Supporting Information:

The anaerobic soil volume as a controlling factor of denitrification: a review

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S.1 Meta-Analysis of Distance Thresholds for Anoxia

The meta-study on controlling factors of the distance threshold for anoxia comprised a total of 90 datasets from 27 experimental studies (16 with microsensors, six with planar optodes, five with X-ray CT) in a range of domains including wet soil aggregates, biofilms, benthic sediments, flooded soils and intact upland soils. Studies from peatland soils were excluded from the analysis. Out of these 90 datasets, 19 were missing O_2 consumption rate information. The complete data set is available in a spread sheet (Data S1) which contains the columns given in Table S1.

Table S1: Headers and explanation, including units, for all columns of data table on the controlling factors of distance thresholds for the formation of anoxia

Column Title	Explanation
Reference	First Author (Year)
doi	Paper reference
DenitrifyingDomain	Short description of the sample, e.g. wet aggregates, intact soil
LabileCarbon	0 – sieved soil with labile carbon removed, e.g. no plant residues 1 – analysis around isolated patches of labile carbon 2 – intact soil or repacked soil with labile carbon mixed in
Method	Microsensor, Planar Optodes, X-ray CT
Distance	Distance threshold for anoxia [mm]. At the scale of observation no information was available distance to air-filled pores, so the values rather refer to distance from the sediment surface, aggregate boundary, POM patch, etc.
RespOrig	Respiration rate for the gas (CO_2 or O_2) with the unit given in the paper
RespO2	Converted O_2 consumption rate in µmol m^{-3} s^{-1}
Radius	Radius of studied aggregates in mm (if applicable)
SOC	Soil organic carbon content in g C kg^{-1}
MatricPotential	Matric potential in hPa
Temperature	Incubation temperature in °C
ExternalO2	Headspace oxygen concentration in % air saturation
External_pO2	Partial Pressure of oxygen in headspace in kPa
Remarks	Treatment name and other auxiliary information

The WebPlotDigitizer Tool (https://apps.automeris.io/wpd/) was used for retrieving numbers from graphics. Unit conversion were done with online catalogs (https://www.ices.dk/data/tools/Pages/Unit-conversions.aspx [last access: Aug 29th 2023]) and online unit converters (https://www.aqua-calc.com/calculate/mole-to-volume-and-weight [last access: Aug 29th 2023], https://unisense.com/unit-converter/#/ [last access: Aug 29th 2023]). A respiratory quotient of 1 was assumed for converting CO_2 emission rates to oxygen consumption rates. In most microsensor studies the O_2 content threshold for anoxia was set at approx. 0.1kPa. In studies with planar optodes, the color range was insufficient at the transition for hypoxic to anoxic conditions so that the O_2 content threshold was approx. around 1-5kPa. For studies with fully saturated or flooded soils, the matric potential was set to -1hPa despite the zero or even positive hydrostatic pressure in order to facilitate the statistical analysis with log-transformed values.

Statistical Analysis was carried out with R (R Core Team 2022). Power-law regression of the distance threshold with O_2 consumption rate or distance threshold with basic soil properties was done with linear regression models of log-transformed values after outlier removal with the robustbase package (Maechler et al. 2023).

S.2 Meta-Analysis of Anaerobic Soil Volume in Oxic-Anoxic incubations

The meta-study on controlling factors of the apparent anaerobic soil volume fraction derived from the ratio of total denitrification at oxic and anoxic conditions comprised 76 datasets from 16 experimental studies. Studies which did not report N2O + N2 at both stages were excluded, e.g. those that reported only N2O during oxic incubation and N2O + N2 with the acetylene method for anoxic incubation. N2O + N2 emissions often varied a lot during the oxic and anoxic incubation stage, typically with an early flush after adding nitrate or labile carbon followed by a steady state (or gradual decline) and ending in a late stage with some kind of limitation (carbon or nitrate). This can lead to a biased oxic-anoxic denitrification ratios, if only cumulative emissions are reported and if the incubation periods are very different. Therefore, the denitrifications rates were estimated in selected time windows based on these heuristic averaging rules:

1. In open systems with a continuous gas flow through the head space, look for steady-state in the oxic stage and anoxic stage, i.e. disregard the late stage with a nitrate or carbon limitation and disregard an N2O+N2 flush after adding nitrate or labile carbon.
2. In addition, omit the first few hours after switching O_2 levels to disregard salt effects or a slow onset of enzyme activity.
3. In closed systems without a continuous gas flow through the head space, use only a short averaging time so that O_2 in oxygenated samples is not taken down too much.

