To what ~~extend~~extent can soil moisture and soil Cu contamination stresses affect ~~greenhouse gas~~nitrous species emissions? ~~An attempt to calibrate~~Estimation through calibration of a nitrification/denitrification model

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Abstract

Continental biogeochemical models are commonly used to ~~prospect~~predict the effect of land use, exogenous organic matter input or climate change on soil greenhouse gas emission. However, they ~~can still not~~cannot be used for this purpose to investigate the effect of soil contamination, while ~~it is known to affect~~contamination affects several soil processes and ~~to concern~~concerns a large fraction of land surface. For that, in this study we implemented a commonly used model estimating soil nitrogen (N) ~~emission~~emissions, the DeNitrification DeCompostion (DNDC) model, with a function taking into account soil copper (Cu) contamination in nitrate production ~~modulation~~control. Then, we aimed at using this model to predict ~~N~~ N_2O~~,~~ ~~-~~N-, NO_2 ~~NOx~~ N, NO-N and ~~N~~ NH_4-N emissions in the presence of contamination and in the context of changes in precipitations. ~~For that,~~Initial incubations of soils were performed at different soil moistures in order to mimic expected rainfall patterns during the next decades and in particular drought and excess of water. ~~The~~ Then, a bioassay was used in the absence or presence of Cu to assess the effect of ~~this~~the single (moisture) or double stress (moisture and Cu) on soil nitrate production ~~was studied using a bio-assay. Then, .~~ Data of nitrate production obtained through a gradient of Cu under each initial moisture ~~treatment~~incubation were used to parameterize the DNDC model and to estimate soil N emission considering the various ~~effect~~effects of Cu. Whatever the initial moisture ~~preincubation~~incubation, experimental results showed a ~~N~~ NO_3-N decreasing production when Cu was added but with different sharpness depending on soil moisture. The DNDC-Cu version we proposed was able to reproduce these observed Cu effects on soil nitrate concentration with r2>0.99 and RMSE<10% for all treatments in the DNDC-Cu calibration range (>40% of the water holding capacity) but showed poor performances for the dry treatments. We modelled a Cu-effect inducing an increase in ~~N~~ NH_4-N soil concentration and emissions due to a reduced nitrification activity, and therefore a decrease in ~~N~~ NO_3~~,~~ ~~-~~N-, N_2O-N and ~~N~~ NOx-N concentrations and emissions. The effect of added Cu predicted by the model was larger on ~~N~~ N_2-N and ~~N~~ N_2O-N emissions than on the other N species and larger for the soils incubated under constant than variable moisture. Our work shows that soil contamination can be considered in continental biogeochemical models to better predict soil greenhouse gas emissions.

Keywords: ~~copper~~bioassay, DNDC modelling, rainfall pattern, ecotoxicology, soil function

1. Introduction

The increase in atmospheric greenhouse gases [GHG] like CO_2, CH_4, or N_2O is expected to induce a global climate change with e.g. higher mean temperature or changes in ~~precipitation~~rainfall patterns with projections of increased precipitations or droughts depending on regions ~~(Knutti and Sedláček 2012).~~(Knutti and Sedláček, 2012). These modifications in rainfall patterns may impact soil moisture which is one of the main drivers of soil microbial activity ~~(Moyano et al. 2013).~~(Moyano et al., 2013; Schimel, 2018; Stark and Firestone, 1995). Microbial communities ensure key activities supporting numerous ecosystem functions, such as those involved in nitrogen (N) cycle influencing N_2O emissions ~~(Jones et al. 2014)~~(Butterbach-Bahl et al., 2013; Galloway et al., 2008) and are at the origin of more than 80% of N_2O fluxes ~~(IPCC 2019).~~(IPCC, 2019). In particular, nitrification/denitrification processes are largely controlled by the local (an-)oxic treatments and therefore by soil moisture~~,~~ (Borken and Matzner, 2009; Fierer et al., 2003; Guo et al., 2014; Schimel, 2018), denitrification being the main source of soil N_2O emission for moist soils whereas for dry ~~soil~~soils N_2O emissions are mainly due to nitrification ~~(Bateman and Baggs 2005). This~~ (Bateman and Baggs, 2005). N soil fluxes dynamics are thus particularly difficult to predict at a large scale because of this strong dependency to local soil O_2 availability ~~(Khalil et al. 2004), by playing on~~(Khalil et al., 2004) disrupting the realization of nitrification/denitrification reactions and N species diffusion ~~(Conrad 1996; Schurgers et al. 2006), makes N soil fluxes dynamics particularly difficult to predict at larger scales.~~(Conrad 1996; Schurgers et al. 2006). Despite this, some continental biogeochemical models have shown improved predictions when N cycle ~~is~~was explicitly represented ~~(Kesik et al. 2005; Butterbach-Bahl et al. 2009; Vuichard et al. 2018)~~(Butterbach-Bahl et al., 2009; Kesik et al., 2005; Vuichard et al., 2019).

In addition to climate change, human activities introduce significant quantities of contaminants into the environment, such as trace elements (TE) which are persistent and can be toxic for soil biota (Bech et al., 1997; Giller et al., 2009). Indeed, the contamination of soils by TE has become a major concern at global scale (De Vleeschouwer et al. 2007; Khan et al. 2008) coming from atmospheric sources (Steinnes et al., 1997) or through the use of pesticides (Nicholson et al., 2003). In particular, TE contaminations are known to largely affect soil microorganisms (Bååth, 1989; Giller et al., 2009) and their activities, such as nitrification/denitrification processes (Broos et al., 2007; Mertens et al., 2010). Therefore, the combined effect of climate change and of soil contamination may largely impact the emissions of NO_x and N_2O from soils (Holtan-Hartwig et al., 2002; Vásquez-Murrieta et al. 2006). However, the effect of the interactions between climate change and soil contamination on the GHG emissions is still poorly documented (Rillig et al., 2019; Zandalinas et al., 2021).

Despite recent progress, the Earth system models (ESMs) used to predict future climate change still don't take into account soil contamination effect on GHG emissions (Anav et al., 2013) whereas at a large spatial scale many soils are listed as contaminated (FAO, 2008; Lado et al., 2008). Furthermore, soil biogeochemical models are often used to estimate loss or accumulation of N species (ammoniac NH_4 volatilization, nitrate NO_3 leaching) (Giltrap et al., 2010) or they respective concentrations under scenarii of organic fertilizer amendments, but do not take into account the contamination which often occurs simultaneously (Wuana and Okieimen, 2011). Thus, there is a growing need to provide continental models combining ecotoxicological/contamination and climate change concerns. Among the biogeochemical models, DeNitrification DeCompostion (DNDC, Changsheng Li et al., 1992) is a relatively simple model handling both biogeochemistry of denitrification and microbial growth (Li et

al., 2000), and on which Land Surface Model-soil N component -a part of ESMs- like ORCHIDEE are built (Vuichard et al., 2019).

In order to improve model outputs, this study combines in an innovative way experimental and modelling approaches to evaluate the impact of soil moisture on the sensitivity of nitrification to copper (Cu) toxicity and consequently on GHG-N emissions. Cu was chosen as a model of soil contamination due to both its relevance in agricultural soils and available data in the literature (Broos et al., 2007; Mertens et al., 2010; Sauvé et al., 1999). It is not straightforward to assess distinct effects between punctual or chronic contamination on microbial structure or soil functions (Brandt et al., 2010; Oorts et al., 2006; Smolders et al., 2009). Here, we designed experiments to assess the conjugated effects of a trace metal contamination and a soil moisture stress on soil N cycle. Soil initial incubations were run during five weeks by applying a given soil moisture from drought to water saturation. Then, a bioassay with a gradient of Cu added by spiking was performed to estimate NO_3^- production. The experimental data were used to calibrate a new model, DNDC-Cu, able to predict NO_x and N_2O emissions with the implementation of new functions considering the effect of Cu concentration ([Cu]) on nitrification/denitrification processes. Our hypothesis is that the building of such a model allows a gain in the understanding of the effect of a soil [Cu] on NO_x and N_2O and NH_4 cycling in a climate change context. Hence, data are also used here to discuss knowledge gaps in such modelling approaches, and to question the matter of soil contamination data in climate change scenarii.

2. Materials and Methods

2.1 Soil sampling

The soil was sampled in January 2017 at the surface layer (0–20 cm) of a control plot at the Qualiagro experimental site (48°87'N, 1° 97'E - https://www6.inrae.fr/valor-pro_eng/Experimental-devices/QualiAgro/QualiAgro-web-site). The soil sample was immediately wet sieved at 5mm and shortly stored at 4°C until microcosm build-up. Aliquots of this sieved soil were used to measure the initial water content in addition to the maximum water holding capacity (WHC) for the further microcosm experiments. This site is located at Feucherolles near Paris, France, and had been designed to evaluate urban compost fertility together with the monitoring of contaminant inputs ~~(Cambier et al. 2019)~~(Cambier et al., 2019). Soil is a luvisol with 15% clay, 78% silt and 7% sand, a pH of 6.9, organic carbon (Corg) and total N contents at 10.5 ± 0.2 and 1.00 ± 0.03 g kg^{-1} soil, respectively, and with a CEC of 7.9 ± 0.8 $cmol^+$ kg^{-1} soil. This soil is not contaminated with Cu, and ~~basal~~geochemical [Cu] background measured by ICP-AES after HF-$HClO_4$ extraction was of 12 mg $Cu.kg^{-1}$ soil.

2.2 Experimental setup

In order to evaluate the impact of soil moisture on the sensitivity of nitrification to Cu toxicity, we carried out a two-step experiment. The first step consisted in initial incubations at 5 different WHC ~~incubation~~ during ~~45 days~~5 weeks, and the second ~~to~~step in a 3-day bioassay with spiked Cu gradient (Fig. 1).

For the 5 weeks' initial incubation, five microcosms were built up with about 5g of sampled soil. Three of them were set up with a constant moisture corresponding to 30%, 60% and 90% of their WHC in order to span

respectively limiting, optimal, and saturating conditions for the microbial activities. These three samples will be called thereafter "30%, 60% and 90%", respectively. Their water contents were verified by weighting every two days and water added if necessary. The two other microcosms were incubated in order to simulate two kinds of drought and Dry-~~rewetting~~Rewet cycles. One, thereafter called "Drought" (or DO), started with one week at 60% WHC and then the soil was left for 3 weeks without added water to mimic a dry period until 10% of the WHC before rewetting at the initial 60% WHC. The other, thereafter called "Dry-~~rewetting~~Rewet" (or DR) encountered ~~alternatives~~2 cycles of one-week ~~dry period (10% of the WHC) followed by one-week~~ near-saturation period (90% WHC~~). The moisture states of microcosms were~~) followed by one-week dry period (10% of the WHC) ending by one week near saturation period. Drying was performed by natural evaporation (gentle air-drying at the laboratory temperature, i.e. 20°C) and controlled by weighting.

