Biology and Fertility of Soils (2022) 58:579–591
https://doi.org/10.1007/s00374-022-01644-x

ORIGINAL PAPER

Greenhouse gas (CO_2, CH_4, and N_2O) emissions after abandonment of agriculture

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Received: 1 December 2021 / Revised: 19 April 2022 / Accepted: 26 April 2022 / Published online: 3 May 2022

Abstract

The GHG (CO_2, CH_4, N_2O) emission potential along a chronosequence of former agricultural soils abandoned for 9 to 32 years were compared to an actively managed (on-going) agricultural soil (reference). The soils were incubated in mesocosms with and without manure amendment, and microbial functional groups involved in nitrous oxide emission were quantitatively assessed. Carbon dioxide emission significantly increased after agriculture abandonment (< 24 years) consistent with higher decomposition rate, but total emission decreased in the long term (> 29 years). With the cessation of agriculture, the abandoned sites generally became a net methane sink. Notably, total nitrous oxide emission showed a significant monotonic decrease over years of abandonment in response to manure amendment, possibly reflecting an altered capacity for (de) nitrification as indicated in the response of the (de)nitrifier abundance. Overall, our findings suggest that the GHG legacy of agriculture diminishes over time (> 29 years), with lowered GHG emissions and global warming potential (GWP) after abandonment of agriculture.

Keywords Agriculture abandonment · Carbon-cycle · Nitrogen cycle · Legacy effect · Ammonium oxidizers · Denitrifiers

Introduction

The conversion of pristine to agricultural lands induces the emission of greenhouse gases (GHG; CO_2, CH_4, N_2O). Vice versa, the reversion of agricultural lands to semi-natural state may reduce GHG emissions (Nazaries et al. 2013; McDaniel et al. 2019). Restoration of former agricultural lands is particularly relevant in industrialized regions, such as in the European Union where up to 11% of agricultural lands (approximately 20 M ha) is anticipated to be abandoned by 2030 (Perpina et al. 2018). The transformation of agriculture into semi-natural sites entails natural succession pattern and an increase in above- and below-ground biodiversity; this transformation thus shows strong temporal dynamics. Therefore, abandoned agricultural lands at different times can be considered as a chronosequence, providing

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an opportunity to capture the gradual shift in soil attributes. Indeed, previous work documented changes in the edaphic properties, as well as the microbial community and interaction network, as a response to the secondary succession of the aboveground plant community using the same chronosequence of agriculture-abandoned fields, as in this study (Kardol et al. 2005; van der Wal et al. 2006; Holtkamp et al. 2011; Van de Voorde et al. 2012; Thomson et al. 2015; Hannula et al. 2017; Morriën et al. 2016). However, still little is known about the persistence of the legacy of agriculture with regard to microbially mediated GHG emissions.

Microorganisms are key drivers for C- and N-cycling in soils, modulating the emission of the primary GHG (CO_2, CH_4, and N_2O; Wagg et al. 2014; Fierer 2017; Kaupper et al. 2020). While microbial respiration (CO_2 production) is a generalized process catalyzed by many soil microorganisms, methane and nitrous oxide emissions are mediated by specific microbial guilds. Methane emission is regulated by methane production and oxidation, catalyzed by methanogens and methanotrophs under anoxic and oxic conditions, respectively (Liesack et al. 2000; Guerrero-Cruz et al. 2021; Kaupper et al. 2022). Well-aerated soils generally act as a methane sink, turning into a methane source with sporadic methane production under anoxic conditions (e.g., increased moisture; Shrestha et al. 2012) or when methanotrophic activity is adversely affected by disturbances (e.g., agricultural activities; Ho et al. 2015a; McDaniel et al. 2019). Traditionally, nitrous oxide emission is determined by nitrification (ammonium oxidation) and denitrification (nitrate reduction), catalyzed by nitrifiers and denitrifiers under oxic and anoxic/micro-oxic conditions, respectively (Kuypers et al. 2018). To date, organisms harboring the Nos-type I and II nitrous oxide reductase are the only known nitrous oxide sink (Hallin et al. 2018; Wrage-Mönnig et al. 2018; Yoon et al. 2019). Many microorganisms capable of reducing nitrous oxide are denitrifiers, which are also drivers for N_2O production. Thus, depending on the environmental conditions (e.g., soil pH, C:N ratio) denitrifiers can act as a source or sink for nitrous oxide (Horn et al. 2006; Baggs et al. 2010; Henderson et al. 2010).

Here, we followed the GHG (CO_2, CH_4, N_2O) emissions along a chronosequence of abandoned agricultural soils with and without manure amendment in a mesocosm study. Soil from field under current agricultural practice was used for comparison. While the actual recovery of GHG emissions after agriculture abandonment were monitored in the un-amended soils, the legacy of agriculture leading to a potential capacity that is still present in the abandoned soil was assessed by determining the response and capacity of the soils to emit GHG following a contemporary manure application. Anticipating that manure amendment will have a direct and pronounced impact on N turnover processes, we enumerated the nitrifiers and denitrifiers based on the abundance of functional genes to provide insights to specific processes associated to nitrous oxide emission. While the impact of on-going agriculture management practices (e.g., fertilization, watering regime, tillage, cover crop cultivation) on GHG emission and the associated microbial communities have been the focus of previous work (e.g., Cuello et al. 2015; Ho et al. 2015a; Hernández et al. 2017; Drost et al. 2020; Kaupper et al. 2020; Brenzinger et al. 2021), less is known on their recovery following agriculture field abandonment. With less readily available N input in the abandoned sites over decades compared to the actively managed agricultural fields (fertilization occurred biannually in our study site), we hypothesized that the abandoned soils will have diminishing potential for (de)nitrification over time resulting from reduced abundances of microorganisms catalyzing these processes. Hence, relatively lower nitrous oxide emission is expected after manure amendment in the foremost (oldest) abandoned soils when compared to soils from the recently abandoned sites.

