1994	March 1994–July 1994	November 1995–March 1996
Activated sludge[b]	10.4±13.2	5.9±2.9	7.7±5.4	n.d.	n.d.	n.d.
Nitrification unit[a,b]	n.d.[c]	936±1354	46±17.3	n.d.	0.8±0.42	23.1±13.1
Denitrifyca-tion unit[b]	n.d.	14.4±17.3	7.1±3.5	n.d.	0.52±0.25	6.1±5.2
Effluent[b]	n.d.	n.d.	3.0±5.8			6.7±2.5

[a] About 150,000 inhabitants deliver their wastewater to the municipal wastewater treatment plant of Giessen, Germany, and this inflowing wastewater has a mean BOD_5 of 170 (10–510) mg L^{-1}, a COD of 196 (32–756) mg O_2 L^{-1}, and NH_4^+-N and NO_3^--N contents of 33 (5.7–52) and 2.5 (0.3–13.8 mg L^{-1}), respectively

[b] The total volumes of the activated sludge-, nitrification- and denitrification tanks were 2080, 11,000, and 11,000 m^3, respectively. The daily effluent of purified wastewater amounted to 52,000 m^3 and the surface area of the discharging channel was 416 m^2

[c] *n.d.* not determined

4 N_2O formation and emission from a constructed wetland

Constructed wetlands, generally having a ground filter, filled with a sandy loam soil peat mixture and planted with reed increasingly purify the wastewater of less populated areas (Leverenz et al. 2010). We were interested in how such wastewater-treating systems work during the winter period when low temperatures interrupt the growth of the ground filter oxygenating reed plants. With an auger, we collected between October 1994 and April 1995 in the 0–20, 20–40, and 40–60 cm soil depth reed bed samples (center and close to the in- and outlet) and quantified COD, BOD_5 C_{H2O}, ammonium, nitrate, nitrite, retained, and emitted N_2O at a soil temperature of around +3 °C, lowest 1 °C below the freezing point in the 10 cm ground filter depth and of around +5,8 °C, lowest +2 °C in the 50 cm ground filter depth (groundfilter, for method and result details, see Fey et al. 1999).

Briefly, N_2O, retained by the 0–20 cm reed bed layer of the constructed wetland, Friedelhausen, Germany (C_{H2O}/NO_3^- ratio 32.1; temperatures between −1 and +5.8 °C) that received farm wastewater was about three times more than the N_2O, retained by the 50 cm ground filter zone (C_{H2O}/NO_3^- ratio 54.8). BOD_5 and COD_{Mn} degradation during the same period were 78.2 and 49.4 %. From the around 159 kg N, the ground filter received during the winter period, around 177 g or 0.11 % was emitted as N_2O-N and 0.02 % retained by the ground filter.

5 Actual and potential denitrification in sewage sludge and/or mineral fertilized Parabrown earth field plots

Actual denitrification measurements provide information about gaseous N surface losses and their atmospheric interactions, while potential denitrification capacity (PDC) quantifications inform about the influence of available carbon on the denitrification process (Burford and Bremner 1975). In both sources of information about denitrification, in 1985, we quantified N_2O in only minerally fertilized and with liquid municipal sewage sludge amended Inceptisol (Parabrown earth) field plots (44.7 % sand, 47.2 % silt, and 8.1 % clay; pH of 6.8 in the upper 20 cm, bulk density 1.52 g cm^{-3}, pore volume 43.5 %, silty sand) at the Federal Research Center of Agriculture, Braunschweig, Germany, with the acetylene inhibition technique (N_2O+N_2). The sewage sludge-amended field received 3500 m^3 ha^{-1} liquid municipal sludge (CN ratio about 10; 620 kg N, 260 kg P, and 40 kg K) in 1971 and from 1980 until the experimental year 1985 annually 300 m^3 ha^{-1}. In auger-collected 40-g Inceptisol samples out of the soil depths of 0–20, 20–40, and 40–60 cm of each experimental field plot, which were in situ filled into dark serum bottles (120 ml; 3 replicates), PDC was quantified. To each bottle 10 ml H_2O was added for approximating a maximal water holding capacity of about 200 % and a KNO_3 solution to have a final N-content of 100 μg NO_3^--N g^{-1} soil. The atmosphere of the airtight-closed flasks was replaced by helium and 5 ml C_2H_2 and after a 48-h incubation the emitting N_2O was expressed in kilograms N_2O-N per ha^{-1} (for further details, see Benckiser et al. 1987).