~~At the end of the incubation period, we performed a nitrification bioassay in triplicate using incubated soils and following an adaptation of the method proposed by Petersen et al. (2012).~~At the end of the initial incubation period, we performed a nitrification bioassay using 3 replicates originating from soils and following an adaptation of the method proposed by Petersen et al. (2012). Bioassay consisted in nitrate production measurement over a short-term aerobic incubation in soil slurries (ratio soil:solution 1:10) with ammonium in excess and in the presence of gradients of Cu. Briefly, 3.5 g of fresh soil (approximately 3 g of soil equivalent dry weight), were mixed in a 50 mL Falcon® tubes with 29mL of a 10 mM HEPES buffer solution (hydroxyethyl piperazineethanesulfonic acid, Sigma-Aldrich, France) to maintain a constant pH under Cu spiking and nitrification activity, and containing the substrate $(NH_4)_2SO_4$ (3 mM) (Sigma-Aldrich, France). Soils were first spiked with a gradient of increasing Cu^{2+} in the presence of an excess of NH_4^+ and the resulting potential nitrification activity (PNA) measured. The microcosms incubated at constant moisture were kept at their moisture level (30, 60 or 90% of WHC) whereas those incubated at variable moisture were set at 60% WHC. The NO_3^- production rates were measured in soil slurries over a short-term aerobic incubation, for each Cu added concentration. Briefly, 1mL of Cu solution at different concentrations ~~were~~was added in soil slurries ~~(soil solution 1:12)~~ to reach ~~final~~ added [Cu] of 50, 100, 250, 500, 750,1000 and 2000 mg Cu.kg soil^{-1} (final soil [Cu] of 62, 112, 262, 512, 762, 1012 and 2012 mg Cu.kg soil^{-1}~~).~~ and control with 12 mgCu.kg soil^{-1}). The pH was adjusted to 7. Then, microcosms were incubated on a rotary shaker (150rpm) under aerobic conditions at 25°C until 72h. After 0, 24 and 72h of incubation, 2 ml aliquots of 3g were transferred in Eppendorf vials and centrifuged. The supernatants were collected and stored in microplates at – 20 °C until analyses of NO_3^- and NO_2^- by colorimetric determinations, following the reduction of NO_3^- in NO_2^- by vanadium(III) and then the detection of NO_2^- by the acidic Griess reaction ~~(Miranda et al. 2001). Finally, PNA (µg N-NO_3 g^{-1} soil h^{-1}) was calculated on the basis of N-NO_3^- + N-NO_2^-~~(Miranda et al., 2001). Finally, PNA (µg NO_3-N g^{-1} soil h^{-1}) was calculated on the basis of NO_3^--N + NO_2^--N concentrations measured at different time steps. In our bioassay, $[NO_2^-]$ were negligible and PNA was thus calculated following Eq. (1), by checking the linear production rate of NO_3^- between 2 h, 24 h and 72h:

$$(1)\ PNA = \frac{[NO_3^-]_{Tfinal} - [NO_3^-]_{Tinitial}}{Tfinal - Tinitial} = \frac{[NO_3^-]_{Tfinal} - [NO_3^-]_{Tinitial}}{Tfinal - Tinitial} \times Vs \div W$$

with *Vs*: Volume of solution

W: Weight of fresh soil ~~(approximately 5 g)~~

T: Time of incubation.

Cu in solution was measured by centrifugation of the soil+solution mixture of each bioassay, followed by a determination of Cu in solution by Flame Atomic Absorption Spectroscopy. Cu in solution values are provided in Suppl. Table 1.

2.3 Nitrification/denitrification model

~~Nitrification and denitrification processes are represented following the DNDC model proposed by Changsheng Li et al. (1992) and Li et al. (2000). In this study, we used a simplified version adapted by Zaehle and Friend (2010) initially calibrated for soil WHC >40%, that we intended here to test for 30% of WHC. This simplified version needs less boundary data but keeps a mechanistic description of the main processes. Modelled N species are expressed in amount of N, i.e. N-NH_4, N-NO_3, N-NOx and N-N_2O. To be able to represent both nitrification and denitrification processes occurring in aerobic and anaerobic sites, the soil is split into aerobic and anaerobic fractions based on an empirical relationship linking O_2 consumption to soil respiration. In aerobic microsites, nitrification takes places following Eq. (2):~~

~~(2) $Nitrification = f(SWC) \times$~~ Nitrification and denitrification processes are represented following the DNDC model proposed by Changsheng Li et al. (1992) and Li et al. (2000). In this study, we used a simplified version of DNDC adapted by Zaehle and Friend (2010) initially calibrated for soil WHC >40%, that we intended here to test for 30% of WHC. This simplified version needs less boundary data but keeps a mechanistic description of the main processes. Modelled N species are expressed in amount of N, i.e. NH_4-N, NO_3-N, NOx-N and N_2O-N. To be able to represent both nitrification and denitrification processes occurring in aerobic and anaerobic sites, the soil is split into aerobic and anaerobic fractions based on an empirical relationship linking O_2 consumption to soil respiration. In aerobic microsites, nitrification takes places following Eq. (2):

(2) $Nitrification = f(SWC) \times f(temp) \times f(pH) \times k_{Nit} \times (1 - anvf) \times NH_4$

with ~~N~~ NH_4-N being the stock of ammonium (in gN.m^{-2}), *(1-anvf)* the aerobic fraction of the soil described thereafter in Eq. (21), k_{Nit} the nitrification rate (day^{-1}), *f(SWC), f(temp)* and *f(pH)* three rate modifiers representing the effect of soil water content (m^3 m^{-3}), temperature (K) and pH as scalar respectively. They are described by the following Eq. (3), (4) and (5):

(3) $f(SWC) = 0.0243 + 0.9975 \times SWC + 5.6358 \times SWC^2 + 17.651 \times SWC^3 + 12.904 \times SWC^4$

~~(4)~~(4) $f(temp) = 0.0233 + 0.3094 \times temp + 0.2234 \times temp^2 + 0.1566 \times temp^3 + 0.0272 \times temp^4$

(5) $f(pH) = 1.2314 + 0.7347 \times pH + 0.0604 \times pH^2$

The ~~N~~ NH_4-N nitrified is transformed into ~~N~~ N_2O, N-, NO-N or ~~N~~ NO_3-N due to ~~bacterial~~microbial processes and chemonitrification following Eq. (6),(7) and (8):

(6) $Nitrification_{N2O} = ftv \times SWC \times k_{Nitri\ \ N2O} \times Nitrification$

(7) $Nitrification_{NO} = ftv \times SWC \times k_{Nit\ \ \ NO} \times Nitrification + 496950 \times e^{-1.62 \times pH} \times e^{-3149\ /(temp \times R)} \times Nitrification$

(8) $Nitrification_{NO3} = Nitrification - Nitrification_{NO} - Nitrification_{N2O}$

with $k_{Nitr\ \ \ NO}$ and $k_{Nitri\ \ N2O}$ two fixed rates (d^{-1}), *ftv* a rate modifier controlled by temperature and given in Eq. (9) and *R* the ideal gas constant.

(9) $ftv = 2.72^{\left(34.6 - \frac{9615}{temp}\right)}$

Then, the ~~N~~ NO_3-N produced during the nitrification process enters the denitrification module where it is reduced sequentially into ~~N~~ NOx, N-, N_2O-N or ~~N~~ N_2-N following Eq. (10) to (12):

(10) $Denitrification_{NOx} = anvf \times \left(\frac{\mu_{NO3}}{0.401} + 0.09 \times \frac{NO_3}{N_{tot}}\right) \times B$

(11) $Denitrification_{N2O} = anvf \times \left(\frac{\mu_{NOX}}{0.428} + 0.035 \times \frac{NO_X}{N_{tot}}\right) \times B$

(12) $Denitrification_{N2} = anvf \times \left(\frac{\mu_{N2O}}{0.151} + 0.079 \times \frac{N_2O}{N_{tot}}\right) \times B$

The anaerobic fraction *anvf* is described following Eq. (13):

(13) $anvf = 0.85 \times \left(1 - \frac{p_{soi\ O2}}{p_{air\ O2}}\right)$

with $p_{air\ O2}$, $p_{soil\ O2}$ being the partial pressure in the air and in the soil respectively. $p_{soil\ O2}$ is calculated following Eq. (14)

(14) $\frac{\partial p_{soil\ O2}}{\partial t} = p_{soil\ O2} - p_{O2resp} \times k \times SOC \times f_{Cu}$

with *SOC* being the soil organic carbon stock (gC m^{-2}), *k* the decomposition rate, p_{O2res} the O_2 partial pressure related to the respiration, and f_{Cu} the effect of Cu on CO_2 emissions as define in Eq.(15), following (Sereni et al., 2021 Eq. (5)):

(15) $f_{Cuco2} = \exp(-0.1 - 0.1 \times \log(Cu) + 0.12 \times pH)$

The relative growth rate of ~~N~~ NO_3, N-, NOx-N and ~~N~~ N_2O-N denitrifiers are described respectively by μ_{NO3}, ~~μ_{NOX}~~ μ_{NOX} , ~~μ_{N2O}~~ μ_{N2O} following Eq. (16), (17) and (18).

(16) $\mu_{NO} = \frac{0.67 \times f_{denit}(temp) \times f_{denit_{NO}3}(pH) \times NO_3}{NO_3 + 166}$

(17) $\mu_{NOX} = \frac{0.34 \times f_{denit}(temp) \times f_{denit_{NOX}}(pH) \times NO_X}{NO_X + 166}$

(18) $\mu_{N2O} = \frac{0.34 \times f_{denit}(temp) \times f_{denit_{N}20}(pH) \times N_2O}{N_2O + 166}$

with $f_{denit}(temp)$, $f_{denit_{NO}3}(pH)$, $f_{denit_{NOX}}(pH)$, $f_{denit_{N}2O}(pH)$ being rates modifiers depending on air temperature and soil pH described in Eq. (19) to (22).

(19) $f_{denit}(temp) = 2^{(temp-22.5)/10}$

(20) $f_{denit_{NO}3}(pH) = 1 - \frac{1}{1+e^{(4.25 \times pH)/0.5}}$

(21) $f_{denit_{NOX}}(pH) = 1 - \frac{1}{1+e^{(5.25 \times pH)}}$

(22) $f_{denit_{N}2O}(pH) = 1 - \frac{1}{1+e^{(6.25 \times pH)/1.5}}$

The denitrifier biomass dynamic B (kg m^{-2}) is described following Eq. (23).

(23) $\frac{\partial B}{\partial t} = (anvf \times (\mu_{NO} + \mu_{NOX} + \mu_{N2O}) - 3.82 \times 10^{-3}) \times B$

Finally, all the gaseous forms of mineral N are emitted into the atmosphere. It is important to note that we did not used directly the DNDC model but a simplified version adapted by Zaehle and Friend, (2010). The original code was in fortran and we translated it in R to facilitate its manipulation. The time step of the model was 30 minutes and most of the parameters were kept to the original values of Changsheng Li et al. (1992) and Li et al. (2000) except k_nit that was modified to 0.1743 instead of 0.2 to better fit the data from the control. Furthermore, the amounts of NH_4-N fixed to the clay were reduced to 0 as the bioassay was performed in excess of NH_4-N (see 2.2.0).

We used measures of N species at the end of initial incubation period as initial values of N species for DNDC (Table 1a and Fig. 2). To estimate the anaerobic volume fraction during the 3 days bioassay, we used a C mineralization rate *k* (Eq. 14) determined on the basis of measurements performed on the same soil (Annabi et al., 2007) and ran DNDC for a 45 days equilibrium period. We then extracted the initial anaerobic volume fraction and partial O_2 pressure.

2.4 Statistical analysis

The dose-response curves of PNA during the bioassay to Cu gradient were plotted and tested with linear, quadratic or cubic functions as fitting models. Our aim was to find, if possible, a similar modelling fit function for all moisture initial incubation treatments. Thus, for each moisture treatment, the two best functions of fit were selected through AIC and R^2 criteria, and compared with ANOVA. After selection of a common type of functions, the permutability of the different functions parameters was tested with the Chow test (gap v.1.2.2 package which tested the regression 1 on the basis of the samples 2 and vice-versa). If the p-value exceeds its critical values, regressions cannot be considered equal ~~(Zhao 2007)).~~(Zhao, 2007).

To estimate the effect of [Cu] and soil moisture on the different variables measured, we used nonparametric Kruskall-Wallis test. The fits between the model and the data of soil nitrate concentration during the bioassays were measured using root mean square error (RMSE, Eq. (24)):

(24) $RMSE = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(X_i - Y_i)^2}$

where i is the number of observations (1 to N), X is the predicted value and Y is the observed value. RMSE was decomposed in standard bias (Eq. (25)), non-unity slope (Eq. (26)) and lack of correlation (Eq. (27)) component following Gauch et al. (2003), with $\overline{X}$ and $\overline{Y}$ the mean modelled and observed values, b the slope of the least square regression of Y on X and r² the square of the correlation:

(25) : $SB = (\bar{X} - \overline{Y})^2$

(26) $NU = (1 - b)^2 \times \sum \frac{x_n^2}{N}$

(27) $LC = (1 - r^2) \times \sum \frac{y_n^2}{N}$

All ~~the~~ analysis were done with R 3.2.3 (R Core Team, 2015).