Materials and methods

Site description, mesocosm setup, and GHG flux measurements

The soils were collected in September 2014 from an actively managed agriculture site (Vredepeel, designated VRE) cropped with leeks at the time of sampling and from former agricultural lands at different stages of abandonment, forming a gradient of agriculture-impacted soils. Soils from the abandoned sites are located in Oud Reemst (designated OR), Mossel (MO), Nieuw Reemst (NR), Mosselse Veld (MV), and Dennenkamp (DK) in the Veluwe, all located in the Netherlands. The selected sites were abandoned since 2005, 1995, 1990, 1985, and 1982, respectively, resulting in a range from 9 to 32 years after abandonment at the sampling time (Table 1; Fig. S1). Prior to abandonment, the sites were cropped with cereals and corn in a rotational scheme, with occasional potato and sugar beet. The abandoned sites are now semi-natural grasslands and heathland; the vegetation as well as the belowground microbial diversity (e.g., microfauna, fungal, and bacterial communities) have been elaborated (van de Voorde et al. 2012; Thomson et al. 2015; Hannula et al. 2017; Morriën et al. 2017). The abandoned sites originated from the same parent material (glacial deposits), typical for the Veluwe region (Hannula et al. 2017). All sites contained well-drained sandy soils. Details on the location of the sites and selected soil properties are given in Table 1.

Soils were collected from the upper 10 cm in four 0.5 m × 0.5 m plots (at least 5 m apart) at random and composited as a bulk soil from each site. After sample collection, the soils were immediately loosely sieved (4 mm)

Table 1 Selected soil physico-chemical parameters

Sites (age)	Coordinates	Soil texture (%)[a]	pH[b]	Water content (%)[c]	Organic matter content (LOI %)	Total C (µg C mg dw soil^{-1})	Total N (µg N mg dw soil^{-1})	C:N	Nutrient contents (µg g dw soil^{-1})		
									NO_x	NH_4^+	PO_4^{3-}
Vredepeel (VRE; agricultural field)	51°32′32″N, 05°50′54″E	1.0, 4.1, 94.8	5.8 ± 0.01 [a]	9.4 ± 0.02 [d]	4.4 ± 0.01 [c]	22.2 ± 3.04 [c]	1.3 ± 0.21 [c]	17.1 ± 0.43 [c]	5.5 ± 0.10 [c]	0.01 ± 0.01 [e]	0.5 ± 0.06 [c]
Oud Reemst (OR; 9a)	52°02′27″N, 05°48′34″E	7.6, 5.1, 87.2	4.7 ± 0.01 [d]	11.4 ± 0.14 [c]	4.4 ± 0.13 [c, d]	29.0 ± 2.23 [b]	1.4 ± 0.15 [c]	20.3 ± 0.67 [b]	5.8 ± 0.21 [b]	0.2 ± 0.06 [d]	0.2 ± 0.07 [e]
De Mossel (MO; 19a)	52°3′40″N, 05°45′8″E	7.9, 4.8, 87.4	5.1 ± 0.01 [b]	11.6 ± 0.08 [c]	4.1 ± 0.22[d,e]	29.0 ± 7.57 [a,b,c]	1.2 ± 0.34 [c]	24.9 ± 1.02 [a]	5.0 ± 0.10 [d]	6.2 ± 0.15 [b]	1.5 ± 0.10 [a]
Nieuw Reemst (NR; 24a)	52°02′33″N, 05°46′29″E	8.7, 6.6, 84.7	4.3 ± 0.01 [f]	13.8 ± 0.99 [b]	4.9 ± 0.09 [b]	28.4 ± 2.12 [b]	1.8 ± 0.17 [b]	16.3 ± 0.39 [d]	9.2 ± 0.14 [a]	5.7 ± 0.40 [b]	0.8 ± 0.15 [b]
Mosselse Veld (MV; 29a)	52°04′23″N, 05°44′13″E	3.4, 2.7, 93.8	4.5 ± 0.01 [e]	11.8 ± 0.68 [c]	3.9 ± 0.17 [e]	34.3 ± 3.12 [a]	3.66 ± 0.23 [a]	9.4 ± 0.29 [e]	2.6 ± 0.10 [f]	3.9 ± 0.08 [c]	0.3 ± 0.02 [d]
Dennenkamp (DK; 32a)	52°01′43″N, 05°48′02″E	5.2, 8.8, 86.0	4.8 ± 0.01 [c]	15.6 ± 0.12 [a]	6.1 ± 0.15 [a]	22.8 ± 8.15 [b,c]	1.3 ± 0.53 [b,c]	17.3 ± 0.91 [c,d]	3.2 ± 0.07 [e]	8.2 ± 0.33 [a]	b.d.l

Lowercase letters indicate a level of significance at $p < 0.05$ between soils

n.d. not determined, *b.d.l.* below detection limit, *LOI* loss on ignition

[a]Given as % clay, % silt, and % sand

[b]pH was determined in 1 M KCl

[c]Water content was determined gravimetrically

without applying pressure through the sieve to remove debris and roots, prior to the mesocosm setup within 48 h. Each mesocosm comprised of 2.5-kg fresh soil (diameter × height, 20 cm × 10–11 cm), with the gravimetric water content adjusted to ~ 16% following the soil with the highest water content (DK; Table 1). This corresponded to 78% of a maximum water-holding capacity in all soils. Commercially available dried manure granules (Pokon Naturado B.V., the Netherlands) were used as soil additive. The manure granules were crushed and sieved (2 mm) before soil amendment. The manure granules (pH 7.9) contained approximately 5.6 µg NO_x g dw^{-1} (sum of NO_2^- and NO_3^-), 955.1 µg NH_4^+ g dw^{-1}, and 225.2 µg PO_4^{-3} g dw^{-1}. Manure was mixed into the soil by hand at a rate of 25 t ha^{-1}, as the typical range of residue input in agriculture practice (Ho et al. 2017a). Un-amended soils served as reference. Six replicate mesocosms were set up per soil and treatment (un- and manure-amended).