Figure 6, comparing N_2O surface fluxes and PDC values, unveils that the PDC-related N_2O formation in the 40–60 cm soil layer of the only minerally fertilized field plots (Fig. 6b), which was about five times less than in the sewage sludge-amended ones (Fig. 6c), surpassed the N_2O-N (N_2O+N_2) surface fluxes (Fig. 6a) and indicates that under the prevailing minerally N fertilized conditions, carbon, rather than the nitrate availability, seems to limit denitrification. The high standard deviations of the emitted N_2O parallels (Fig. 6a) suggest the compost pile (Fig. 5), wastewater plant (Table 1), and the constructed wetland measurements (Fey et al. 1999) hot spot denitrification. About 5 to 10 % of the added mineral

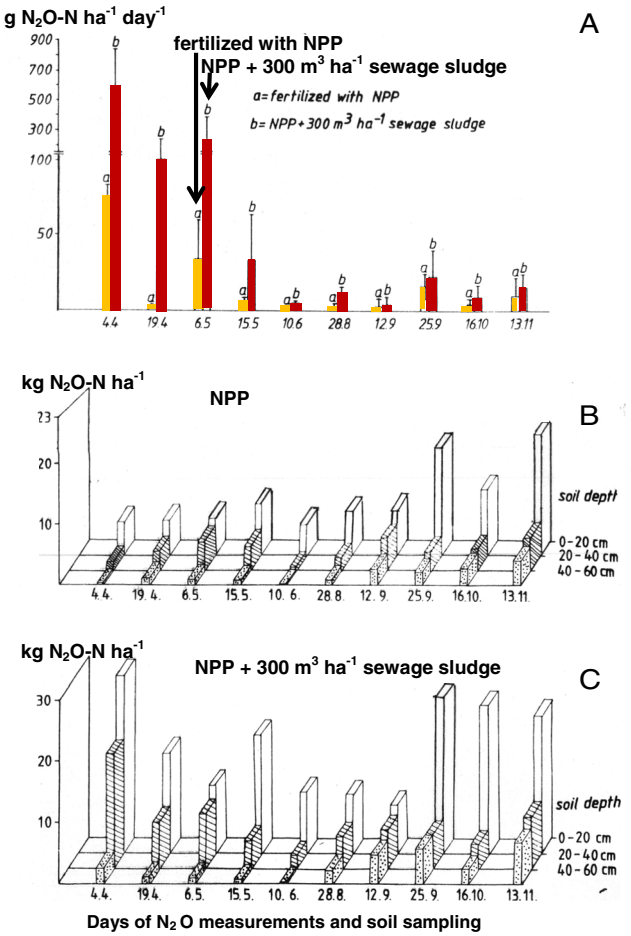

Fig. 6 Daily acetylene inhibition technique measured N_2O-surface fluxes from minerally and/or sewage sludge amended Parabrown earth (Inceptisol) field plots, planted to spring wheat during 1985 (**a**) in comparison to potential N_2O-production in the soil depths of 0–20, 20–40 and 40–60 cm (**b** and **c**)

fertilizer N to the Inceptisol soil was lost as emitted N_2O. Though it is meanwhile well known that gaseous N loss estimates with the acetylene inhibition technique are questionable, because C_2H_2 not only blocks the N_2O-reductase but also impairs the nitrification process and favors NO_2 formation (Bollmann and Conrad 1997), measurements with this technique provide at least hints which of the denitrification determining parameters need to be controlled when a N_2O emission reduction is aimed. Very recent slurry spreading experiments with different application techniques to field soils differing in clay, silt organic matter contents, pH, bulk density, and water content at field capacity revealed that broadcast spreading favors NH_3 emission and slurry injection in the release of N_2O (Langevin et al. 2014). Sewage sludge spreading in such high amounts on agricultural fields, even deployed with sophisticated techniques, is nowadays less tolerated, especially when greenhouse gas emissions shall be kept in a tolerable range.

6 Nitrification inhibitor effects on N_2O emissions from wastewater and soil

In wastewater, liter-wise sampled on March 02, 1996, in different depths of the earlier mentioned units of the wastewater treatment plant, Giessen, Germany, which were thoroughly mixed and in 200-ml subsamples filled in airtight closable 500-ml flasks (six replicates of each unit), to which 30 mg nitrate L^{-1} as KNO_3 was added before the liquid culture nitrification inhibiting efficiency of nitrapyrin, allylthiourea, dimethylether, and 3,4-dimethylpyrazole phosphate were tested. Nitrapyrin and allylthiourea were added in a concentration of 5 mg L^{-1} (Powell and Prosser 1986; Wagner et al. 1995), dimethylether in a concentration of 25 % (*v*/*v*; Miller et al. 1993) and 3,4-dimethylpyrazole phosphate in a concentration of 0.01 mg L^{-1} wastewater (Weiske et al. 2001). After inhibitor addition, the flasks were airtight closed (dimethylether was added to the airtight-closed flasks) and incubated. Nitrapyrin and dimethylether effects were only studied in activated sludge wastewater and are reported in the text, while allylthiourea and 3,4-dimethylpyrazole phosphate effects are shown in Fig. 7. The mean initial headspace pO_2 of all flasks was 460 mg O_2 L^{-1}.