~~2~~3 Results

3.1. Effect of Cu on potential nitrification activity (PNA): statistical model selection

The soil N species measured at the end of the soil ~~pre-incubation~~initial incubations in each soil moisture treatment were used to initialise the DNDC model (Table 1). Two anomalous points leading to anomalous calculated ~~N-~~NO_2-N values were excluded from the experimental results because of technical problems during measurements (the C replicates in the DR and DO cases).

The bioassay experiments performed at the end of the soil ~~pre-incubation~~initial incubations allowed us to determine the rate of nitrate production as a function of soil [Cu] for each soil moisture (Fig. 1). In all cases, the PNA ~~was~~values were found to decrease with the increase in soil [Cu] but at different rates depending on the moisture treatment. Based on AIC values (suppl. Table ~~1) values,~~2), we first selected ~~the~~one model per moisture incubation that better fitted the data. For 30 and 60% of WHC, a quadratic model was found to provide the better compromise between the number of parameters and the prediction capacity ~~for incubation.~~. For 90% WHC, no significant difference was found between the cubic and the quadratic models (ANOVA, p.v=0.07). For DR, no significant difference was found between linear and quadratic models (supplementary Tables ~~1a~~2a and ~~1b~~2b) whereas for DO the cubic model provided a substantially better fit than the quadratic model (AIC and adj. R2 score, suppl. table ~~1~~2). Finally, we found that the quadratic model fitted correctly all the sets of data, allowing to be homogeneous across the ~~moisture's~~initial moisture incubation treatments (Fig. 2b). The quadratic function was thus chosen to quantify the Cu effect on PNA ~~also for~~including the DO treatment.

The parameters of the ~~5~~five quadratic functions (one for each moisture treatment) were found different from each other, except ~~between~~for 60 and 90% WHC (p.v=0.001, Chow test). A single function was thus used to adjust PNA to soil [Cu] curves at 60 and 90% WHC but with different intercepts for these two WHC treatments (suppl. Table ~~2~~3 and Fig. 2).

The final 4 quadratic equations ~~(~~are as follow: Eq. (28) for 30% WHC, Eq. (29) for 60 and 90% WHC, Eq. (30) for DR, and Eq. (31) for DO~~,~~. (Fig. 2).

(28) $F_{Cu30} = 0.782 - 0.000451 \times Cu + 9.49 \times 10^{-8} \times Cu^2$

(29) $F_{Cu60/90} = b - 0.000342 \times Cu + 4.30 \times 10^{-8} \times Cu^2$

with b= 0.795 for 60% WHC and b= 0.796for 90% WHC

(30) $F_{CuDR} = 0.552 - 0.000164 \times Cu + 6.09 \times 10^{-8} \times Cu^2$

(31) $F_{CuDO} = 0.625 - 0.000192 \times Cu + 2.82 \times 10^{-8} \times Cu^2$

~~)~~According to the fitted equations, the decrease in nitrate production rates as a function of soil [Cu] depended on initial incubation treatment. Decreases were found steeper following 30% WHC > 60-90% WHC > DO>DR.

These 4 equations were then added in the DNDC model, allowing to adjust the Eq. (2) which regulates the nitrate production to soil Cu contents~~:~~.

3.2. Modelling soil nitrate concentrations in Cu contaminated treatments using a DNDC-Cu model.

3.2.a. Set up of the DNDC-Cu model

The DNDC model was originally constructed to model both C and N soil cycles. The relative proportion of nitrification and denitrification processes thus depends on soil aerobic fraction determined both by soil C respiration and soil moisture (Eqs. (13) and (14)). Before any addition of Cu function in DNDC, we estimated this soil aerobic fraction~~, arising from~~ using C mineralisation ~~and the aerobic fraction. Therefore, we used~~ . Previous data from a 366 days incubations made on the same uncontaminated soil ~~(Annabi et al. 2007) to~~ (Annabi et al., 2007) were first used to fit a C mineralisation coefficient rate, k. The resulting k coefficient (k =1.234 ~~e~~.10^{-4} gC.m^{-2}.$30min^{-1}$) was introduced in the DNDC model and forced to equilibrium (45 days) without soil Cu contamination effect. This provided ~~us~~ a basal aerobic volume fraction for each soil moisture through Eq. (13~~)~~), corresponding to ~~0.00352~~3.52 .10^{-3} at 30%, ~~0.006167~~6.167.10^{-3} at 60% (and DR/DO to which bio assays were performed at 60% WHC) and ~~0.02705~~2.705 .10^{-2} at 90% of the WHC ~~and~~. The partial O_2 pressure ~~to~~was calculated as 211.4 hPa at 30% WHC, 210.7 hPa at 60% WHC, DR and DO and 205.4 hPa at 90% WHC. These values were ~~then~~ used to initiate ~~our~~the DNDC-Cu version. We then ran the DNDC-Cu version for a 3-day ~~modelisation~~simulation. The constant rate of C mineralisation, k, was adjusted to take into account ~~Cu through~~ the ~~eq 13 and~~Cu contents with the Eq ~~28-31 adjusted N-~~.(14) while the Eqs. (28)-(31) were used to adjust NO_3-N production rate (Fig. 1) to Cu.

3.2.b. DNDC-Cu model validation

~~A validation of~~ Our DNDC-Cu model ~~was made~~has been evaluated by comparing experimental data of soil nitrate concentration measured after 1 and 3 days of the bioassay incubation with the model outputs. A ~~better~~good fit was provided for 60 and 90% of WHC ~~which are~~ in the range of the DNDC calibration compared to 30 ~~%~~% WHC where the nitrate production is largely underestimated (more than twice after 3 days of incubation, Fig. 3a). The regression slopes between modelled and measured soil [nitrate] for 60 and 90% WHC were respectively 0.94 ±0.01 and 0.91±0.01 (R2=0.99 in both cases, Fig. 3a.) whereas for 30% WHC the regression slope was 1.21 ± 0.08 (R2=0.92) (Fig. 3a). For DR, the soil nitrate stocks were either overestimated (at 762 mgCu.kg soil^{-1} soil ~~and~~) or underestimated (at 2012 mgCu.kg soil^{-1} ~~(~~, Fig. 3b~~) (respectively 389.7 gN.m-2 and 310.5 gN.m^{-2} mean modeled nitrate against 375.3 and 290.6 gN.m^{-2} mean measured nitrate)~~ but overall modelling adequately fitted the data with a regression slope at 0.95 ± 0.02 and R2=0.99. For DO, the regression slope between modelled and measured soil nitrate stocks was 0.95± 0.02 too. The Supplementary Fig. 1 shows the improvement of the DNDC-Cu version to model NO_3-N soil concentration for contaminated soils with the differences between modelled and measured [NO_3-N] using the default DNDC version compared to our DNDC-Cu version for each [Cu].

Considering all ~~incubations~~the moisture treatments, RMSE was about 57.3 as a mean (46.4 ~~gN-NO3~~gNO_3-N.m^{-2} standard error) for a mean soil nitrate measured at 390 ~~gN-NO3~~gNO_3-N.m^{-2} (69 ~~gN-NO3~~gNO_3-N.m^{-2} standard error) after 3 days of incubation. However, for the 30% WHC, RMSE was 139.9 thus 3.7 times more than for the other treatments (Suppl. Fig. ~~1~~2). Despite the reduction ~~of~~in nitrate production rate from 0.~~200~~020 to 0.~~175~~318 gN.hour^{-1} (see material and methods), soil nitrate stock was still slightly overestimated in the 90% WHC as shown by the largest lack of correlation in this case compared to the 60% WHC treatment (Fig. 3a, Suppl. Fig. ~~1~~2). Lack of correlation was ~~small~~reduced for all tested moisture ~~incubation~~ treatments (mean $\sqrt{LC}$ = 23.0, standard error =5.4 which is roughly 1/20 of the produced nitrate in 3 days in uncontaminated treatment). Results showed that our DNDC-Cu version was able to reproduce the variability observed in Cu contaminated soils except for the 30% WHC treatment where soil nitrate stocks were largely underestimated. The following results thus focused on the use of DNDC-Cu for DR, DO, 60 and 90% of WHC treatments to predict soil N emissions.

3.3 Use of DNDC-Cu to predict N fluxes in contaminated soils.

3.3.a. Effect of soil [Cu] on soil N stocks.

The soil Cu function we included in the DNDC-Cu model modified specifically the default nitrification equation in complement to pH, soil moisture and O_2 availability (Eq (2.)). In the presence of low [Cu] (12-512 mgCu.kg soil^{-1}), the predicted ~~N-~~NO_3-N soil stocks were found equivalent between 60% WHC and DO and, to a less ~~extend~~extent, DR treatments (Suppl. Fig. ~~2~~3). When soil [Cu] increased, soil [~~N-~~NO_3-N] decreased but with different rates depending on the moisture of initial incubations (~~eq~~Eqs. 28-31). The evolutions of concentrations in soils and emissions fluxes of each species in response to [Cu] gradient were also found highly different depending on the species and on the moisture of initial incubations. However, the relative evolution in term of both soils concentration and emissions fluxes were identical for each species and each initial incubation treatment

and are represented in table 2. Largest variations were modelled for N_2O-N decrease (around -63% for the constant moisture treatments and -54% for the DR at 2012 mgCu.kg soil^{-1}) while smallest variations were modelled for NH_4-N increase (8-10% for the 60 and 90% WHC against 5-7% for the DR and DO initially incubated soils at 2012 mgCu.kg soil^{-1}). Due to the different evolutions with Cu gradient, concentrations or intensities of fluxes for a given specie may reversed between two moisture treatment with an increase in soil [Cu].

For instance, up to 548 mgCu.kg soil^{-1}, we modelled the lowest ~~N-~~NO_3-N stocks in DR incubated soils. Above it, ~~N-~~NO_3-N soil stocks were the smallest for the 60% WHC treatment as a result of the sharpest decrease in ~~N-~~NO_3-N production due to soil [Cu]. ~~N-~~NO_3-N soil stock for initial incubation at 90% WHC were the highest for soil [Cu] below 1432 mgCu.kg soil^{-1}. Between 1432 and 2000 mgCu.kg soil^{-1}, ~~N-~~NO_3-N soil stocks were similar for 90% WHC, DR and DO (Suppl. Fig. ~~2~~3).

~~The decrease in the nitrification rates with~~In the absence of Cu, NO_3-N/NH_4-N ratios were similar among soil moisture treatments. However, the variations in NH_4-N and NO_3-N stocks in response to Cu gradient were different across soil moistures. Indeed, the increase in soil [Cu] resulted in a decrease in nitrification rate, thus in an increase in soil ~~N-NH$_4$ stocks. Our model also predicted largest N-NH$_4$~~NH_4-N stocks ~~in the DR and DO soils than in the 60 and 90% WHC soils~~ (Suppl. Fig.~~3~~ 4). The ~~variations in~~ NO_3-N-/NH_4~~ and -N-NO$_3$~~ stocks ~~were however different across soil moistures so that at the highest [Cu], the N-NH$_4$ soil stocks were modelled similar between all moisture treatments whereas the N-NO$_3$ stocks were modelled smallest for the 60% WHC treatment. The N-NO$_3$/ N-NH$_4$ stocks ratios thus varied between soil moistures with Cu levels whereas in the absence of Cu, N-NO$_3$/ N-NH$_4$ ratios were similar among soil moisture treatments. The ratio of N-NO$_3$/ N-NH$_4$~~ ratios decreased faster for 60~~/~~-90% WHC than for DR~~/~~ and DO with an increase in soil [Cu] (suppl Fig. ~~4~~5; Table 2). .