The mesocosms were incubated in the dark at 15 °C, approximating the average air temperature, for 56 days in a climate-controlled chamber. Throughout the incubation, deionized water was sprayed three times weekly onto the surface of the soil to maintain the water content. At designated intervals (days 1, 7, 14, 22, 28, 42, 56), the mesocosm was placed in a gas tight chamber equipped with a fan (diameter × height: 24 cm × 40 cm) for GHG (CO_2, CH_4, N_2O) measurements using the Innova 1412-5i photoacoustic infrared gas analyzer (lumaSense Technologies, Denmark) connected to an automated sampler with a moisture trap (Innova 1309 multiplexer, lumaSense Technologies, Denmark). Each mesocosm was left in the chamber for 30 min to equilibrate the soil-atmosphere gas exchange before the first measurement was performed. The gas flux rate was determined by linear regression ($R^2 > 0.98$) from at least four time intervals over approximately 2.0–2.5 h. The total GHG consumed during the incubation (56 days) was determined by integrating the area below the curve for the gas flux rate over time (Ho et al. 2017a). After the flux measurement on days 7, 14, 22, and 56, the soil (~ 100 g) was sampled with an auger (diameter × height: 3 cm × 10–11 cm), homogenized, and stored at − 20 °C in aliquots for the molecular analyses and at 4 °C for the determination of soil physico-chemical parameters. A plastic tube of the same diameter was used to replace the sampled core to minimize disturbance to the surrounding soil.

To assess plant growth properties of the soils, the soil was homogenized by hand, and cropped with two common wheat (*Triticum aestivum*) seedlings (pre-germinated) in the greenhouse at 22–24 °C after the final sampling (day 56). The crop was frequently watered until harvest after approximately 3 months. Crop growth was assessed by determining the aboveground biomass after drying in the oven at 60 °C until constant weight.

In parallel, a litter bag assay ($n = 6$ per soil) was performed using the same mesocosm setup (without crop) and incubation conditions to assess manure decomposition. In this assay, manure was contained in a litter bag with a 0.4-mm mesh size and was buried in the soil. Manure weight loss (%) was determined after the incubation.

Determination of selected soil physico-chemical properties

Inorganic nitrogen (NO_x; exchangeable NH_4^+) and PO_4^{3-} concentrations in the soil were determined in 1 M KCl (1:5 dilution) using the SEAL QuAAtro SFA autoanalyzer (Beun de Ronde B.V., the Netherlands). Total C and N concentrations were determined using the Flash EA1112 CN analyzer (Thermo Fisher Scientific, the Netherlands) from oven-dried (40 °C for 5 days) and sieved (0.4 mm) soil. The organic matter content was determined by loss on ignition (LOI %). Detailed soil characteristics are given elsewhere (Morriën et al. 2017).

DNA extraction and quantitative PCR (qPCR)

DNA was extracted from ~ 0.3 g soil in triplicate from the starting material, and from three randomly selected replicates out of six per time (days 7, 14, 22, and 56) and treatment (un- and manure-amended) in the VRE, OR, and DK soils using the PowerSoil DNA Isolation kit (MOBIO, Uden, the Netherlands) according to the manufacturer's instructions.

The qPCR assays were performed to enumerate the abundance of nitrifiers and denitrifiers based on functional genes for the respective functional groups, including bacterial and archaeal *amoA* genes for ammonium oxidizers (AOB and AOA), *nirS* and *nirK* genes for nitrite reducers, and *nosZI* gene for nitrous oxide reducers. These genes were quantified in the starting material and over time for the on-going agricultural soil (VRE), and the abandoned soils since 2005 (OR) and 1982 (DK). The qPCR assays were performed using a Rotor-Gene Q real-time PCR cycler (Qiagen, Venlo, the Netherlands). For each DNA extract ($n = 3$), duplicate qPCR reactions were performed, giving a total of six reactions per time and treatment. The primer pair and primer concentrations, as well as the PCR thermal profiles, are provided in Table 2. Each qPCR reaction consisted of 10 µl 2 × SensiFAST SYBR mix (BIOLINE, Alphen aan den Rijn, the Netherlands), forward and reverse primers each, 1 µl bovine serum albumin (5 mg ml^{-1}; Invitrogen, Breda, the Netherlands), and 2 µl template DNA. DNase- and RNase-free water was used to top up to a total volume of 20 µl per reaction. The template DNA was diluted 20–50-fold (for the AOA and AOB *amoA*-targeted qPCR) or 100-fold (for the *nirS*-, *nirK*-, and *nosZI*-targeted qPCR). Preliminary qPCR

Table 2 qPCR assays used in this study

Target gene	Primer pair (forward/reverse)	Primer concentration (nM)	PCR thermal profile[a]	No. of cycles	Standards[b]	Reference
amoA (AOA)	AOA-amoA1F/amoA2R	100/100	95 °C, 15 s; 56 °C, 45 s; 72 °C, 45 s	40	*Nitrosomonas viennensis*	Francis et al. (2005)
amoA (AOB)	AOB-amoA1F/amoA2R	100/100	95 °C, 15 s; 55 °C, 45 s; 72 °C, 45 s	40	*Nitrosomonas europea*	Rotthauwe et al. (1997)
nirS	nirScd3aF/nirSR3cd	500/500	95 °C, 15 s; 58 °C, 30 s; 72 °C, 30 s	45	*Pseudomonas fluorescens*	Kandeler et al. (2006)
nirK	nirK876/nirK1040	500/500	95 °C, 15 s; 60 °C, 30 s; 72 °C, 30 s	45	*Pseudomonas stutzeri*	Henry et al. (2006)
nosZI	nosZ2F/nosZ2R	500/500	95 °C, 15 s; 65 °C, 30 s; 72 °C, 30 s	40	*Azospirillum irakense*	Henry et al. (2006)

[a]PCR thermal profile given in the following steps: denaturation, annealing, and elongation. Initial denaturation was at 95 °C for 3 min

[b]Source of the qPCR standards

runs have shown that these dilutions yielded the optimum gene copy numbers. DNA obtained from a gene library or isolates harboring the functional gene fragment was used to generate the calibration curve (10^1 to 10^8 copy numbers of targeted gene) (Veraart et al. 2014; Ho et al. 2020). Amplicon specificity was assessed from the melt curve determined from 70 to 95 °C at 1 °C increment, and further confirmed on 1% agarose gel electrophoresis, showing a single band of the correct size.

Statistical analyses

The statistical analysis was performed in Sigmaplot version 12.5 (Systat Software Inc.). A Shapiro–Wilk test was performed to test for normal distribution, while the homogeneity of variance was determined using Levene's test. Where normality and homogeneity of variance were met, an ANOVA with Tukey post hoc test (level of significance, $p < 0.05$) was performed to compare the GHG fluxes and qPCR data between sites. Where normality and homogeneity of variance were not met, a Kruskal–Wallis ANOVA and Dunn's post hoc test ($p < 0.05$) were performed. Additionally, total nitrous oxide emission was correlated to time since agriculture abandonment by linear regression using Origin (OriginLab Corporation, Northampton, MA, USA).