Rates were calculated from original data or were retrieved from figures with the WebPlotDigitizer Tool (https://apps.automeris.io/wpd/). In addition to the denitrification rates at oxic and anoxic conditions, many more site and soil properties were reported. The complete data set is available in a spread sheet (Data S2) which contains the columns given in Table S2. The set of meta-information is an extension of the standard reporting framework of denitrification research (Almaraz et al. 2020). Denitrification rates and respiration rates were reported in different units (e.g. per weight or per area) and not converted to a unified unit. Therefore, only ratios of these rates between the oxic and anoxic stage were considered for the data analysis. Respiration ratios were only reported for 34 out of 72 datasets.

Statistical analysis was carried out with R (R Core Team 2022). Linear correlations regression of the apparent anaerobic soil volume fraction with bulk soil properties was done with linear regression models after outlier removal with the robustbase package (Maechler et al. 2023). Seven out of 72 datasets with anaerobic soil volume fractions greater than 1 were disregarded from the analysis.

Table S2: Headers, units and explanations for all columns of data table on the controlling factors the anaerobic soil volume fraction estimated with oxic-anoxic incubations

column	unit	explanation
paper	-	first author
doi	-	without https
ecosystem	-	desert, grassland, pasture, cropped agriculture, woodland, shrubland, other
continent	-	

latitude	-	N/S followed by number in decimal units, e.g. N50.12
longitude	-	W/E followed by number in decimal units, e.g. E14.52
MAT	°C	
MAP	mm	
manipulations	-	type of manipulation experiment, e.g. nitrogen, carbon, water, oxygen, pH, temperature, other
sieved	-	yes or no
soil depth	cm	upper and lower end of sampling depth
soil volume	cm^3	
sample height	cm	in the incubation vessel
method	-	acetylene, 15N tracer, direct, other
rates	-	instantaneous (open system, e.g. Roflow), cumulative (closed, e.g. N-FARM)
incubation_mode	-	sequential (ox and anox with same sample), parallel (ox and anox with separate samples)
preincubation	days	
oxic_concentration	%	
oxic_phase	days	
anoxic_phase	Days	
measured gases	-	CO_2,N_2O,N_2,O_2
land use	-	
treatment	-	which of the manipulation treatments, e.g. control
added_N	-	yes or no, add exact number in column NO3- or NH4+
clay	g kg-1	
silt	g kg-1	
sand	g kg-1	
bulk density	g cm-3	
saturation	-	volumetric water saturation = WFPS
temperature	°C	during incubation
SOM	g kg-1	
C:N	-	
pH	-	
DOC	mg C kg-1	
NH4+	mg N kg-1	
NO3-	mg N kg-1	
resp_aerobic	-	
resp_anaerobic	-	
resp_unit	variable	e.g. µmol C d–1 cm–3
resp_ratio	-	Resp_anaerobic/Resp_aerobic
denit_aerobic	-	
denit_anaerobic	-	
denit_unit	variable	µg N kg-1 d-1
ANSV	-	apparent anaerobic soil volume fraction: denit_aerobic/denit_anaerobic
Notes	-	

S.3 Model comparison of the anaerobic soil volume fraction

The semi-empirical nature of anaerobic soil volume (ANSV) fractions makes it challenging to derive suitable experimental data for validating approaches of ANSV quantification used in models, which might be one reason for the diversity of approaches introduced in Table 3. The empirical estimation of ANSV by the oxic-anoxic denitrification rate concept (see section 3.5) would be an obvious approach to derive such validation data because the ANSV concept was implemented in models to better describe denitrification rates in soils.