~~With~~ The decrease in soil ~~N-~~NO_3-N stocks at high [Cu], ~~we predicted~~induced a decrease in the modelled growth of denitrifying bacteria that is directly related to [NO_3-N] (Eq. (13)). Consequently, the modelled denitrifying bacterial pool was reduced when soil [Cu] increases (Fig. 4). Whatever the soil [Cu], denitrification was ~~however~~ modelled roughly twice larger in the soils incubated at 90% WHC than in the other treatment as this moist treatment is defined as perfect condition for denitrifying bacteria in the DNDC model ~~(Changsheng Li et al. 1992). Soils incubated at 60% WHC were modeled~~(Changsheng Li et al., 1992). Soils incubated at 60% WHC were modelled with the lowest denitrifying bacterial pool. No difference between the DR and DO soils was found due to uncertainties in the modelled denitrifying bacterial pool which resulted from the different concentrations in N species used to ~~initialize~~initialise DNDC-Cu (Table 1). The soil ~~N-~~N_2O-N stocks and dissolved ~~N-~~NOx-N being directly related to denitrifying bacteria ~~and its~~, they followed similar trends than soil ~~N-~~NO_3-N stocks with a global decrease in soil stocks with an increase in soil [Cu] (table 2) and larger stocks at the wetter treatment.

3.3.b. Estimation of soil N emissions under various moistures

Large differences are predicted in the ~~N-~~NH_4~~, -~~N-~~,~~ NOx-N and ~~N-~~N_2O-N fluxes between the 90% WHC soil and the 3 other soil moisture treatments (Fig. 5). ~~For instance, we modelled a decrease comparable in N-NOx emissions between DR/DO and 60-90% WHC for soil [Cu] about 112 mgCu.kg soil^{-1} (2-3% respectively - Table 2a and 2b.) but with~~ Due to the ~~increase in soil [Cu], the variation~~different evolutions of ~~emissions between soil moisture became larger. Hence, around~~

~~2012 mgCu.kg soil^{-1} we modelled more than 50% decrease in N-NOx and 62% decrease in N-N_2O emission~~ fluxes ~~for soils at 60% WHC against only 40% decrease in N-NOx and 54% in N-N_2O emission for soils previously exposed to DR (Tables 2a and b.). Thus, intensities of fluxes between two moisture treatments reversed with an increase in soil Cu contamination.~~

~~The smallest~~ in response to Cu, NH_4-N fluxes were ~~predicted~~ modelled smallest for the DR soils than for the ~~wetter treatment despite higher modelled N-N_2O stocks at 90% WHC whatever [Cu]. N-NH_4 fluxes were modelled higher for the DR soils than in the~~ 60% WHC incubated for soil Cu ~~higher than~~below 1774 mgCu.kg soil^{-1} and ~~smallest below.~~higher above 1774 mgCu.kg soil^{-1}(Fig .5(a)). The emissions of ~~N-~~NH_4-N in the DO treatment were predicted to be higher than those of the DR treatment for soil Cu higher than 1290 mgCu.kg soil^{-1} and smallest below 1290 mgCu.kg^{-1} (Fig. 5a). In the studied range of added Cu, ~~N-~~NOx-N fluxes predicted by the model are largest from 60% WHC to DO, DR and 90% WHC (Fig. 5(b.)) for a moderate Cu input (~ below 1380 mgCu.kg soil^{-1}). The decrease in ~~N-~~NOx-N emission with the increase in soil [Cu] was however steeper for soils incubated at 60% WHC~~.~~ (Tables 2 (a.) and 2(b.)). Hence, at 2012 mgCu.kg soil^{-1} ~~N-~~NOx-N fluxes in soil incubated at 60% WHC were similar to those in the soils incubated under drought treatment (Fig. 5 (b.)). ~~N-~~The smallest fluxes of N_2O-N were predicted for the wetter treatment despite higher modelled N_2O-N stocks at 90% WHC whatever the [Cu] (Table 2 (a.) and Fig 5 (c.)). The N_2O-N emissions fluxes in the presence of Cu were predicted to be 4 times smallest in the 90% WHC treatment compared to the others. N_2O-N fluxes had similar trends than ~~N-~~NOx-N for moderate Cu inputs but fluxes were still largest from 60% WHC to DO, DR and 90% WHC (Fig. 5 (c~~.))~~).

~~The N-N_2O emissions fluxes in the presence of Cu were predicted to be 4 times smallest in the 90%WHC treatment compared to the others, whereas N-.)~~), and N_2-N emissions were larger at ~~this~~the wettest treatment (Fig. 5 (d.)). The ratio of emitted ~~N-~~N_2O-N per denitrification products (i.e.~~g~~ N_2O-N~~-~~ / N_2O~~/ -~~N~~-N_2O+ N-~~ + N_2-N) was hence smallest in the moistest soils, which means that the largest soils ~~N-~~N_2O-N stocks in the case of 90% WHC had more chance to be transformed rather than emitted (Fig. 6).

4 DISCUSSION

4.1. From laboratory experiment to soil N emission modelling

Thanks to our laboratory experiments, we were able to define a function modulating the soil NO_3-N production rates in relation with soil [Cu] and depending on soil moisture. Our results showed that soil nitrate decreases with an increase in soil [Cu]. Initial incubation treatment significantly affects the response of soil nitrate production rate to subsequent Cu stress with steeper decrease in the order 30% WHC > 60-90% WHC > DO > DR for the Cu range studied. The lowest sensitivity of Cu in soils initially incubated with dry-rewet events suggest that it might have selected more resistant communities (Barnard et al., 2013; Gleeson et al., 2008). More complex dose response functions have been used in (Sereni et al., 2022) to assess thresholds and loss of functions after such a double stress. These results are in relatively good agreement with those presented here using the quadratic fit, especially for the highest half of [Cu]. However, they also presented a limited increase in nitrification rate for small Cu input that we weren't able to emphasize in the present study. In the present article we used simple functions of fit to describe the response of soil nitrate production to Cu gradient after the first moisture stress as they further have to

be included in the DNDC model. After implementing these quadratics Cu modulating functions into the DNDC-Cu model, we were however able to reproduce the observed soil nitrate stock particularly for the soils incubated at 60 and 90% of WHC. The variability around the mean due to the Cu effect was also reproduced by our DNDC-Cu version at 30% of WHC despite strong underestimation of mean soil nitrate stocks due to model moisture-limit (Changsheng Li et al., 1992). In the case of the DR and DO incubated soils, the so-called "Cu function" also accounted for the effect of drought stress. In fact, our Cu functions were defined on the basis of soil nitrate production against the whole gradient of Cu thus also considering the control without Cu. ~~N-NO_3 production rates in relation with soil [Cu] and depending on the soil moisture. Our results showed that soil nitrate decreases with an increase in soil [Cu].~~However, the double stress effect was also well reproduced in nitrate production.

4.2. Expected ecological implications of soil Cu contamination

Based on nitrate production measurements, we modelled a decrease in denitrifying activities with an increase in soil [Cu] as a consequence of the decrease in soil nitrate stocks. However, the experiments performed here did not allow us to determine if the soil Cu contamination rather affects nitrifying bacteria (e.g. decrease in nitrifying activity and in NO_3-N production) or denitrifying bacteria (e.g. increase in denitrifying activities and NO_3-N consumption). The effect of soil contamination on N_2O-N production is debated because i) microbial species involved are not clearly identified (Wrage-Mönnig et al., 2018), ii) species richness is not necessary related to soil functions (Ruyters et al., 2013) and iii) denitrifying communities could be differently sensitive than the nitrifying to soil contamination (Hund-Rinke and Simon, 2008; Vásquez-Murrieta et al., 2006). Also, our approach to model N_2O-N, N_2-N and NOx-N production in the contaminated context could have been more constrained with measurement of denitrification rate to assess the effect of Cu on proportion of production and consumption of NO_3-N.

Based on our simulations, the soil Cu contamination was expected to substantially modify the proportion of available N in soils with the increase in NH_4-N stock at the expense of NO_3-N. NH_4-N accumulation and the large expected decrease in NO_3-N /NH_4-N ratio in contaminated soils (around 50% for the 60% WHC) may lead to shift in plant community structures with different preferences in N assimilation (Cui and Song, 2007; Peacock et al., 2001). Therefore, Cu stress could not only have implications in microbial community patterns as a stressor, but could also induce further shifts due to N species redistributions in soils.

~~After implementing these Cu modulating functions into the DNDC-Cu model, we were able to reproduce the observed soil nitrate stock particularly for the soils incubated at 60 and 90% of WHC. The variability around the mean due to the Cu effect was also reproduced by our DNDC-Cu version at 30% of WHC despite strong underestimation of mean soil nitrate stocks due to model moisture-limit (Changsheng Li et al. 1992). In the case of the DR and DO incubated soils, the so-called "Cu function" also accounted for the effect of drought stress. In fact, our Cu functions were defined on the basis of soil nitrate production against the whole gradient of Cu thus also considering the control without Cu. However, the (double) stress effect was also well reproduced in nitrate production.~~

4.4.4.3. From ~~N-~~N_2O-N, N-~~N_2~~ $_2$-N and ~~N-~~NOx-N soil stocks to emissions

~~In the present study, we predicted highest soil N-N_2, N-N_2O and N-NOx stocks in the moistest treatments as they are produced by the denitrifying bacteria expected to behave optimally at 90% WHC or after DR cycles (Changsheng Li et al. 1992; Homyak et al. 2017). However, N-N_2O and N-NOx emissions were modelled higher in the driest soils, whereas numerous studies (Dobbie and Smith 2003; Xiong et al. 2007; Manzoni et al. 2012) reported high measured N-N_2O emissions with high moisture. In the present version of DNDC-Cu, the soil N emissions were directly controlled by their diffusion in soil, calculated on the basis of clay and soil moisture content. The diffusion of each species would hence be 11 times smaller under 90% WHC (D_s= 0.00357) than under the 60% WHC treatment (D_s=0.0306) because the model described the diffusion as a whole and do not separated pores with or without water. Diffusion was hence slower in the water than in the air. Thus, the weighted mean diffusion was lower in the high moisture treatment. Without Cu soil nitrous stocks being roughly 1.6 times and soils N-N_2 stocks 11.1 larger under 90% WHC treatment than the other, the emission of N-N_2O were larger under driest treatment even if stocks were smaller.~~

~~Several studies also reported flushing event with dry rewetting cycles which would enhance C mineralisation, known as the Birch effect (Birch 1958; Göransson et al. 2013), hence reducing soil O_2 concentration. Moreover, soil [O_2] is closely related to the pore size distribution, being of major importance in nitrification/denitrification control (Khalil et al. 2004) with a dominating nitrification for aggregates up to 0.25cm (Kremen et al. 2005). Pore size distribution under dry/rewet events is controlled by cracking, (des)aggregation (Denef et al. 2001; Cosentino et al. 2006) or gas displacement (Kemper et al. 1985) that we weren't able to take into account in the present study. In DNDC, the calculation of denitrification rate and diffusion was based on a rough description of anaerobic zone with approximation of soil pore space distribution (Li et al. 2000; Blagodatsky et al. 2011). The soil pore space distribution approach has been demonstrated to be more generally applicable (Arah and Vinten 1995; Schurgers et al. 2006) whereas soil aggregates have been shown to control the extend of nitrification and denitrification (Kremen et al. 2005; Schlüter et al. 2018). However, if models have been proposed to take O_2 availability at the aggregate size into account in the nitrous oxide production (Leffelaar 1988; Kremen et al. 2005), they also point out the difficulty in parametrization which need a large panel of soil measurements. Moreover, they are rarely transposable at the meso and regional scale due to high spatial variations in soil structure (Butterbach-Bahl et al. 2013). The DNDC-Cu version we used here particularly pointed out the difficulty in dealing on biogeochemistry model with physical processes, with large discrepancies between modelled soils stocks and emissions. The validation we performed focused on soil nitrates stocks and a second step to go further on would be the measure of gaseous species to ensure that emissions were also impacted by soils treatment. Moreover, we assumed here that soil [Cu] affected the C mineralisation with a decrease in soil O_2 production leading to an increase in denitrification and N-N_2O, N-NOx. Nevertheless, the present DNDC-Cu version didn't take into account the retroaction between C and N cycles. Further research would thus be required to include Cu contamination into C and N interacting cycles.~~