Results

GHG emissions (CO_2, CH_4, N_2O)

Carbon dioxide emission was significantly lower in the un-amended agricultural soil compared to the abandoned soils (Fig. 1; Fig. S2). In particular, the more recently un-amended abandoned soils (OR, MO, NR; < 24 years since abandonment) emitted significantly ($p < 0.05$) higher total carbon dioxide than the older abandoned soils (MV, DK; Fig. 1). Following manure addition, carbon dioxide emission in all soils appreciably increased. The discrepancy in carbon dioxide emission among the abandoned soils became less apparent, but the foremost abandoned soil (DK) still showed significantly lower total carbon dioxide emission comparable to the agricultural soil (Fig. 1). Consistently, the litter bag assay exhibited significantly ($p < 0.05$) higher manure mass loss in the more recently abandoned soils (OR, MO; Fig. S3), corroborating with the higher carbon dioxide emissions, indicating a higher capacity for decomposition in these soils.

The agricultural soil was a net source of methane, whereas the abandoned soils were net methane sinks during the incubation, with an exception; in MO, methane emission was detected < 21 days during incubation (Fig. 1; Fig. S4). Soon after manure amendment (day 7), methane emission increased in the agricultural soil, effectively turning the soil into a stronger net methane source by approximately tenfold (Fig. 1). Similarly, the abandoned soils either became a net methane source (OR) or a weaker methane sink (NR, MV, DK) after manure amendment, with the exception of MO whereby the soil turned from a net methane source to sink (Fig. 1). Generally, manure amendment weakens the methane sink function in the abandoned soils, but showed no apparent trend in response to the years of abandonment.

Nitrous oxide emission was appreciably higher ($p < 0.05$) in the recently un-amended abandoned soils (OR, MO), which occurred mostly at < 14 days during the incubation (Fig. 1; Fig. S5). In the other un-amended soils, including the agricultural soil, nitrous oxide emission was low with flux rates ranging from 0 to < 0.5 μmol m^{-2} (Fig. S5). However, nitrous oxide emission increased (< 21 days; Fig. S5) after manure amendment, coinciding with a decrease in

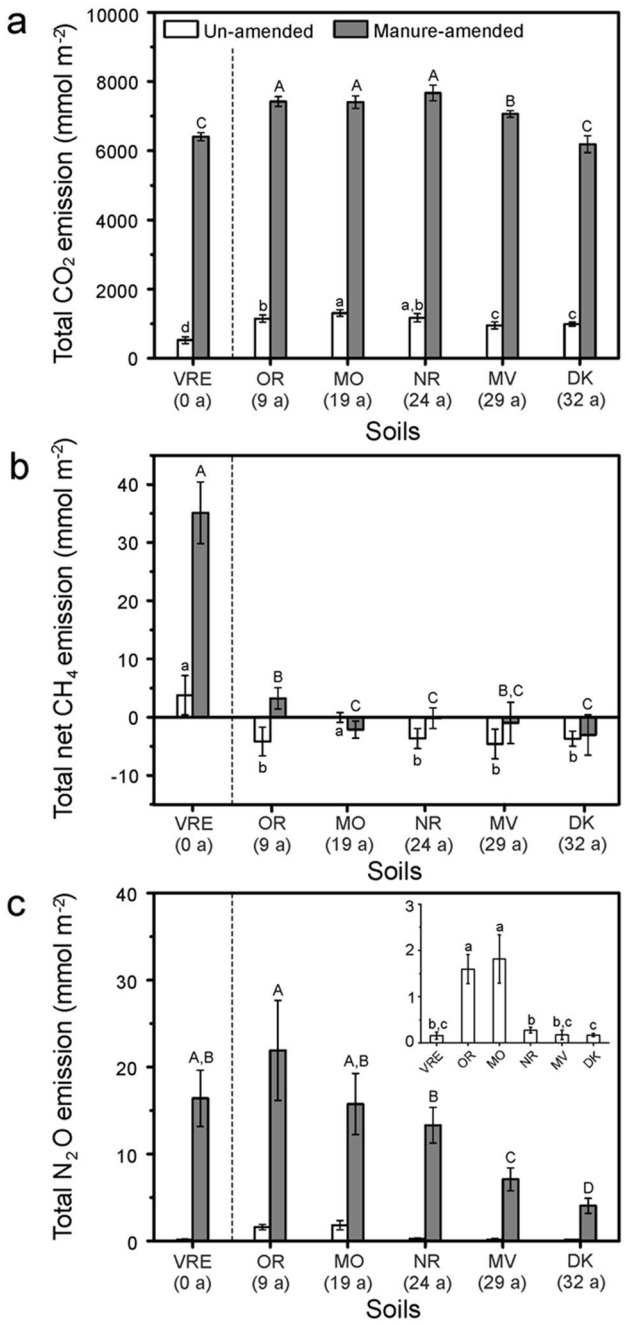

Fig. 1 Total CO_2 (**a**), CH_4 (**b**), and N_2O (**c**) emissions in the un- and manure-amended agricultural soil (VRE) and abandoned soils (OR, MO, NR, MV, DK) (mean ± s.d.; $n = 6$). In **c**, the inset figure shows N_2O emission in the un-amended soil. Lowercase and uppercase letters indicate the level of significance ($p < 0.05$) among the un-amended and manure-amended soils, respectively. Temporal dynamics in the CO_2, CH_4, and N_2O flux rates are given in Figs. S2, S4, and S5, respectively