The used activated sludge wastewater had a BOD_5 and COD_{Mn} of 1629 and 3498 O_2 L^{-1} and contained 19.9, 4.75, 0.01 mg NH_4^+-, NO_3^--, NO_2^--N L^{-1}, the nitrification tank

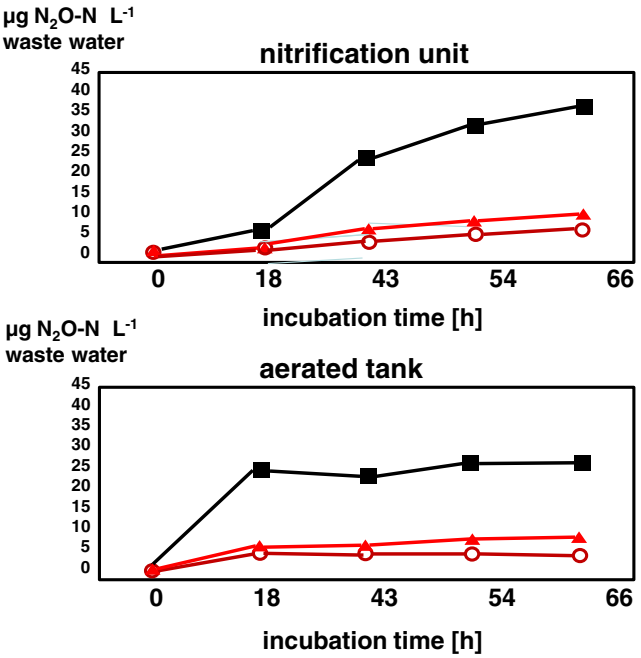

Fig. 7 Nitrification inhibitor 3,4 dimethylpyrazolephosphate and N-allylthiourea added in a concentration of 1 or 5 mg L^{-1} to waste water collected on March 02, 1996, in the aerated nitrification unit and activated sludge tank, public waste water treatment plant Giessen, Germany, in comparison to controls without the addition of nitrification inhibitors. The initial flask headspace pO_2 was 460 mg O_2 L^{-1}

wastewater a BOD_5 and COD_{Mn} of 1386 and 4549 O_2 L^{-1} and contained 3.1, 3.3, >0.01 NH_4^+-, NO_3^--, NO_2^--N mg L^{-1} (analysis, Benckiser et al. 1996). The driven out N_2O after five incubation days (water bath: 80 °C; 80 min; Weiske et al. 1995) was, as O_2, gas-chromatographically analysed and expressed in milligrams N_2O-N per liter wastewater.

The mean flask headspace pO_2 declined from 460 mg O_2 L^{-1} to 33±1.87 mg O_2 L^{-1} and the emitted N_2O in the presence of nitrapyrin and dimethylether was 97.1 or 97.3 % lower (51±9 without/1.5±0.4 mg N_2O-N L^{-1}×100 with nitrapyrin or 51±14 without/1.4±0.6 mg N_2O-N L^{-1}×100 with dimethylether). Similar effects showed 3,4-dimethylpyrazole phosphate and N-allylthiourea (Fig. 7). In summary, all four tested nitrification inhibitors exhibited as promising N_2O formation reducing candidates under liquid (wastewater) conditions.

Under soil conditions, the AMO inhibiting efficiency of 3,4-dimethylpyrazole phosphate and dicyandiamide was tested in a 3-year agricultural field experiment, in which ASN–N fertilized spring barley, *Hordeum vulgare* L (90 kg N ha^{-1}), maize, *Zea mays* L. (160 kg N ha^{-1}), and winter wheat, *Triticum aestivum* L.(180 kg N ha^{-1}) plots (26 % N, 18.5 % NH_4^+–N and 7.5 % NO_3^--N) were compared with plots having received DMPP and DCD ASN–N-stabilized fertilizers (DCD: 16 % to NH_4^+–N; DMPP: 1.6 % to NH_4^+–N). The trace gas emissions (CO_2, CH_4, and N_2O), converted into CO_2-equivalents (Table 2) were evaluated by the closed-chamber method (Weiske et al. 2001). Besides plant growth, soil parameters and the disappearance of 3,4-dimethylpyrazole phosphate and dicyandiamide were measured (for method details see Rajbanshi et al. 1992; Weiske et al. 2001; Benckiser et al. 2013).