~~4.5.4.1. Expected ecological implications of soil Cu contamination~~

~~Based on nitrate production measurements, we modeled a decrease in denitrifying activities with an increase in soil [Cu] as a consequence of the decrease in soil nitrate stocks. However, the experiments performed here did not allow us to determine if the soil Cu contamination rather affects nitrifying bacteria (e.g. decrease in nitrifying activity and in N-NO_3 production) or denitrifying bacteria (e.g. increase in denitrifying activities and N-NO_3 consumption). The effect of soil contamination on N-N_2O production is debated because i) species involved are not clearly identified (Wrage Mönnig et al. 2018), ii) species richness is not necessary related to soil functions (Ruyters et al. 2013) and iii) denitrifying communities could be differently sensitive than the nitrifying to soil contamination (Vásquez-Murrieta et al. 2006; Hund-Rinke and Simon 2008). Also, our modeling approach of N-N_2O, N-N_2 and N-NOx production in the contaminated context could have been more constrained with measurement of denitrification rate to assess the effect of Cu on proportion of production and consumption of N-NO_3~~

~~Based on our simulations, the soil Cu contamination wass expected to substantially modify the proportion of available N in soils with the increase in N-NH_4 stock at the expense of N-NO_3. N-NH_4 accumulation and the large expected decrease in N-NO_3/N-NH_4 ratio in contaminated soils (around 50% for the 60% WHC) may lead to shift in plant community structures with different preferences in N assimilation (Peacock et al. 2001; Cui and Song 2007). Therefore, Cu stress could not only have implications in microbial community patterns as a stressor, but could also induce further shifts due to N species redistributions in soils.~~

In the present study, we predicted highest soil N_2-N, N_2O-N and NOx-N stocks in the moistest treatments. Indeed this species are produced by the denitrifying bacteria expected to behave optimally at 90% WHC or after DR cycles (Changsheng Li et al., 1992; Homyak et al., 2017). However, N_2O-N and NOx-N emissions were modelled higher in the driest soils, whereas numerous studies (Dobbie and Smith 2003; Xiong et al. 2007; Manzoni et al. 2012) reported high measured N_2O-N emissions with high moisture. In the present version of DNDC-Cu, the soil N emissions were directly controlled by their diffusion in soil, calculated on the basis of clay and soil moisture content. The diffusion of each species would hence be 11 times smaller under 90% WHC (D_s= 0.00357) than under the 60% WHC treatment (D_s=0.0306) because the model described the diffusion as a whole and do not separated pores with or without water. Diffusion was hence slower in the water than in the air. Thus, the weighted mean diffusion was lower in the high moisture treatment. Without Cu soil nitrous stocks being roughly 1.6 times and soils N_2-N stocks 11.1 larger under 90% WHC treatment than the other, the emission of N_2O-N were larger under driest treatment even if stocks were smaller.

Several studies also reported flushing event with Dry-Rewet cycles which would enhance C mineralization, known as the Birch effect (Birch, 1958; Göransson et al., 2013), hence reducing soil O_2 concentration. Moreover, soil [O_2] is closely related to the pore size distribution, being of major importance in nitrification/denitrification control (Khalil et al., 2004) with a dominating nitrification for aggregates up to 0.25cm (Kremen et al., 2005). Pore size distribution under dry/rewet events is controlled by cracking, (des)aggregation (Cosentino et al., 2006; Denef et al., 2001) or gas displacement (Kemper et al., 1985) that we weren't able to take into account in the present study. In DNDC, the calculation of denitrification rate and diffusion was based on a rough description of anaerobic

zone with approximation of soil pore space distribution (Blagodatsky et al., 2011; Li et al., 2000). The soil pore space distribution approach has been demonstrated to be more generally applicable (Arah and Vinten 1995; Schurgers et al. 2006) whereas soil aggregates have been shown to control the extend of nitrification and denitrification (Kremen et al., 2005; Schlüter et al., 2018). However, if models have been proposed to take O_2 availability at the aggregate size into account in the nitrous oxide production (Kremen et al., 2005; Leffelaar, 1988), they also point out the difficulty in parametrization which need a large panel of soil measurements. Moreover, they are rarely transposable at the meso-and regional scale due to high spatial variations in soil structure (Butterbach-Bahl et al., 2013). The DNDC-Cu version we used here particularly pointed out the difficulty in dealing on biogeochemistry model with physical processes, with large discrepancies between modelled soils stocks and emissions. The validation we performed focused on soil nitrates stocks and a second step to go further on would be the measure of gaseous species to ensure that emissions were also impacted by soils treatment. Moreover, we assumed here that soil [Cu] affected the C mineralisation with a decrease in soil O_2 production leading to an increase in denitrification and N_2O-N, NOx-N. Nevertheless, the present DNDC-Cu version didn't take into account the retroaction between C and N cycles. Further research would thus be required to include Cu contamination into C and N interacting cycles.

4.6.4.4. Climate change could substantially modify contaminated soil N emission

~~It is well known that climate change and rainfall patterns could substantially modify the soil N balance and its GHG emissions (Galloway et al. 2003, 2008; Butterbach-Bahl et al. 2013). Our results showed that increased Cu contamination as well might affect soil N emissions with smallest emissions of N-NOx and N-N_2O. These two gases are of major importance in GHG mitigation with a warming potential per mass 300 and 40 times greater than CO_2, respectively. Agricultural soils being the dominating source of N-N_2O (Beauchamp 1997; Signor and Cerri 2013), even a limited decrease in their emissions could have major implication for climate. Based on our modelling, the join effect of soil moisture and [Cu] wass particularly important with larger differences in N-N_2O and N-NOx emissions between rainfall patterns at high [Cu] (3.3.b.). We (Sereni et al. in press) previously/also showed that soil Cu contamination differently affect soils nitrification depending of primary soil moisture stress. Here we showed that the N-N_2O and N-NOx emission variations are significantly more sensitive to the combined effect of Cu and precipitation regime than the nitrate stock. Based on these results, soil Cu inputs on moistest soils would lead to a largest decrease in soil N-N_2O and N-NOx emission compared to that on driest soils, and even more than on soils submitted to abrupt and intense shifts in rainfall patterns as the DR and DO soils.~~

~~5. CONCLUSION~~

It is well known that climate change and rainfall patterns could substantially modify the soil N balance and its GHG emissions (Galloway et al. 2003, 2008; Butterbach-Bahl et al. 2013). Despite limitation in DNDC accuracy for nitrous emissions (Foltz et al., 2019), our results tend to showed that increased Cu contamination as well might affect soil N emissions with smallest emissions of NOx-N and N_2O-N. These two gases are of major importance in GHG mitigation with a warming potential per mass 300 and 40 times greater than CO_2, respectively.

Agricultural soils being the dominating source of N_2O-N (Beauchamp, 1997; Signor and Cerri, 2013), even a limited decrease in their emissions could have major implication for climate. Based on our modelling, the join effect of soil moisture and [Cu] was particularly important with larger differences in N_2O-N and NOx-N emissions between rainfall patterns at high [Cu] (3.3.b.). Sereni et al., (2022) also showed that soil Cu contamination differently affect soils nitrification depending of primary soil moisture stress. Here we showed that the N_2O-N and NOx-N emission variations are significantly more sensitive to the combined effect of Cu and precipitation regime than the nitrate stock. Based on these results, soil Cu inputs on moistest soils would lead to a largest decrease in soil N_2O-N and NOx-N emission compared to that on driest soils, and even more than on soils submitted to abrupt and intense shifts in rainfall patterns as the DR and DO soils.

5. CONCLUSION

In the present study, we aimed at combining ecotoxicological experiments and biogeochemical modelling focusing on the effect of soil Cu contamination on soil N emission under different soil moisture treatments~~.~~ constant moisture (30, 60 or 90% WHC) or to a single long drought period (DO) or several Dry-Rewet cycle (DR). Based on a 3-day bioassay measuring soil ~~N-~~NO_3-N over time, we were able to adjust the DNDC model to take into account the Cu effect on soil N emission. ~~Experiments were performed under different moisture treatments to create a DNDC-Cu model taking account the effect of an exposure to a constant moisture (30, 60 or 90%WHC) or to a single long drought period (DO) or several dry rewetting cycle (DR).~~ The DNDC-Cu version we proposed was able to reproduce the observed Cu effect on soil nitrate stock with R2>0.99 and RMSE<10% for all treatments in the DNDC calibration range (>40% WHC).

We modelled a Cu effect inducing a decrease in denitrifying bacterial pool leading to an increase in ~~N-~~NH_4-N soil stocks at the expense of ~~N-~~NO_3~~,~~ ~~-~~N-, N_2O-N and ~~N-~~NOx-N stocks. ~~Emissions of N-NH_4 were expected to slightly increase with soil Cu contamination whereas those of N-N_2O and N-NOx are expected to decrease. We also~~We showed that the effect of soil Cu contamination was different among moisture treatment and N species. ~~In fact~~For instance, we modelled that the largest [Cu] (2012mg Cu.kg soil^{-1}) provoked a decrease in soil nitrate stocks from -28% in the DR case to -44% in the 60% WHC whereas ~~N-~~N_2O ~~emission~~ N emissions were expected to decrease up to 63% in the 90% WHC (-62% in the 60% WHC case, -54% in the DO case). However, our results tended to show that the amount of ~~N-~~N_2O-N emitted from denitrification would decrease with an increase in soil [Cu] and from 60% WHC to DR, DO and 90% WHC, so that less ~~N-~~N_2O-N produced would be converted to ~~N-~~N_2-N. This result points out two main difficulties in biogeochemical modelling: i) the difficulty to take into account hydrological dynamics (produced ~~N-~~NO_3-N and ~~N-~~NH_4-N could be expected to leach) and soil structures at different spatial scale (denitrification is estimated based on rough estimation on anaerobic soil volume which also controlled emissions rates through diffusion processes) and ii) linking soil function to microbial dynamics, in particular in this case were only the ~~N-~~NO_3-N stock was measured (without dealing between production and consumption for instance). Despite these two main points of uncertainty, the combination of incubations and of modelisation we conducted here emphasize the need to account for soil contamination when dealing with soil GHG emission modelling and climate change, as both contamination and rainfall patterns affect in a different way the soil ~~N-~~NOx-N and ~~N-~~N_2O-N emissions.

Data availability: Data are available at: ~~https://doi.org/10.15454/ZUKN90~~https://doi.org/10.15454/ZUKN90

Authors Contributions: the authors contributed as follows:

Laura Sereni: Methodology, formal analysis, data processing, writing original draft.

Bertrand Guenet: Methodology, conceptualization, writing review and editing, supervision

Charlotte Blasi: Experimentations and draft initialization

Olivier Crouzet: Methodology, conceptualization, writing review and editing, supervision

Jean-Christophe Lata: writing review and editing, supervision

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Table 1: N species measured in the soils at the end of pre-initial incubation period further used to initialise the DNDC model, mean modelled N-NO_3-N stocks and mean emissions of N-NH_4-N, N_2-N, N_2O-N, NOx-N modelled without Cu. 90 = 90% WHC; 60= 60% WHC; DO =Dry-Only; DR =Dry Rewet conditiontreatment during pre-initial incubation. A, B and C are replicates.

	Measured (µg.g soil^{-1})			Modelled (gN.m^{-2}h^{-1} for emissions, gN.m^{-2} for stocks)					
Ech	N-NH_4-N	N-NO_2-N	N-NO_3-N	N-NH_4-N emissions	N-N_2-N emissions	N-N_2O-N emissions	N-NOx-N emissions	N-NO_3-N stocks	N-NO_3/-N-/NH_4-N stocks
30_A	4.3	0.1	15.3	NA	NA	NA	NA	NA	NA
30_B	4.0	0.2	14.4						
30_C	4.5	0.2	14.3						
60_A	6.9	0.1	18.8	2.28.10^{-10}	2.26 .10^{-7}	1.3 .10^{-4}	1.3. .10^{-3}	456.3	0.21
60_B	6.9	0.2	18.8						
60_C	6.7	0.2	18.7						
90_A	8.2	0.2	23.6	2.64. .10^{-11}	6.21 .10^{-7}	2.7 .10^{-5}	2.7 .10^{-4}	509.8	0.24
90_B	12.6	0.9	24.0						
90_C	8.8	0.2	24.2						
DO_A	5.4	0.2	26.1	2.35 .10^{-10}	4.3 .10^{-7}	1.1 .10^{-4}	1.1 .10^{-3}	432.0	0.19
DO_B	5.9	0.3	29.8						
DO_C	7.4	0.9	26.4						
DR_A	3.7	0.2	28.4	2.36.10^{-10}	3.72 .10^{-7}	9.4 .10^{-5}	1.1 .10^{-3}	454.5	0.21
DR_B	3.4	0.2	29.8						
DR_C	5.0	0.3	29.9						

Table 2: Percentage of variation in soil N-NO_3-N stocks, soil N-NO_3/-N-/NH_4-N stocks, N-NH_4-N, N_2-N, NOx-N and N-N_2O-N emissions in response to soil [Cu] in the various initial incubation conditionstreatments for a 3-day modelisation.

a.