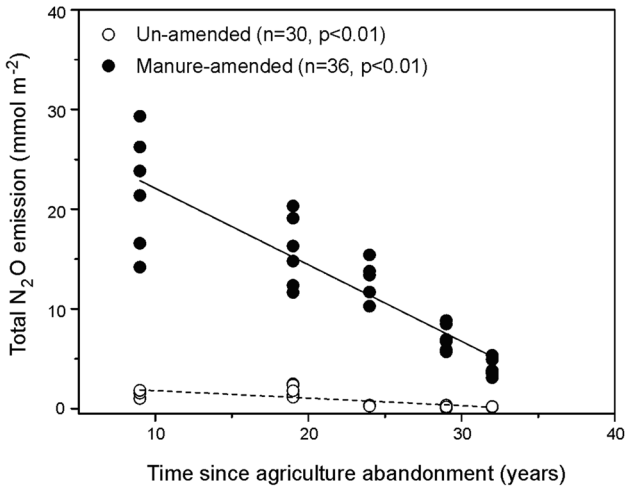

Fig. 2 The relationship between total nitrous oxide emission and time (years) since the abandonment of agriculture in the un-amended and manure-amended soils. All replicates with detected nitrous oxide flux were included in the linear regression, showing a significant correlation ($p < 0.05$) between the total nitrous oxide emitted and time since agriculture abandonment for both un-amended and manure-amended soils

exchangeable ammonium concentrations and an increase of NO_x (sum of NO_2^- and NO_3^-) over time (Fig. S6), indicating nitrification. Total nitrous oxide emitted showed a monotonic decrease over time of abandonment ($p < 0.05$; Figs. 1 and 2), more pronounced in the manure-amended than in the un-amended soils, indicating reduced nitrous oxide production and/or higher nitrous oxide sink capacity with increasing abandonment of soils.

The abundance of nitrifiers and denitrifiers

The abundance of the *amoA* gene for AOA was below or close to the detection limit of the qPCR assay in all sites at the start of the incubations ($< 4.2 \times 10^4$ gene copy no. g dw $soil^{-1}$). In contrast, copy number of the *amoA* genes for AOB was numerically predominant, and was significantly higher ($p < 0.05$) in the agricultural than abandoned soils (Fig. 3). Among the genes indicative of the denitrifiers, the abundance of the *nirS* gene was below or close to the detection limit of the qPCR assay, while the *nirK* gene was numerically predominant, detected at 2–3 orders of magnitude higher than the *nirS* gene in all sites and over time. Considering this, and that both *nirS* and *nirK* genes encode for nitrite reductase, the copy numbers of these genes were summed (*nirS* + *nirK*) to be used as a proxy for the abundance of nitrite reducers (Harter et al. 2014). The initial *nirS* + *nirK* gene abundance at all sites ranged between 1.0 and 2.5×10^8 gene copy no. g dw $soil^{-1}$, with the exception of DK where values were significantly lower ($p < 0.05$) relative to the recently abandoned and agriculture soils (Fig. 3). Although average *nosZI* gene abundance was within a narrow range in all soils (4.7×10^5–1.6×10^6 gene copy no. g dw $soil^{-1}$), copy numbers were significantly higher ($p < 0.05$)

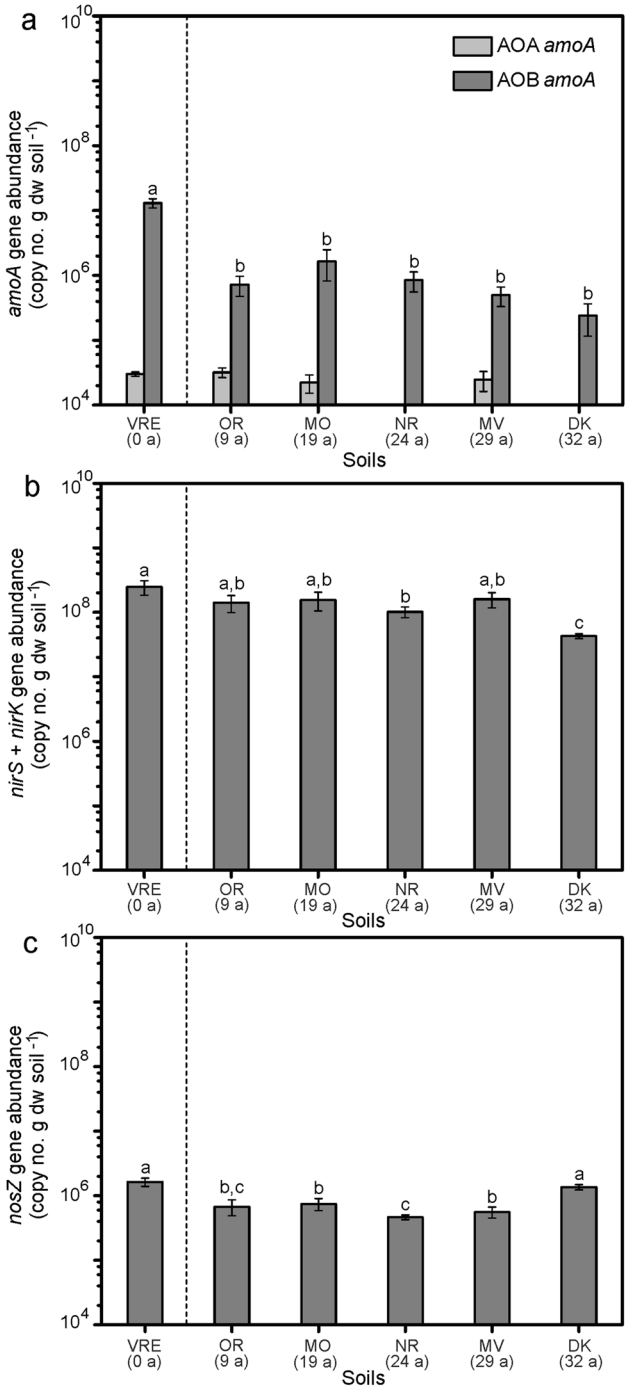

Fig. 3 The abundance of the bacterial and archaeal *amoA* (**a**), *nirS* + *nirK* (**b**), and *nosZI* (**c**) genes in the on-going agricultural and agriculture-abandoned soils (mean ± s.d.). Duplicate qPCR reactions were performed for each DNA extract ($n = 3$) from the starting material, giving a total of 6 replicates per soil. The detection limit of the qPCR assays was at ~ 3×10^4 (**a**) and 8×10^4–9×10^4 (**b** and **c**) copy numbers of the target genes g dw soil^{-1}. In **a**, archaeal *amoA* gene abundance was at (in VRE and OR) or below (in MO, NR, MV, and DK) the detection limit. Lowercase letters indicate the level of significance at $p < 0.05$ between sites

in DK than in the more recently abandoned soils, and was comparable to agricultural soil abundance (Fig. 3).