In the presence of dicyandiamide and 3,4-dimethylpyrazole phosphate, the emission of N_2O was reduced by 25.8 and 48.7 %, respectively, and was, in field soil, compared to the N_2O reduction efficiency of nitrification inhibitors (NI) in liquid culture (Fig. 7), considerably lower (Table 2). NI diffusion and adsorption phenomena in soils certainly more distinct than in wastewater may explain the inhibition efficiency difference. Fortunately, not affected was the AMO resembling mono methane oxidase (MMO) by DMPP and DCD, indicating a high inhibition specificity of both NI. Nevertheless, the observed CO_2 emission decline in the presence of DMPP (28.3 %) and DCD (6.6 %; Table 2) is presently not understood.

Table 2 Influence of the nitrification inhibitors 3,4-dimethylpyrazole phosphate (*DMPP*) and dicyandiamide (*DCD*) control on the CO_2, CH_4 and N_2O emissions and the global warming potential caused by the DMPP treated summer barley (1997), corn (1998) and winter wheat (1999) cropped plots in comparison to control plots, which never have seen DMPP. For the database see Weiske et al. (2001)

	Σ g CO_2-C ha^{-1} day^{-1}	Σ g CH_4-C ha^{-1} day^{-1}	Σ g N_2 O-N ha^{-1} day^{-1}	CO_2-equivalents[a]
Control	5840594	−130	828	6094553 (100 %)
DCD	5456339	−130	614	5643788 (93 %)
DMPP	4185470	−166	425	4313752 (70 %)

[a] CO_2+(CH_4×21)+(N_2O×310)

7 CN ratio-dependent N_2O emissions

In addition to the nearly annually and globally produced 4 billion metric tons of crop residues, which are incorporated into agricultural land or burnt on fields, carbon sequestration favoring, soil fertility ameliorating composts, and animal manures with varying CN ratios are spread and all these organic amendments shift the electron donator/electron acceptor ratio (C_{H2O}/NO_3^- ratio) towards electron surplus. Sewage sludge spreading on fields is nowadays less recommended; rather, what is recommended is the addition of sewage sludge to biogas plants, or its deposition in waste dumps, or making compost out of it. Under natural conditions, only 0.1 to 2 % of the microbial soil community seem to be continually active but when soils are organic amended, substrate is added and their activity can increase within minutes to around 40 % (Blagodatskaya and Kuzyakov 2013). Dormant microbial cells in contrast need several days before their activity regains, because at this living status, nutrient availability to the producer cell enzymes determines the transition length from the dormant to the active status. Substrate turnover and cycling through the food web and chemical, physical, and biological soil properties changes are thereby also of relevance (Huang et al. 2004; Klemedtsson et al. 2005; Lal 2005; Toma and Hatano 2007; Steffen et al. 2011; Blagodatskaya and Kuzyakov 2013; Burns et al. 2013).

A low NO_3^- availability at electron surplus (Figs. 5, 6, 7, and 8) forces the denitrifying community to reduce under such circumstances also N_2O efficiently to N_2 (Fig. 8). Plant residues have a CN ratio spectrum from 8 to 118 (Huang et al. 2004). If residues with a CN ratio >75 are incorporated into soils then it is observed that less N_2O and CO_2 are emitted, because of an assumed protein-N complexation and polyphenol N immobilization. The low N_2O emissions from forested histosol sites substantiate this observation (Klemedtsson et al. 2005; Zhang et al. 2011). A soil CN ratio above ca. 30, shaped by factors as climate, pH, groundwater tables, oxygen depletion, carbon availability, to mention a few, is threshold for net N immobilization. Incorporated onion leaf, soybean stem and leaf, rice, and wheat straw residues of 11.6, 14.5, 62.3, and 110 CN ratios, respectively, into Gray Lowland soil, Mikasa, Hokkaido, Japan, exhibited, as also found by Bremner and Blackmer (1981), a negative correlation between residue CN ratio and the emission of N_2O (Toma and Hatano 2007). Organic residue amendments plus urea placement lead to