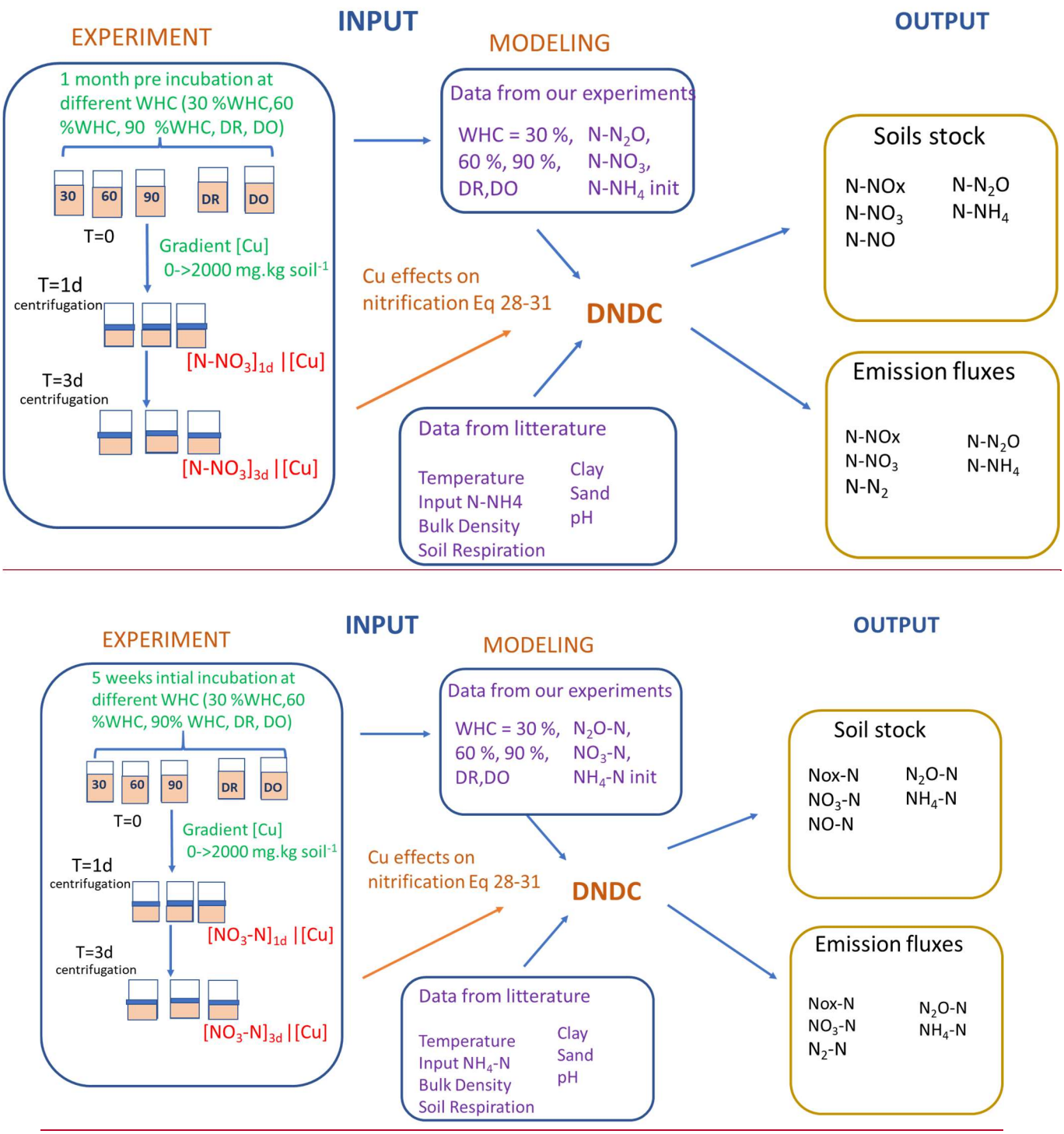
Moisture conditiontreatment	Added Cu (mgCu.kg soil^{-1})	N-NO_3-N soils stocks	Emission N-NH_4-N	Emission N-N_2-N	Emission N-NOx-N	Emission N-N_2O-N	Soil stocks N-NO_3/-N-/NH_4-N
60	0	0.0	0.0	0.0	0.0	0.0	0.0
60	50	-1.3	0.3	-17.9	-3.5	-2.1	-1.5

60	100	-2.6	0.6	-24.4	-5.5	-4.1	-3.2
60	250	-6.7	1.5	-35.0	-10.5	-9.8	-8.0
60	500	-13.3	2.9	-45.6	-17.8	-19.0	-15.7
60	750	-19.5	4.3	-53.4	-24.5	-27.7	-22.8
60	1000	-25.4	5.5	-59.8	-30.6	-35.8	-29.3
60	2000	-44.5	9.7	-78.0	-50.5	-62.3	-49.4
90	0	0.0	0.0	0.0	0.0	0.0	0.0
90	50	-1.0	0.3	-16.4	-6.7	-3.1	-1.2
90	100	-2.2	0.6	-22.4	-9.4	-5.3	-2.7
90	250	-6.0	1.5	-32.3	-14.5	-11.1	-7.3
90	500	-12.1	3.0	-42.7	-20.8	-20.1	-14.7
90	750	-18.0	4.5	-50.7	-26.1	-28.4	-21.5
90	1000	-23.6	5.8	-57.4	-30.8	-36.2	-27.8
90	2000	-41.8	10.3	-76.4	-46.0	-61.6	-47.2

b.

Moisture ~~conditiont~~ reatment	Added Cu (mgCu.kg soil^{-1})	NO_3 ~~soil~~-N soils stocks	Emission NH_4-N	Emission N_2-N	Emission NOx-N	Emission N_2O-N	Soil stocks NO_3-N /NH_4-N
DO	0	0.0	0.0	0.0	0.0	0.0	0.0
DO	50	-0.7	0.2	-17.7	-3.2	-1.7	-0.8
DO	100	-1.5	0.3	-23.9	-4.8	-3.2	-1.8
DO	250	-3.9	0.8	-33.5	-8.4	-7.6	-4.7
DO	500	-8.1	1.7	-42.8	-13.6	-14.8	-9.6
DO	750	-12.3	2.6	-49.8	-18.4	-22.1	-14.5
DO	1000	-16.5	3.5	-55.8	-23.1	-29.3	-19.3
DO	2000	-33.3	7.0	-75.7	-41.6	-58.3	-37.7
DR	0	0.0	0.0	0.0	0.0	0.0	0.0
DR	50	-0.6	0.1	-17.6	-3.6	-1.6	-0.7
DR	100	-1.3	0.3	-23.8	-5.3	-3.1	-1.6
DR	250	-3.5	0.7	-33.3	-9.1	-7.3	-4.2
DR	500	-7.2	1.4	-42.4	-14.2	-14.3	-8.6
DR	750	-10.9	2.2	-49.1	-19.0	-21.2	-12.8
DR	1000	-14.5	2.9	-54.8	-23.5	-27.9	-16.9
DR	2000	-28.6	5.7	-73.2	-40.7	-54.1	-32.5

875



880

Fig. 1: Schematic representation of the experimental and modelling procedures. Left refers to the experimental part and ~~enter~~centre to right to the modelling part. Soils were first ~~pre-~~incubated 5 weeks at different constant percentage of the water holding capacity (WHC~~, N-NO_2, N-NO_3~~) or at two variable moistures, Dry-Only (DO) and ~~N-~~Dry-Rewet (DR). Then NO_2-N, NO_3-N and NH_4-N soil concentrations were ~~then~~ measured after this

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~~preincubation~~initial incubation, and values were used to initialise DNDC~~.~~, while a bioassay was ~~then~~also applied on soil aliquots. The 3 days bioassay included NH_4^+ in excess and copper (Cu) ~~was spiked~~spikes at 0, 50, 100, 250, 500, 750, 1000, 2000 mg Cu.kg $soil^{-1}$ of soil ~~to reach concentrations of 12, 62, 112, 262, 512, 762, 1012 and 2012 mg Cu.kg $soil^{-1}$ and left for incubation.~~. After 1 and 3 days of bioassay incubation, ~~N-~~NO_3-N production was
890 measured in the supernatant. ~~N-~~NO_3-N productions against [Cu] gradients were used to define the functions of eq. 28 to 31 in §3.1~~.~~ (see text). Soil respiration values were extracted from the curve C_i of Fig 1 in ~~Annabi et al. (2007)~~Annabi et al. (2007).