The response to manure addition was monitored over time in the agricultural soil (VRE), as well as in the most recently (OR) and foremost (DK) abandoned soils (Fig. 4). The abundance of bacterial *amoA* genes (AOB) increased by 1–2 orders of magnitude after manure amendment in all soils. In contrast, the archaeal *amoA* gene (AOA) abundance did not change in response to manure amendment (> 7 days; Fig. S7). Particularly, the AOA *amoA* gene abundance in DK remained below the detection limit of the qPCR assay during incubation, like in the starting material. This suggests that nitrification was predominantly mediated by the AOB. Similarly, the *nirS* + *nirK* gene abundance increased by around an order of magnitude after manure amendment in all soils during incubation. The *nosZI* gene abundance was also stimulated by manure amendment, exhibiting an increase by around an order of magnitude in the VRE and OR soils, and to a lesser extent in the DK soil when compared to the un-amended soil (Fig. 4). Overall, manure amendment markedly stimulated the abundance of the nitrifiers (AOB), and to a lesser extent, the denitrifers and (denitrifying) nitrous oxide reducers. Generally, the stimulatory effect occurred at < 21 days coinciding with the nitrous oxide emission peak (VRE, OR), with the exception of the DK soil where the increase in the *nirS* + *nirK* and *nosZI* gene abundances was more pronounced later during the incubation (Fig. 4; Fig. S5), indicating a delayed response to the manure amendment.

Discussion

The legacy of agriculture on CO_2 and CH_4 emissions following agriculture abandonment

Carbon dioxide emission significantly increased after the abandonment of agriculture, more pronounced in the recently (< 24 years) than older (> 29 years) abandoned soils, indicating a shift towards higher soil respiration in the un-amended restored agricultural lands. Although the soils contained largely comparable total C and organic matter content (Table 1), the trend in carbon dioxide emission

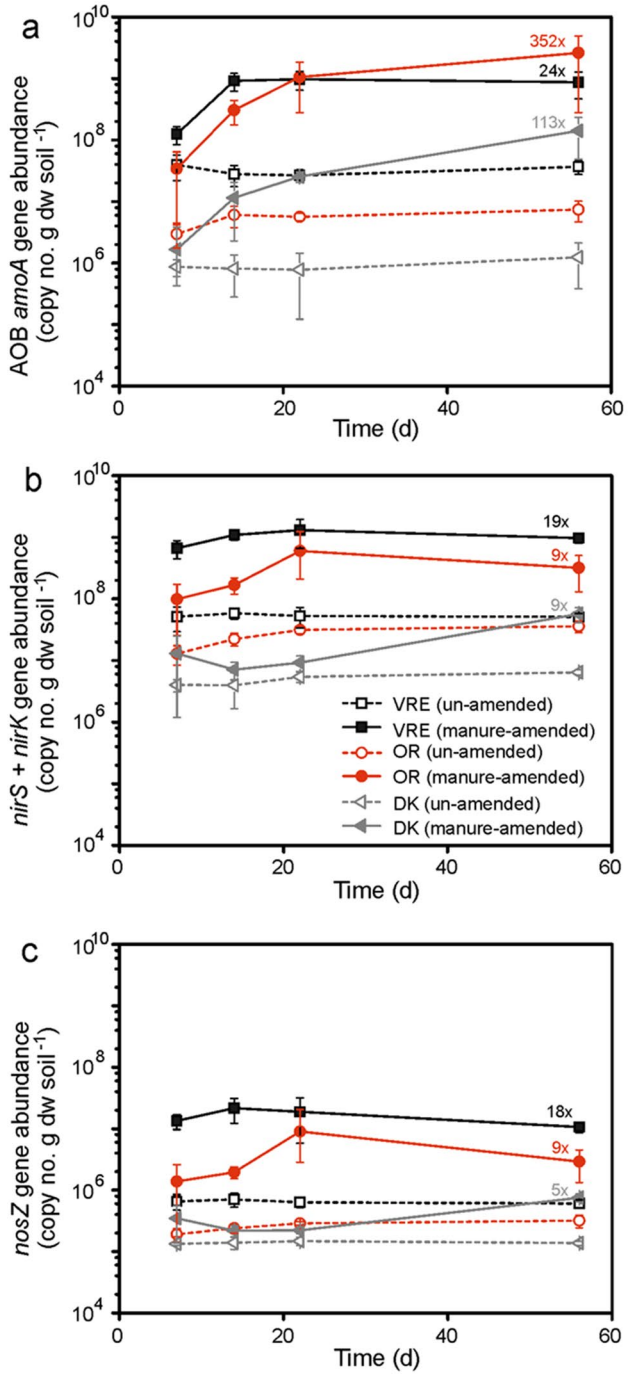

Fig. 4 Temporal changes in the bacterial *amoA* (**a**), *nirS*+*nirK* (**b**), and *nosZI* (**c**) gene abundances in the un- and manure-amended on-going agriculture soil (VRE), and recently (OR) and foremost (DK) agriculture-abandoned soils during incubation (mean ± s.d.). Duplicate qPCR reactions were performed for each DNA extract ($n=3$), giving a total of 6 replicates per soil, treatment, and time. All gene abundances were significantly higher ($p<0.05$) after manure amendment relative to the un-amended soil with the exception of the bacterial *amoA* gene abundance at day 7, and *nirS*+*nirK* and *nosZI* gene abundances at days 7 and 14 in DK. The values in the figure indicate the number of fold increase in the gene abundances at the end of the incubation comparing the un- and manure-amended soils. AOA *amoA* gene abundances remained relatively unchanged during the incubation (> 7 days; Fig. S7)