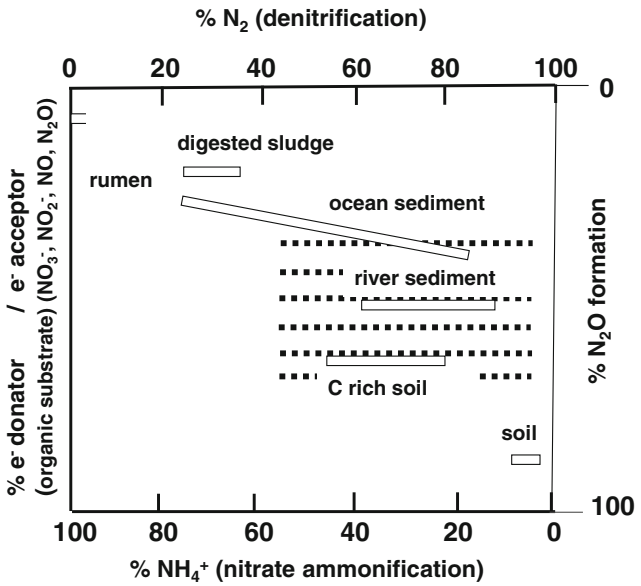

Fig. 8 Terrestrial and aquatic, energy gaining nitrate reduction processes in dependency of the electron donator ((easy available carbon)/acceptor (nitrate) ratio (adapted from Poth and Focht 1985; Poth 1986; Tiedje 1988; Benckiser 1997; Strous et al. 1997; Philips et al. 2002; Strohm et al. 2007)). N_2 formation and nitrate ammonification occur preferred in biotops with electron surplus (rumen, digested sludge, rhizosphere), less in only minerally (NO3-) fertilized soils

uneven nutrients distributions. Nitrification inhibitors tentatively enhance this effect and spot-wise nitrification (Azam et al. 2001). A negative correlation between N_2O/NO_3^- and residue CN ratio is favored (Bremner and Blackmer 1981; Benckiser 1997; Klemedtsson et al. 2005; Chen et al. 2013; Xu et al. 2014). Urea combined with low CN ratio organic residues fertilization (CN ratio below 15–20) stimulates the activity of chemolithotrophic, urease producing, and ammonia oxidizing bacteria. The soil nitrate concentration increases. The less economically working denitrifying community will prefer to release N_2O instead of N_2 (Fig. 7; Huang et al. 2004; Koper et al. 2004; Chen et al. 2013). All these dependencies make the prediction of N_2O emissions not easy. Low in nitrate but rich in sun-energized electrons are present in terrestrial ecosystems rhizospheres, such as anaerobically functioning grass-fed rumens, earthworm guts or soil crumbs after residue incorporation. Such carbon surplus environments exemplify par excellence the CN ratio influence on N_2O emissions and where available NO_3^- may even be reduced to NH_3 (Figs. 1 and 8). In soil zones enriched with nitrate by nitrification or fertilization, much of the produced N_2O escapes after rainfall events from the cell membranes and reach the surrounding atmosphere before it is reduced to N_2 (Fig. 8; Hungate 1966; Depkat-Jakob et al. 2010; Carvalhais et al. 2011; Fungo et al. 2014). Once N_2O reached the atmosphere, a reduction to N_2 could be possible but is unlikely (Vieten et al. 2008; Chen et al. 2013). The difficulties in predicting nitrification–denitrification events, in predicting N_2O emission, also the soil mosaic formation of well and less aerated, well and less organically enriched patches, created by long-term environmental drivers as climate, plant exudation, soil animal activity, microbial and fertilizer N inputs, O_2 diffusion, or ammonium fixation by biomass and clay–humus complexes, soil texture changes, root-hyphae effects, the fact that in air-, water-filled soil pores aerobic, anaerobic respiration, and fermentation occur in close neighborhood, the steadily varying $C_{H2O}/NH_4^+/NO_3^-$ ratios contribute and make the modeling of N-cycling a challenge (Fig. 8; Scherer 1993; Syring and Benckiser 1990; Benckiser 1997; Taroncher-Oldenburg et al. 2003; Huang et al. 2004; Wallensten et al. 2006; Henry et al. 2008; Benckiser 2010; Carvalhais et al. 2011; Benckiser and Bamforth 2011; Hangs et al. 2013). During Earth's genesis, plants have learnt to attract inorganic nutrients extracting and N supplying microbes by phloem exudation and this strategy helped to adapt on fluctuating N availabilities (Nacry et al. 2013). Ruminants have learnt to select among grassland plants and tried to avoid those containing nitrate <1 % per dry weight, because ruminants' mouth/rumen microflora reduces nitrate mostly only to NO_2^- that causes chronic nitrite intoxication and not further to NO, nitrosamines (RN-NO), N_2O, and N_2 (Purcell et al. 1971; Braker et al. 2012).