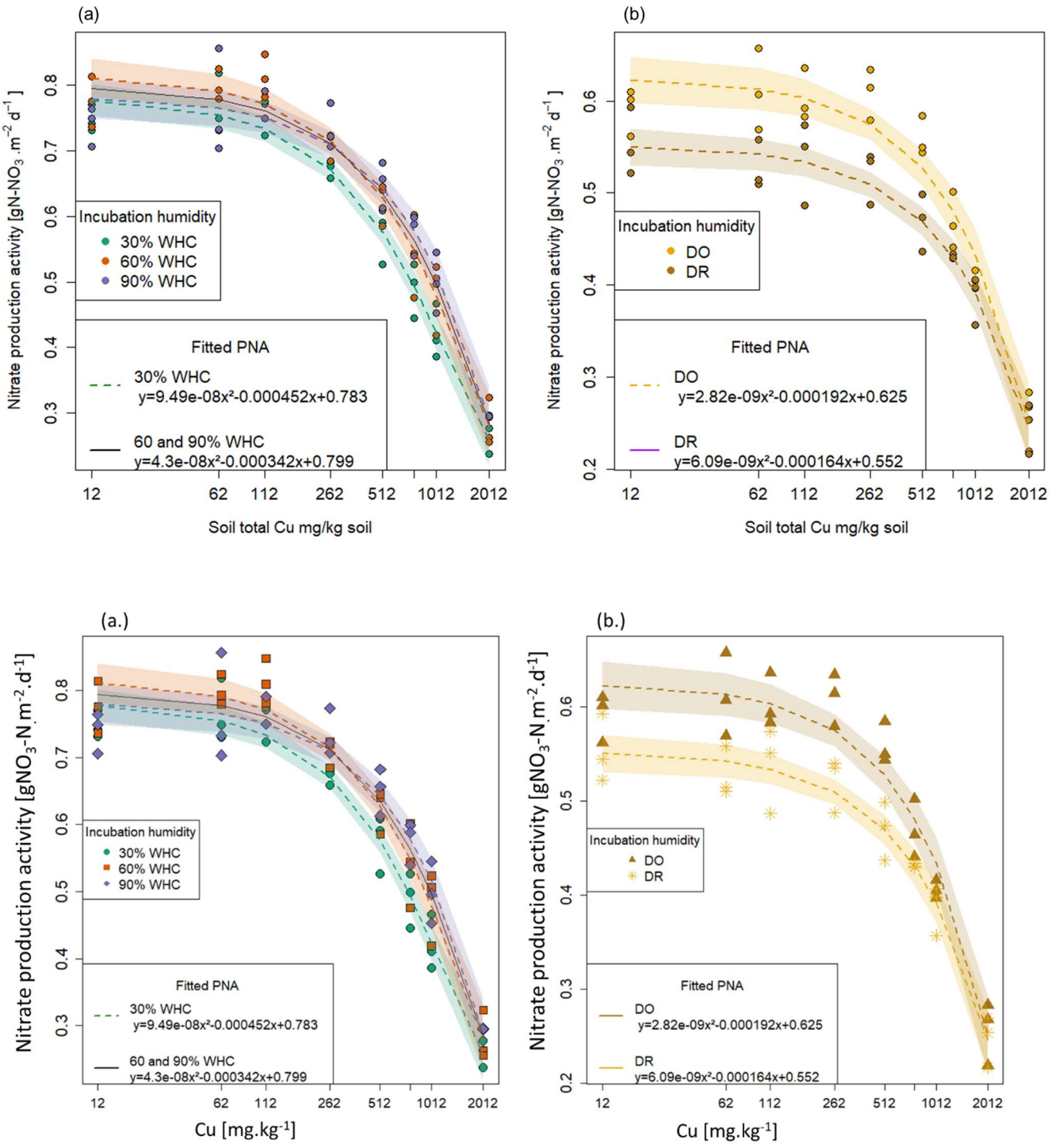
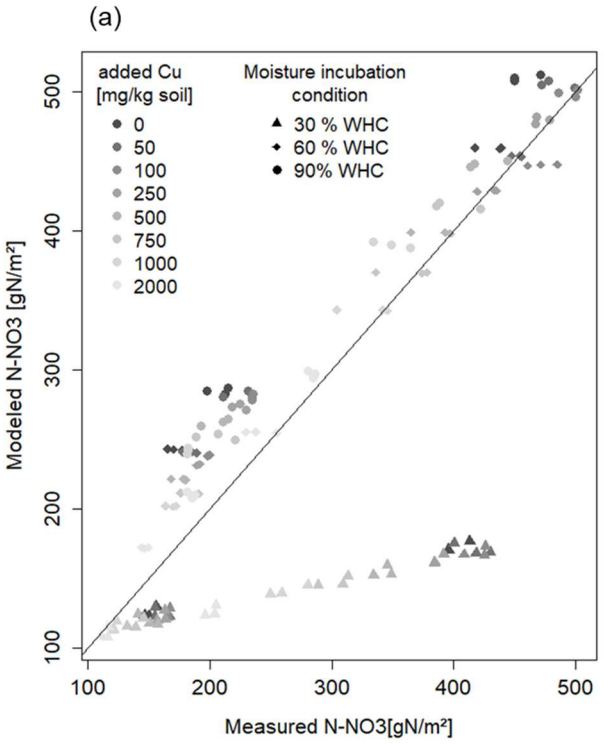
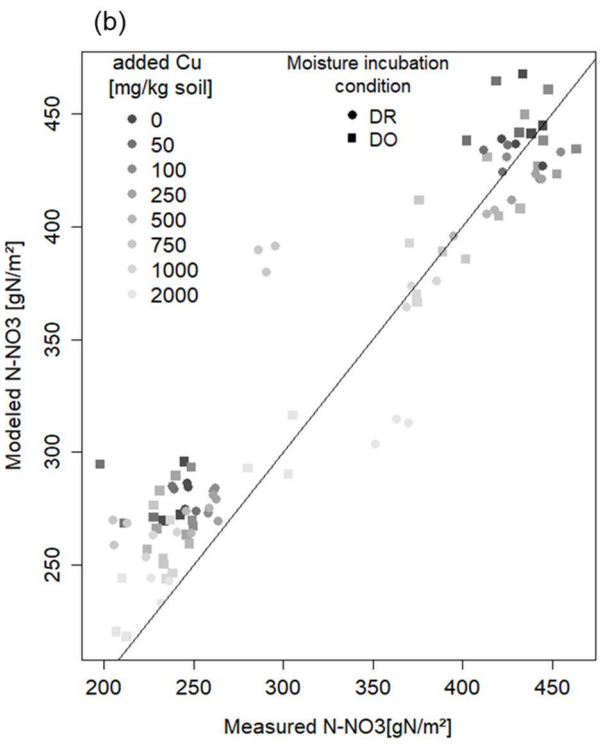
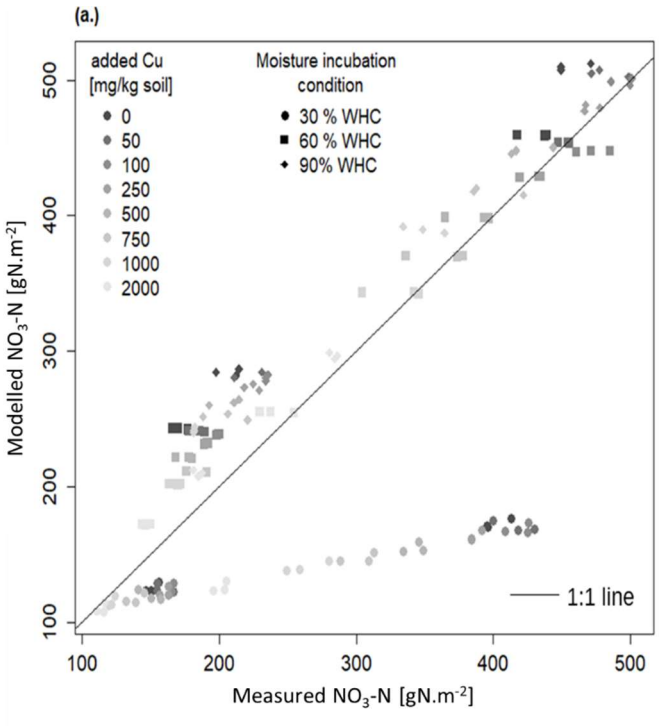


Fig. 2: Fitted functions of potential nitrifying activities (PNA) against total soil copper concentrations [Cu] for each initial moisture incubation ~~condition~~treatment. Points are the measured nitrate production and lines the fitted quadratic function with their 95% confidence interval. (a). Constant moisture treatments: green circle is for 30% WHC, red square for 60% WHC and purple diamond for 90% WHC. The black line is the common fitting function used for 60 and 90% WHC ~~incubation conditions.~~moisture treatments. (b). Variable initial moisture treatments: brown star is for Dry-~~rewetting~~Rewet (DR) and yellow triangle for Dry-Only (DO).

905


(a)
added Cu
[mg/kg soil]
0
50
100
250
500
750
1000
2000
Moisture incubation
condition
30 % WHC
60 % WHC
90% WHC
Modeled N-NO3 [gN/m²]
Measured N-NO3[gN/m²]
100
200
300
400
500


(b)
added Cu
[mg/kg soil]
0
50
100
250
500
750
1000
2000
Moisture incubation
condition
DR
DO
Modeled N-NO3 [gN/m²]
Measured N-NO3[gN/m²]
200
250
300
350
400
450


(a.)
added Cu
[mg/kg soil]
0
50
100
250
500
750
1000
2000
Moisture incubation
condition
30 % WHC
60 % WHC
90% WHC
1:1 line
Modelled NO3-N [gN.m-2]
Measured NO3-N [gN.m-2]
100
200
300
400
500

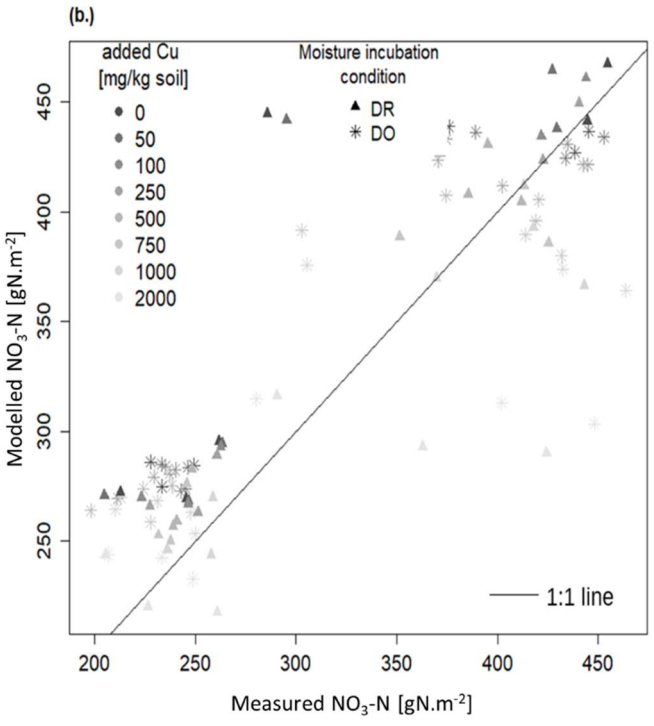
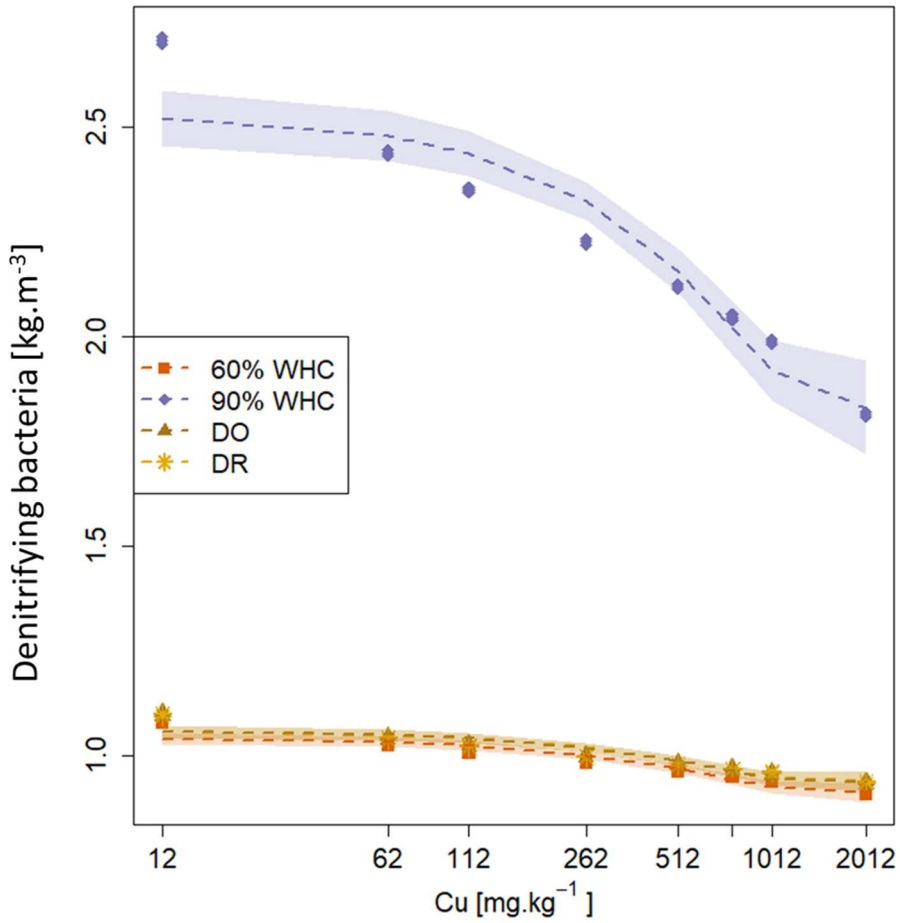
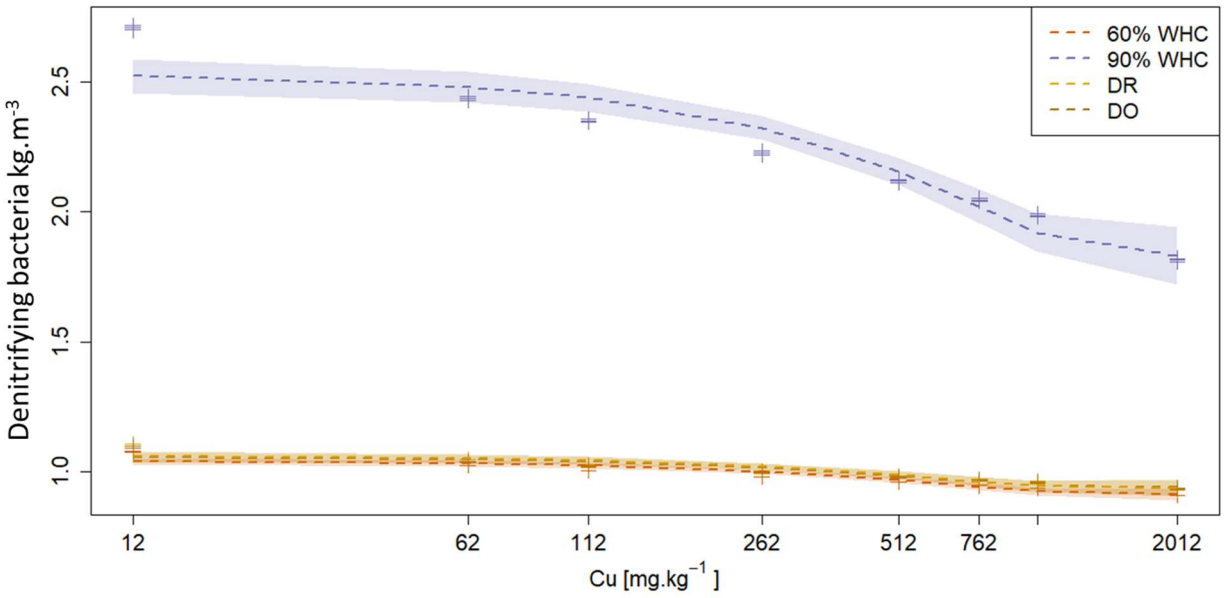