could be related to the organic fraction of the total C, which warrants further attention. Admittedly, we cannot exclude that preparation of soils (i.e., homogenization while sieving) enhanced the degradation of organic matter which may partly explain the higher carbon dioxide emission. However, it is noteworthy that all soils were prepared similarly and incubation conditions were standardized. Expectedly, carbon dioxide emission was appreciably stimulated after manure input in all soils, suggesting a relief of C/nutrient limitation, and/or priming of the recalcitrant C fraction with the input of more easily accessible manure-derived C which can account for up to 50% of carbon dioxide emitted (Fontaine et al. 2003; Arcand et al. 2017). Like in the un-amended incubations, the recently abandoned soils emitted significantly higher carbon dioxide after manure addition, consistent with the higher manure weight loss in the litter bag assay (Fig. 1; Fig. S3), indicating a relatively higher capacity for manure decomposition. This is in agreement with previous work, showing relatively higher C mineralization up to 22 years after agriculture abandonment (Holtkamp et al. 2011). However, decomposition rate returned to lower levels after > 29 years of agriculture abandonment (Fig. 1; Fig. S3). The increased manure decomposition in the recently abandoned soils had no apparent effect on crop yield (biomass) but crop yield was significantly lower in the agricultural soil compared to the abandoned soils after manure addition (Fig. S3). This is not entirely surprising as microorganisms in agricultural soils compete with crops for nutrients. Such competition likely favored more copiotrophic fast-growing microorganisms (Ho et al. 2017b), which would rapidly exhaust any extraneous labile energy source and may partly explain the significantly lower crop yield in the agricultural relative to the abandoned soils. Over time, more oligotrophic microbial communities likely emerged with reduced labile C input during restoration, consistent with the documented distinct shift in the microbial community composition along this chronosequence (Thomson et al. 2015; Hannula et al. 2017). Therefore, the altered carbon dioxide emission and potential decomposition persisted at least in the short term (< 24 years) after agriculture abandonment.

Methane emission significantly increased (agricultural soil) or the methane sink function was adversely affected (agriculture-abandoned soils), in response to manure addition. Previously, the addition of specific bio-based residues (e.g., compost) transiently, but significantly stimulated potential methane oxidation rate, resulting in agricultural soils turning into a methane sink (Ho et al. 2015a, 2019). However, not all soil additives imposed a stimulatory effect on the soil methane uptake (Brenzinger et al. 2018). Manure addition to agricultural soils had been shown to stimulate methanogenesis in anoxic incubations, which likely increased total methane emission and/or weakened the soil methane sink function (Ho et al. 2015b; Kim et al. 2018),

while seemingly having marginal effects on the methanotrophic abundance (Zhang et al. 2018). Generally, the abandoned soils act as a methane sink, with no apparent effect of time since abandonment in response to manure amendment. Therefore, we focused instead on the N-cycling microorganisms, where a distinct trend in nitrous oxide emission was detected following agriculture abandonment. Moreover, manure addition may have elicited a stronger effect than legacy-induced changes (Krause et al. 2018; van Kruistum et al. 2018) to methanogenic and methanotrophic activities.

The legacy of agriculture on N_2O emission, linking changes in the functional gene abundances to (de) nitrification and nitrous oxide reduction

Interestingly, total nitrous oxide emission showed a continuous decrease over time in the abandoned soils in response to manure amendment, possibly reflecting a shift in the underlying processes (i.e., nitrification, denitrification) leading to decreased nitrous oxide production or/and increased nitrous oxide sink capacity (i.e., nitrous oxide reduction) at different stages of abandonment. To this end, the potential capacity of the soils to (de)nitrify and reduce nitrous oxide was assessed by the response of the functional gene abundances (magnitude of change) to manure amendment, comparing the actively managed agricultural soil to the most recently and foremost abandoned soils (Fig. 4). Most pronounced changes were detected in the abundance of bacterial *amoA* genes after manure amendment, indicating a high capacity for nitrification in all soils, particularly in the recently abandoned soil (352-fold increase) coinciding with the highest mean total nitrous oxide emitted (Figs. 1 and 3). With the concurrent increase in *amoA* gene abundance and nitrous oxide emission, the relevance of nitrifier denitrification cannot be excluded, but the mechanism for this process is poorly understood (Prosser et al. 2020). Notably, archaeal *amoA* gene abundance was below the detection in soils being longer abandoned from agriculture. While it is not surprising that AOB showed a stronger response to abrupt labile N availability (Ouyang et al. 2016; Angell et al. 2018; Katulanda et al. 2018; Ho et al. 2020; Meyer et al. 2021), the detected low abundance of AOA was not expected as affinities for ammonia are comparable (Martens-Habbena et al. 2009), but their role under more oligotrophic conditions comparable to abandoned soils have been frequently described (Prosser et al. 2020). Hence, other overriding (a) biotic factors may have driven niche differentiation among the ammonium oxidizers in the abandoned soils (e.g., pH, oxygen concentration; Berg et al. 2015; Ouyang et al. 2017; Prosser et al. 2020; Yoon et al. 2019). Besides the AOB and AOA, comammox (complete ammonia oxidizers; Daims et al. 2015; van Kessel et al. 2015) may also play a role in ammonium oxidation in this agricultural soil, which warrants attention in future studies.

Denitrification potential, as indicated by the significant change in the abundances of *nirS*+*nirK* genes in response to manure amendment (Fig. 4), was comparable in the abandoned soils (OR and DK; ninefold increase), and highest in the agricultural soil (19-fold increase). This suggests reduced capacity for denitrification after agriculture abandonment. Although the *nirS*- and *nirK*-targeted qPCR assays do not have a complete coverage of the diversity of the denitrifiers (Wei et al. 2015; Ma et al. 2019), they nonetheless may indicate the potentially relevant processes leading to nitrous oxide production. Besides clade I, other *nirK*-containing denitrifier clades may also play an important role, contributing to nitrous oxide production in agricultural soils (Wei et al. 2021). Particularly in our sampling sites, a shift towards enhanced role of fungi associated to the rhizosphere in soil processes was documented over time after land abandonment (Hannula et al. 2017). Therefore, fungal denitrification contributing to nitrous oxide emission, particularly after fertilization, cannot be completely excluded (Rütting et al. 2013; Wei et al. 2014; Yoon et al. 2019). Like the response of the *nirS*+*nirK* gene abundances, the increase in the *nosZI* gene abundance was most strongly detected in the agricultural soil following manure amendment, and decreased from the recently to the foremost abandoned soils (Fig. 4). Evidently, the capacity to act as a nitrous oxide sink was offset by the even higher capacity for (de)nitrification (VRE and OR), resulting in high nitrous oxide emission, in agreement with previous work suggesting that (de)nitrification were the more important microbial controls of nitrous oxide emission in agricultural soils (Cavigelli and Robertson 2000; Soares et al. 2016; Linton et al. 2020).