8 Denitrification strategies

The denitrification forerunner, nitrification, converts fertilized NH_4^+ to NO_3^- by consuming O_2 (Fig. 2). If O_2 is consumed and sufficient C_{H2O} (electron donors) is available, denitrifying communities reduce nitrate to NO_2^-, NO, N_2O preferred to N_2 (Zumft 1997). At each NH_4^+ oxidation and reduction step, energy is conserved, under optimal growth and denitrifying conditions per mole of glucose up to 90 % of the energy (ATP) obtainable by O_2 respiration (Figs. 1 and 2; Burth et al. 1982; Zumft 1997; Cabello et al. 2004; Zhang et al. 2011; Braker et al. 2012). A key enzyme is the nitrite reductase of which two forms exist. In the same bacterial strain, only one of these two nitrite reductases is present, either the *nir*S gene encoded cytochrome cd_1 or the non-heme copper-dependent *nirK* and nosZ gene encoded nitrite reductase. Ammonia oxidizing bacteria, for example, possess a *nirK*-encoded nitrite reductase, while N_2O-emitting denitrifiers or halobenzoate degraders possess a *nirS*-gene encoded nitrite reductase. Bacterial species as *E. coli*, a well-known representative among intestinal bacteria, and some of the archaea reduce NO_3^- only to NO_2^-, the key role player in heterotrophic nitrifiers' denitrification or nitrate ammonification (Figs. 3 and 8). Microorganism and C_{H2O}-dependent NO_2^- is further reduced to NO and the greenhouse gas N_2O (Fig. 1). If N_2O escaped from the cell membrane and entered earth's atmosphere only in traces, it will be further reduced to N_2 (Vieten et al. 2008); and in holding N_2O on the membrane, the element ruthenium (Ru) seemingly

plays a crucial role ($[(RU)(NH_3)_5(N_2O)]^{2+}$ (Zumft 1997; Fungo et al. 2014). Ru is like iron and copper, a d-block element in denitrification enzymes, and may at least partly explain why in soil biochar experiments the N_2O emission reduction varied between 10 and 41 %.

The highly mobile nitrate ion, if not taken up by plants or denitrified to N_2O and N_2, will reach the ground (drinking) water and may cause health problems. If it is reduced to N_2O, it is an atmosphere-threatening 300-times-more-potent greenhouse gas than CO_2 and an ozone-layer depleter (Michel and Wozniak 1998; Crutzen et al. 2008). Thus, it is of general interest to keep the formation of both compounds in an acceptable range all the more because presently, annual costs of around 100 billion US dollars caused by NO_3^- and N_2O needs to be avoided (Ravishankara et al. 2009; Steffen et al. 2011; Sutton et al. 2011). Increasingly identified as N_2O and CH_4 emission sources are converted primary forests and permafrost soils, and ecologists have started to think about how ecosystem-change-related greenhouse gas emissions can be slowed in their magnitude. Also, turned around compost heaps and aerated wastewaters emit N_2O and force waste managers to think in a similar direction. In wastewater treatment systems, aeration is necessary for minimizing C and N overloads but favors concomitantly nitrate formation; thus, a reduction of N_2O emissions is not easy to achieve. Ploughing soils and organic amendments with varying wide CN ratios intend to stimulate O_2 diffusion and SOM degradation for reaching satisfying yields, which are easier realized by overloading agricultural land with mineral N fertilizers. An uncontrolled denitrification results instead of a more controlled one by organic amendments. Yet, high groundwater NO_3^- contents and increasing N_2O emissions force farmers to overthink their fertilization concepts whereby nitrification inhibitor stabilized fertilizers are seen as the solution (Zerulla et al. 2001). The concept behind this is to follow nature's C_{H2O}/NO_3^- ratio managing strategy, which also includes nitrification-inhibiting compounds. The aim is to prevent NO_3^- formation especially during the plant-free period, to balance the C_{H2O}/NO_3^- ratio towards C_{H2O} surplus. Despite all endeavors undertaken by ecologists, waste managers, farmers, and the industry in replacing oil based by renewable energy sources, the atmospheric N_2O concentration continues to increase by annually 0.25 % (Michel and Wozniak 1998; Eurostat yearbook, statistical guide to Europe, European Commission 2013). New, politically claimed models must be developed for being more precise in predicting NO_3^- and N_2O impacts on ground waters, ecosystems, and climate (Crutzen et al. 2008; PICC MAT 2011; Lienhard et al. 2014). Climate change alters ecological strategies of soil bacteria and thus triggers shifts within taxa, C_{H2O}/NO_3^- ratio and soil community composition (Evans and Wallenstein 2014).