(b.)
added Cu
[mg/kg soil]
0
50
100
250
500
750
1000
2000
Moisture incubation
condition
DR
DO
1:1 line
Modelled NO3-N [gN.m-2]
Measured NO3-N [gN.m-2]
200
250
300
350
400
450

Fig 3: Comparison ~~of modeled against~~between modelled and measured soil [nitrate] incubated in different moisture~~.~~ with 1:1 line (a) = the 3 initial incubations under constant ~~humidity~~moisture. (b) = the two initial incubations under variable moisture Dry-~~rewetting~~Rewet (DR) and Dry-~~-~~Only (DO) ~~conditions~~treatments. For 30% WHC, Model=1.84 * Measure and R2=0.93; for 60% WHC Model=0.93 *measure. R2=0.99; for 90% WHC Model=0.90 * measure. R2=0.99 ; for Dry -rewetting (DR) model = 0.96*measure. R2=0.98; for Dry-~~-~~ Only (DO)Model=0.95*measure. R2=0.99


Denitrifying bacteria [kg.m-3]
2.5
2.0
1.5
1.0
60% WHC
90% WHC
DO
DR
12
62
112
262
512
1012
2012
Cu [mg.kg-1]


Denitrifying bacteria kg.m-3
2.5
2.0
1.5
1.0
60% WHC
90% WHC
DR
DO
12
62
112
262
512
762
2012
Cu [mg.kg-1]

920

Fig 4. Modelled soil denitrifying bacterial pool after 3 days ($gN.m^{-3}$ soil) for the 4 moisture treatments. Purple diamond is for 90% WHC, red square for 60% WHC, brown star for Dry ~~rewetting~~-Rewet (DR) and yellow triangle for Dry ~~-~~Only (DO). Red, brown and yellow curves being superposed. Pools were modelled for 12, 62, 112, 262, 512, 762, 1012 and 2012 mg Cu,kg soil $^{-1}$ as represented by cross. Quadratic fits were used for representation.

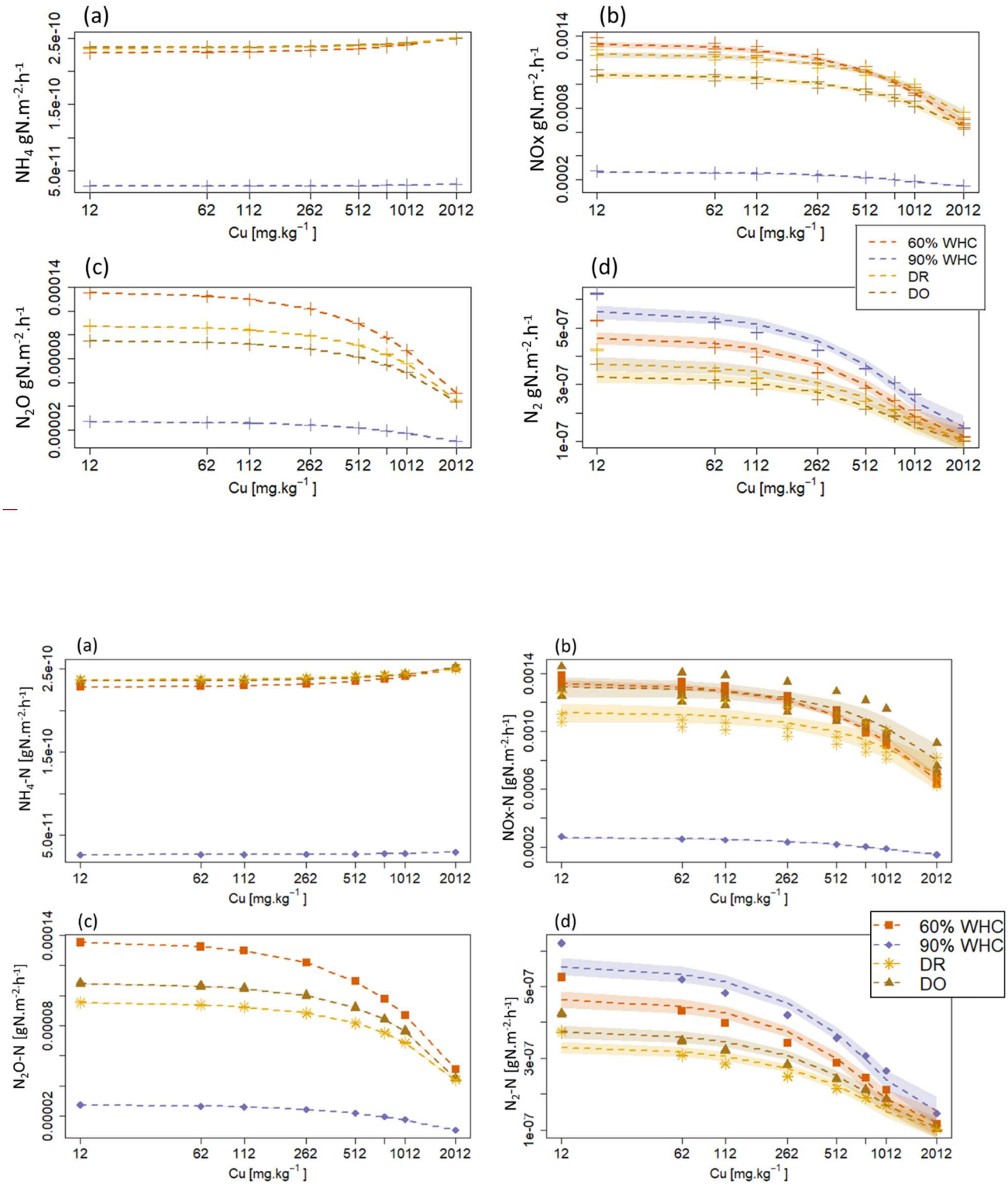
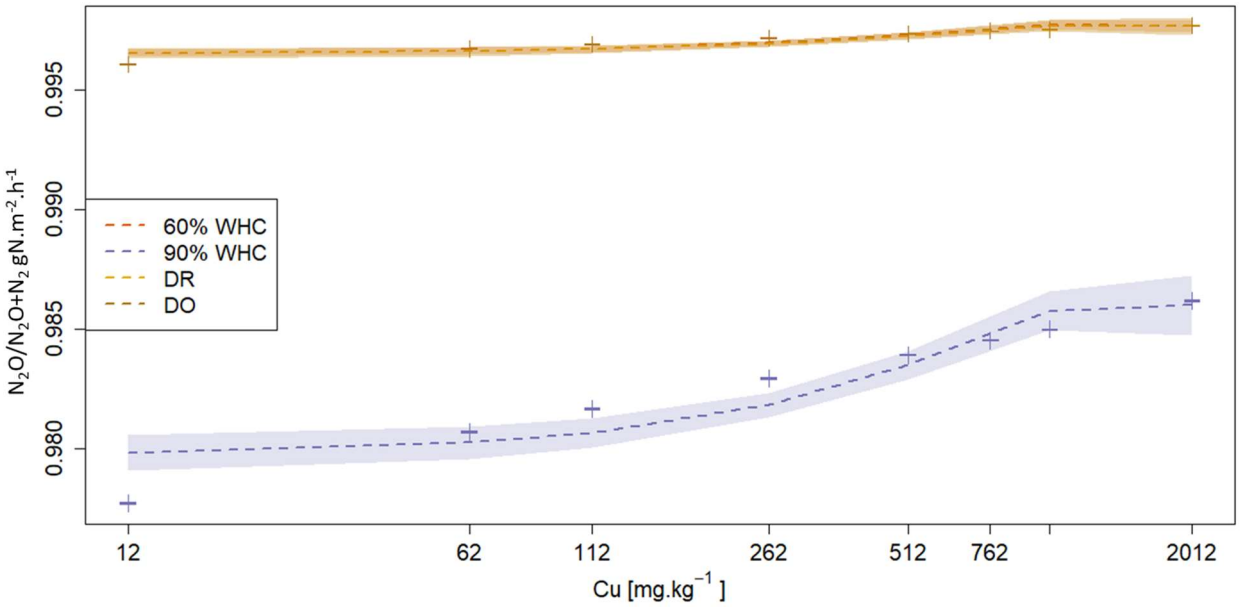


Fig 5: Modelled N emission fluxes at 3 days in gN.m^{-2}.30min^{-1} under the different moisture conditionstreatments. a.) N-NH$_4$-N emission fluxes. b.) N-NOx-N emission flues c.) N-N$_2$O-N emission fluxes and d.) N-N$_2$-N emission fluxes. Purple diamond is for 90% WHC, red square for 60% WHC, brown star for Dry

~~rewetting~~ Rewet (DR) and yellow triangle for Dry ~~-~~ Only (DO). Fluxes were modelled for 12, 62, 112, 262, 512, 762, 1012 and 2012 mg Cu.kg soil $^{-1}$ as represented by cross. Quadratic fits were used for representation.



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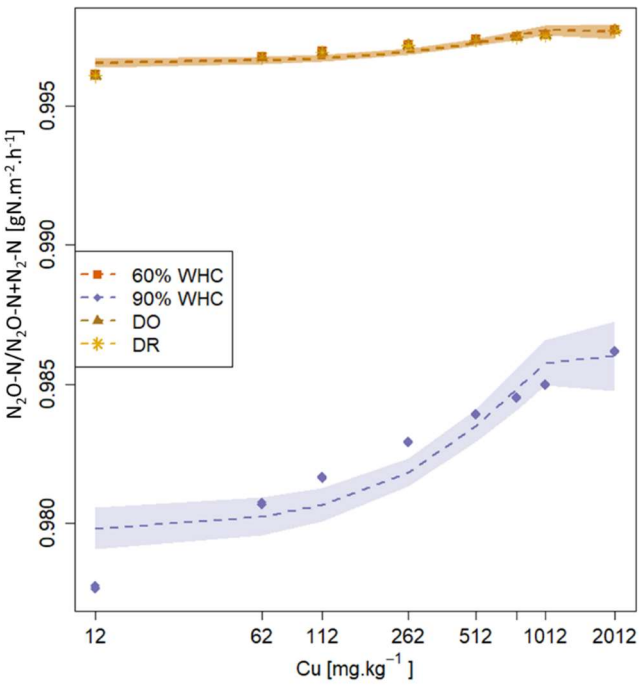

Fig. 6: Proportion of N_2O-N emitted arising from the denitrification calculated as N_2O-N /N_2O-N +N_2-N modelled fluxes in response to soil Cu concentration for the various moisture ~~conditions~~treatments. Red square is for 60 % WHC, purple diamond is for 90% WHC, yellow circle for ~~the~~ Dry-Rewet (DR) and brown star for ~~the~~ Dry-Only (DO~~.~~). Red, yellow and brown curves are superposed. Fluxes were modelled for 12, 62, 112, 262, 512, 762, 1012 and 2012 mg Cu.kg soil $^{-1}$ as represented by cross. Quadratic fits were used for representation.