Insights into the underlying processes driving nitrous oxide emission, in response to manure amendment

Nitrous oxide emission in the agricultural soil was likely to be driven by nitrification and denitrification, as indicated by the comparable magnitude change in the AOB *amoA* and *nirS*+*nirK* gene abundances, respectively, following manure amendment (Fig. 4). On the other hand, significantly higher nitrous oxide emissions were probably driven by nitrification in the recently abandoned soil, given that the capacity for denitrification was relatively low. In the foremost abandoned soil, the initial abundances of nitrifiers and denitrifiers were significantly lower than in the other soils, which may explain the reduced nitrous oxide emission (Fig. 3). However, the appreciable increase in the nitrifier abundance (113-fold; Fig. 4) after manure amendment indicates that the soil maintained a heightened potential for nitrification. While harboring significantly lower initial denitrifier abundance,

the foremost as well as the recently abandoned soils showed comparable response in terms of GHG emissions to manure addition (OR and DK, ninefold increase; Fig. 4). Therefore, the significantly lower nitrous oxide emission in the foremost abandoned soil cannot be explained by changes in the abundances of the specific microbial guilds alone. This suggests that other denitrifiers not captured by the qPCR assays (besides *nirK* clade I) were involved, and/or the community composition of the different microbial guilds, consisting of members with different ecological traits, may also be relevant controls of nitrous oxide emission (Cavigelli and Robertson 2000; Sadet-Bourgeteau et al. 2019; Chang et al. 2021). Although environmental study documented a predominance of *nosZ* clade II over clade I gene abundance in an agricultural soil (Xu et al. 2020), the reason for this observation remains ambiguous (Conthe et al. 2018). Nevertheless, the community members harboring the *nosZII* gene may also act as a nitrous oxide sink in this study (Linton et al. 2020). Previously, compositional shifts in the denitrifier community could be linked to changes in nitrous oxide emissions (Highton et al. 2020), which likely occurred with agriculture abandonment (Thomson et al. 2015). Hence, we anticipate that a detailed qualitative analysis of the (de)nitrifier diversity may further shed light on the microbial controls of nitrous oxide emission.

Decreasing global warming potential (GWP) with the abandonment of agriculture

To allow broader inferences of the effects of land abandonment, the GWP was determined, incorporating the total emitted GHG during the incubation. The GWP was determined based on a 34-fold and 298-fold stronger radiative forcing effect of methane and nitrous oxide over a 100-year time frame with climate-carbon feedbacks, respectively, whereas the GWP for carbon dioxide is regarded as 1 (IPCC 2013). The GWP, expressed as carbon dioxide equivalent (mg CO_2 kg $soil^{-1}$), peaked after abandonment of agriculture (< 24 years), but significantly decreased over time (> 29 years) in the un-amended and manure-amended soils (Fig. 5). Carbon dioxide primarily contributed to the GWP, but the relative contribution was lower in the manure-amended soils (on average, > 70%, un-amended; 54–84%, manure-amended). After manure amendment, the relative contribution of nitrous oxide to the GWP increased (on average, 3–30%, un-amended; 16–46%, manure-amended) following the trend in nitrous oxide emission (Fig. 1). Methane contributed marginally to the GWP, given that the abandoned soils were largely methane sinks. Overall, the lower GWP in the foremost abandoned soil, in response to manure amendment, indicates a significantly reduced capacity for GHG emissions over time.

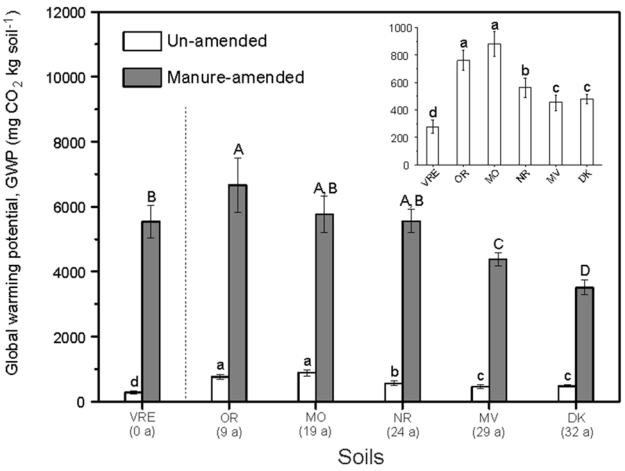

Fig. 5 Global warming potential (GWP) in the un-amended and manure-amended agricultural soil (VRE) and abandoned soils (OR, MO, NR, MV, DK) (mean ± s.d.; $n = 6$). The GWP was derived from the cumulative carbon dioxide, methane, and nitrous oxide fluxes (Fig. 1) over the incubation (56 days). The GWP values for methane and nitrous oxide are considered to be 34 and 298 over a 100-year time frame, respectively. The GWP value for carbon dioxide is considered to be 1

Conclusions

Overall, the impact of agriculture decreased over time (> 29 years), characterized by the significantly lower GHG emissions and GWP after agriculture abandonment, with increased carbon dioxide and nitrous oxide emissions occurring < 24 years after abandonment. Supporting our hypothesis, the abandoned soils exhibited diminishing capacity for nitrous oxide emission over time, in response to manure input. The reduced nitrous oxide emission could be related to the significantly lower (de)nitrifier abundances particularly in the foremost abandoned soil, but this was not consistent with the capacity of the soils for (de)nitrification, as indicated by the magnitude change in the functional gene abundances in response to manure amendment. Although we determined the persistence of the legacy of agriculture on the GHG emissions, the microbial controls of the underlying processes of the GHG warrant further investigations.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-022-01644-x.

Acknowledgements We are grateful to the "EcoFINDERs" (EU-FP7-264465) consortium for providing the soil texture of our sampling sites. This publication is publication no. 7419 of the Netherlands Institute of Ecology (NIOO-KNAW).

Author contribution All authors have seen and approved the final version submitted.

Funding Open Access funding enabled and organized by Projekt DEAL. A. H. is financially supported by the BE-BASIC grant F03.001

(SURE/SUPPORT) and Deutsche Forschungsgemeinschaft (grant no. HO6234/1–2). K. B. was financially supported by a DFG PostDoc stipend BR 5535/1–1.

Declarations

Conflict of interest The authors declare no competing interests.

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