The validation of simulated ANSV fractions against experimental values is challenging as the estimation from measurements depend on semi-empirical approximations valid only for some rather strong assumptions. For this model comparison, we use as validation data the apparent anaerobic soil volume fractions derived from total denitrification rates (N2O+N2) under sequential oxic-anoxic mesocosm incubations of repacked soil under otherwise constant laboratory conditions (see section section 3.5). The anoxic stage is not relevant for modelling, but only required to estimate the ANSV fraction experimentally. We included some of the models listed in Table 3 (L-DNDC, CoupModel and DNDC.vCAN) in the model comparison

Soil material for the mesocosm experiment was taken from three different sites: the silty loam cropland soil and the clayey grassland soil from Figure 2, and in addition a sandy cropland soil. The basic soil properties of each soil are given in the Supporting Data (Data S2). The repacked soils were amended with $^{15}N\text{-}NO_3^-$ and incubated at four different WFPS levels (60/65/70/75 %) for 26 days under aerobic (20 % O_2) and 16 days under anaerobic (0 % O_2) conditions. The CO_2 and N_2O fluxes were measured three times a day and N_2 fluxes two times a week.

Table S3: Estimated and modeled anaerobic soil volume (ANSV) fractions of three soil materials in a laboratory mesocosm experiment. Soils were incubated at four water-filled pore space (WFPS) levels. The estimated anaerobic soil volume fraction during oxic incubation was compared with the simulated values of three biogeochemical models (Landscape-DNDC, Coup, DNDC.vCan).

			Anaerobic soil volume fraction [-]			
Soil texture	**Land use**	**WFPS [%]**	**Measured**	**L-DNDC**	**DNDC.vCan**	**Coup**
sand	cropland	60	0.002	0.127	0.000	0.019
		65	0.002	0.134	0.000	0.024
		70	0.018	0.146	0.000	0.028
		75	0.088	0.194	0.000	0.033
silt loam	cropland	60	0.004	0.118	0.000	0.028
		65	0.003	0.119	0.000	0.025
		70	0.003	0.122	0.000	0.025
		75	0.531	0.126	0.000	0.026
clay	grassland	60	0.014	0.124	0.000	0.029
		65	0.001	0.129	0.000	0.052
		70	0.007	0.136	0.000	0.055
		75	0.449	0.156	0.000	0.062

The estimated ANSV fractions ranged from 0.002 to 0.09 for the sandy soil, 0.003 to 0.53 for the silt loam soil, and 0.001 to 0.45 for the clay soil. The estimated ANSV fraction did not show a clear increase in response to WFPS change from 60% to 70%. However, a large increase was observed when WFPS increased to 75%, particularly for the fine-textured silt loam and clay soils. This is in line with the findings that WFPS is a weak predictor of variability in the anaerobic soil volume fraction, but that O_2 availability modulated by the diffusivities of the soils is relevant.

In general, DNDC.vCan and Coup simulated the magnitude around the estimated ANSV fraction close to zero in the WFPS range of 60-70%, whereas for L-DNDC they were above 0.1 (Table S3), indicating that independent of modeled O_2 concentrations (Figure S1) formulas shown in Table 3 result in highly variable ANSV fractions between models. The algorithm linking O_2 concentration to ANSV fraction in DNDC.vCAN (Table 1) had a steep increase at very low O_2 concentrations, so that the soil does not turn anoxic irrespective of WFPS (Table S3), even if DNDCv.CAN modeled the most pronounced decrease of O_2 for WFPS at 75% compared to the other two models (Figure S1). In DNDCv.CAN, it is assumed that rain events cause anoxic conditions (ANSV=1), so at the field scale, there are always suitable conditions for denitrification during rain events. However, a better description of the O_{2-}-ANSV relationship is desirable and would probably improve the description of short-term denitrification processes in DNDCv.CAN.

A common feature for all the three models was that none of them reproduced the steep increase of the estimated anaerobic soil volume fraction around 75% WFPS, irrespective of soil (Table S3, Fig. S1). While in DNDCv.CAN this is caused by the functional relationship between ANSV and O_2, the other two models show no distinct effect of WFPS increase from 70% to 75% on O_2 concentrations in soil which remain close to atmospheric levels around 20kPa (Figure S1). Therefore, it is not astonishing that the also modeled ANSV is not responding. Modeled O_2 concentrations in the soil profile depend on the modeled O_2 consumption by respiration and by O_2 transport, and those are highly sensitive to the initial distribution of SOC among model pools on the one hand and the definition of diffusion coefficients for those repacked soil cores in this experiment on the other hand.