9 C_{H2O}/NO_3^- ratio managing possibilities

Nature has shaped a soil C_{H2O}/NO_3^- ratio concept inter alia to control nitrification and denitrification. The concept includes plant diversity, animal activity, the distribution of organic matter (energy source) and electron acceptors, inorganic nutrients, and nitrification inhibiting compounds. The intention is to force denitrifying bacteria and archaea residing in water-filled soil crumb zones, compost clots, wastewater flocks or earthworm guts to use the electron acceptors NO_3^-, NO_2^-, NO, and N_2O economically (Figs. 3 and 8; Table 2; Simarmata et al. 1993; Horn et al. 2006; Benckiser 2010). Have nitrate ions reached the groundwater, the chance to be reduced decreases. There is also little chance that escaped N_2O molecules from the cell membrane into the surrounding atmosphere are further reduced to N_2 (Lin et al. 2008; Vieten et al. 2008). Compost with a mean CN ratio of around 15 and a C_{H2O}/NO_3^- ratio of around 1000 and spread on agricultural land shapes the nitrification–denitrification activities in soils and is thus a possibility to reduce N_2O emissions (Barth et al. 2008; Beylich et al. 2010; PICCMAT 2011; Langevin et al. 2014). Though laboratory experiments with soil C/NO_3^- ratios between 84 and 130 exhibited that as soon as NO_3^- is widely exhausted N_2O is reduced to N_2 even in the presence of C_2H_2, a N_2O emission reduction with organic amendments is not easy to achieve (Simarmata et al. 1993; Chen et al. 2013). A controlled nitrate availability is easier achieved with nitrification inhibitors as nature is exemplifying it with compounds like karanjin, humic acids, or neem (Hassett et al. 1987; Majumder et al. 2001; Abasi et al. 2011). Nitrification inhibitors not only downsize the nitrification but also control the follower process, denitrification (Fig. 2). Nitrification inhibitors as dicyandiamide or 3,4-dimethylpyrazole phosphate unveil a N_2O reduction efficiency of 25.8 or 48.7 % especially during the plant's free and early growth periods when only little of the forming or fertilized nitrate is taken up (Table 2; Weiske et al. 2001; Chang et al. 1998; Akiyama et al. 2010). In liquid culture, the N_2O reducing efficiency by the nitrification inhibitors 3,4-dimethylpyrazole phosphate, *N*-allylthiourea, dimethylether, or nitrapyrin is around 95 % and thus significantly higher than in soils (Fig. 7; Table 2), presumably because the soil clay–humus complex fixes parts of the added NH_4^+.

An ideal inhibitor, environmentally applied, should penetrate the low concentrated target cell and only influence the envisaged enzyme. It should not too rapidly degrade, not suffer from a high degree of adsorption, not affect other processes and organisms, and not interfere with analytical procedures required during the experiment (Miller et al. 1993). In field experiments with the nitrification inhibitors dicyandiamide and 3,4-dimethylpyrazole phosphate, it was found that the AMO resembling MMO was not curtailed (Table 2; Holmes et al. 1995). That signalizes a highly specific enzyme blocking

of both inhibitors. The existing analysis for dicyandiamide and the analysis for 3,4-dimethylpyrazole phosphate developed by us enabled to show that both N_2O emissions reducing nitrification inhibitors disappeared, the 3,4-dimethylpyrazole phosphate less rapidly than dicyandiamide (Rajbanshi et al. 1992; Weiske et al. 2001; Benckiser et al. 2013). Thus, both NI seemingly fulfill the criteria for an ideal inhibitor. The still unexplained CO_2 emission reduction, especially in the presence of 3,4-dimethylpyrazole phosphate (Table 2), relativizes the conclusion drawn before.