This small model comparison based on estimated ANSV fraction from oxic-anoxic denitrification rate experiments shows that there is a high diversity between models for the conceptual variable ANSV, making model evaluations on empirical quantified ANSV for constrained experimental settings desirable, since ANSV is a key variable for the modeling of denitrification rates. Similar to those proposed in Grosz et al. (2021), these model comparisons would profit from the extension of available data sets with well-defined initial conditions (distribution of SOC in pools), soil properties (diffusivity), and boundary conditions (WFPS, O2, and CO2 concentrations, applied organic substrate, etc.). In addition, improving the models requires integrating the processes that affect their estimated O2 concentrations, including the missing priming effect on carbon mineralization, and calibrating denitrifier microbial dynamics (Grosz et al. 2021). The estimation of ANSV based on oxic-anoxic denitrification rates is based on several assumptions (see 3.5), questioning the reliability of this approach to evaluate modeled ANSV. However, it would be interesting to investigate the usefulness of this concept with respect to modeled denitrification. This could be done by calibrating models on observed O_2 and CO_2 fluxes and formulating O_2-ANSV relationships based on estimated ANSV by the oxic-anoxic denitrification rate approach for a range of experimental conditions. The usefulness could then be assessed by comparing N_2O and N_2 fluxes.

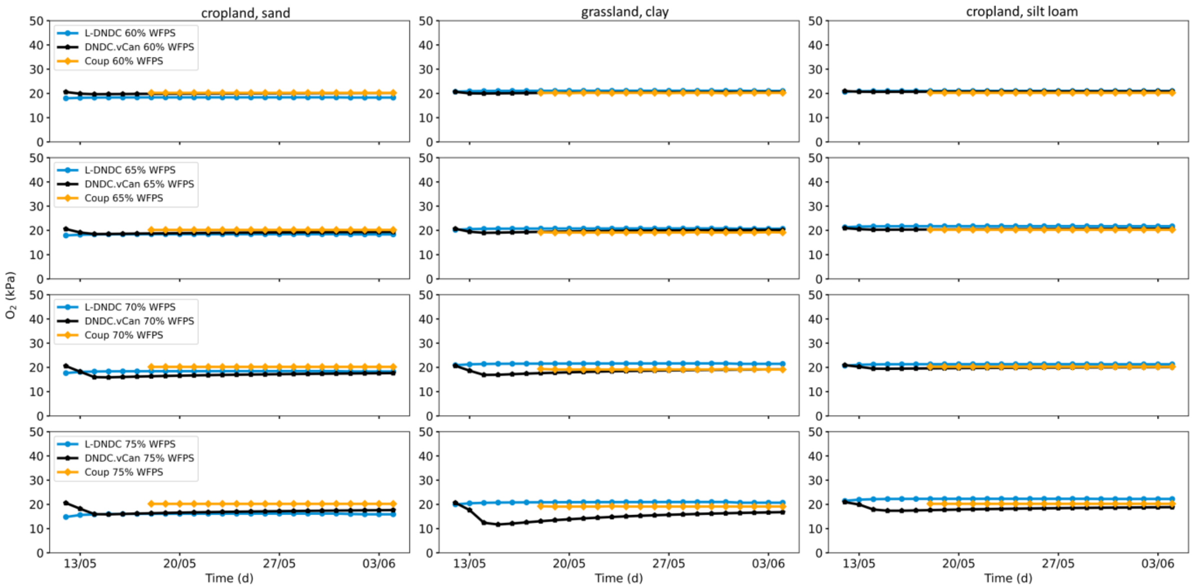

Figure S 1: Modeled (Landscape-DNDC, Coup, and DNDC.vCan) O2 contents for a soil incubation study with three different soils (Fuhrberg, Rotthalmunster, and Gießen). Per soil type four water-filled pore space levels (60/65/70/75%) were kept constant during the 42-day experiment.

References

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