In a 10-day laboratory incubation experiment with 3,4-dimethylpyrazole phosphate, stabilized ASN granules placed on a silty clay soil, adjusted to a water holding capacity of 24 %, showed that 3,4-dimethylpyrazole phosphate remained in this time frame to 80 % within the 0- to 5-mm region around the fertilizer granule (Azam et al. 2001). Only 5–15 % moved 5–20 mm away from the granule and <3 % into the 25–40 mm region. In the same time frame, ammonium diffused 4 cm away from the fertilizer granule and nitrate showed a fairly uniform distribution. In conclusion, 4 cm away from the field-applied 3,4-dimethylpyrazole phosphate stabilized fertilizer granule ammonium oxidizing bacteria, archaea, and denitrifying bacteria and archaea can continue oxidizing NH_4^+ to NO_3^- and reducing NO_3^- to N_2O and N_2, while the proximate granule neighborhood is protected. Dicyandiamide and 3,4-dimethylpyrazole phosphate, applied at recommended rates, evidently do not affect the essential soil processes in temperate climate regime conditions (Tindaon et al. 2012), but this might be different at soil temperature regimes above 30 °C (Ali et al. 2008; 2012). Under hot climate soil conditions, dicyandiamide and also 3,4-dimethylpyrazole phosphate are less resistant to degradation, in an alkaline calcareous soil dicyandiamide even seems to increase the fertilizer N loss and a satisfying AMO inhibition is first achieved when the application rate of dicyandiamide and 3,4-dimethylpyrazole phosphate is at least 10 times higher than the recommended one for temperate climate regimes (16 %, dicyandiamide, or 1.6 % 3,4-dimethylpyrazole phosphate to NH_4^+–N; Mahmood et al. 2011). A 10 times higher 3,4-dimethylpyrazole phosphate application rate than the recommended one for temperate climate regimes given to a liquid medium growing nitrifying bacteria consortium exhibited that after a 75-day incubation even the target organisms not recovered (Benckiser et al. 2013). A 10 times higher 3,4-dimethylpyrazole phosphate soil applied rate than the recommended one for temperate climate regimes will affect the nitrifying microbial community less drastically because of absorption phenomena and low diffusion velocity (Azam et al. 2001; Tindaon et al. 2012). Dicyandiamide and 3,4-dimethylpyrazole phosphate-related soil (field) studies carried out by Di et al. (2007) and Kleineidam et al. (2011) indicate that both nitrification inhibitors may insufficiently block the archaeal AMO. But these first hints need to be proven and a continuing nitrification inhibitor stabilized fertilizer development may improve the inhibition efficiency in combination with an advancing ploughing technology (inorganic–organic mixing and O_2-diffusion improvement), intercropping (improved NO_3^- uptake), N requirement adapted fertilization, or a crop, field properties adapted precision farming farmers (Bouma 2014; Langevin et al. 2014). This bouquet of possibilities helps to influence and control nitrate formation and N_2O emissions in agricultural landscapes better in future. In a present cost/benefit analysis of farming practices of the European Commission (EC), the EC hesitates to recommend the use of nitrification inhibitor stabilized N fertilizers in spite of a 26 to 49 % N_2O emission reduction, because they are still relatively costly and their side effect behavior is insufficiently tested (PICCMAT 2011). Also, the crop yield improvement of cereals and maize in presence of nitrification inhibitors is not conclusively documented. In this context, a better comprehension of modern analysis such as the $^{15}N^{14}N$ isotope ratio technique would be helpful in shaping C_{H2O}/NO_3^- ratio-dependent N_2O emissions more satisfyingly in our industrializing world by combining in a plant N demand adapted the application of animal manure, plant residues, compost, and nitrification inhibitor stabilized N fertilizers (Sturm and Lojen 2011; Decock and Six 2013). Plants growing on animal manure-enriched fields accumulate due to the animal transaminase activity in such fertilizers ^{15}N in their tissue to around +10±4‰, while plants grown in fields receiving different mixtures of inorganic plant residue fertilizers may enrich in their tissues ^{15}N by around 4‰ and plants grown on fields receiving legume residues or exclusively mineral fertilizers show no accumulation of ^{15}N. Precision farming and waste management escorted by ecologists and advising agencies, and continually improving analysis-based techniques may in the future enable waste managers and farmers to sustain satisfying yields at reduced N inputs or satisfying organic load degradation at well-balanced C_{H2O}/NO_3^- ratios and reduced N_2O emissions.

10 Conclusion

Converted primary forest and permafrost soils into monoculture agroecosystems, industrially organized waste recycling systems, and industries are sources for increasing atmospheric N_2O emissions. Landscape managers have two options for reducing the N_2O increase:

a. To suppress denitrification by enriching the system with O_2, whereby nitrification and organic matter degradation are favored, and
b. Balancing the water soluble carbon (C_{H2O})/NO_3^- soil ratio appropriately with animal manure, compost, and/or nitrification inhibitor stabilized N fertilizers.

The aim of all measures is to organize energy conservation hierarchically and in a way that anaerobic NO_3^- respiration majorly ends as N_2. C_{H2O}/NO_3^- ratio balancing towards a surplus of electron donors at anaerobic denitrifying conditions force denitrifying bacteria, archaea, and fungi to use the electron acceptors NO_3^-, NO_2^-, NO, and N_2O economically by reducing them mostly to N_2. Such balanced C_{H2O}/NO_3^- ratios are easier to achieve with nitrification inhibitor stabilized N fertilizers than with the application of organic materials. Nitrification inhibitors control nitrification specifically and consequently the following process, denitrification. In shaping C_{H2O}/NO_3^- ratios, analysis such as the $^{15}N^{14}N$ isotope ratio technique may be helpful. They allow better insights in N-transformations. From a climate point of view, complete prevention of NO_3^- leaching and N_2O emissions is not a reality and even not desirable.

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