Stratification of reactivity determines nitrate removal in groundwater

Tamara Kolbe,a,1,2 Jean-Raynald de Dreuzya,b, Benjamin W. Abbott,c,d, Luc Aquilina,a,e, Tristan Babeya,a,3 Christopher T. Green,a,3 Jan H. Fleckenstein,d,a,3 Thierry Labasque,a,3 Annett M. Lavermana,3 Jean Marçaisb,a,3 Stefan Peiffer,a,3 Zahra Thomas,d,4 and Gilles Pinaya,b

aCentre National de la Recherche Scientifique (CNRS), Géosciences Rennes - UMR 6118, Université de Rennes, 35042 Rennes, France; bCentre National de la Recherche Scientifique (CNRS), Institut National de la Recherche Agronomique (INRA), Observatoire des Sciences de l’Univers de Rennes (OSUR) - UMR 3343, Université de Rennes, 35042 Rennes, France; cDepartment of Plant and Wildlife Sciences, Brigham Young University, Provo, UT 84604; dCentre National de la Recherche Scientifique (CNRS), ECOBIO - UMR 6553, Université de Rennes, 35042 Rennes, France; eWater Mission Area, US Geological Survey, Menlo Park, CA; fDepartment of Hydrogeology, Helmholtz Centre for Environmental Research - Zentrum für Umweltforschung (UFZ), 04318 Leipzig, Germany; gDivision of Hydrologic Modeling, University of Bayreuth, 95447 Bayreuth, Germany; hEcole Nationale du Génie Rural, des Eaux et des Forêts (ENGREF), Agroparistech, 75231 Paris, France; iDepartment of Hydrology, Bayreuth Center of Ecology and Environmental Research, 95447 Bayreuth, Germany; jInstitut National de la Recherche Agronomique (INRA), Sol Agro et Hydrosystème Spatialisation, UMR 1069, Agrocampus Ouest, 35042 Rennes, France; kUniversité de Rennes (OSUR) - UMR 3343, Université de Rennes, 35042 Rennes, France. We applied it to five other aquifers in different geological settings located in the United States and compared results among 44 locations across the six aquifers to assess the generality of reactivity trends. Of the sampling locations, 79% showed pronounced increases of reactivity with depth. This suggests that previous estimates of denitrification have underestimated the capacity of deep aquifers to remove nitrate, while overestimating nitrate removal in shallow flow paths. Oxygen and nitrate reduction likely increases with depth because there is relatively little organic carbon in agricultural soils and because excess nitrate input has depleted solid phase electron donors near the surface. Our findings explain the long-standing conundrum of why excess nitrate input has depleted solid phase electron donors near the surface because of inorganic and organic electron donors (10–17); however, the spatial distribution of these reactivity with depth. This stratified reactivity framework is promising for understanding the dynamics, including nitrogen, carbon, and silica, with existing datasets for ecosystems around the globe. Understanding the subsurface structure of reactivity would also improve estimates of recovery time frames of polluted ecosystems and inform sustainable limits for anthropogenic activity.

Significance

Although groundwater is a critical source of drinking water and irrigation, it has been polluted worldwide by agriculture, industry, and domestic activity. Because assessing groundwater quality and recovery rates is challenging, we developed a method for determining where and how quickly nitrate is removed in aquifers using just a few points of groundwater chemistry. This methodology opens new avenues for characterizing catchment-scale nutrient dynamics, including nitrogen, carbon, and silica, with existing datasets for ecosystems around the globe. Understanding the subsurface structure of reactivity would also improve estimates of recovery time frames for polluted ecosystems and inform sustainable limits for anthropogenic activity.

Biogeochemical reactions occur unevenly in space and time, but this heterogeneity is often simplified as a linear average due to sparse data, especially in subsurface environments where access is limited. For example, little is known about the spatial variability of groundwater denitrification, an important process for removing nitrate originating from agriculture and land use conversion. Information about the rate, arrangement, and extent of denitrification is needed to determine sustainable limits of human activity and to predict recovery time frames. Here, we developed and validated a method for inferring the spatial organization of sequential biogeochemical reactions in an aquifer in France. We applied it to five other aquifers in different geological settings located in the United States and compared results among 44 locations across the six aquifers to assess the generality of reactivity trends. Of the sampling locations, 79% showed pronounced increases of reactivity with depth. This suggests that previous estimates of denitrification have underestimated the capacity of deep aquifers to remove nitrate, while overestimating nitrate removal in shallow flow paths. Oxygen and nitrate reduction likely increases with depth because there is relatively little organic carbon in agricultural soils and because excess nitrate input has depleted solid phase electron donors near the surface. Our findings explain the long-standing conundrum of why excess nitrate input has depleted solid phase electron donors near the surface because of inorganic and organic electron donors (10–17); however, the spatial distribution of these reactivities in aquifers remains almost completely unknown. Consequently, new methods to efficiently characterize the vertical and lateral spatial pattern of subsurface reactivity are urgently needed to determine sustainable limits of anthropogenic activity and predict recovery time frames of polluted ecosystems (18, 19).

Denitrification is the primary removal pathway of reactive nitrogen in aquifers (20), and it occurs when three factors coincide: occurrence of denitrifying microorganisms, presence of anoxic conditions, and availability of electron donors (20–24). Denitrification rates in groundwater can be inferred from NO3− disappearance and N2 production during transport along flow paths from aquifer recharge to discharge zones. These overall, or bulk, rates are generally interpreted as apparent reaction rates integrating flow, transport, and reactive conditions over large aquifer widths and depths (24–26). Consequently, the same apparent rates could result from either slow reactions over long flow paths or fast reactions in localized zones. The assumption of continuous reactivity may be appropriate for some sedimentary aquifers, but structured vertical patterns of groundwater denitrification or retained in the subsurface because of inorganic and organic electron donors (10–17); however, the spatial distribution of these reactivities in aquifers remains almost completely unknown. Consequently, new methods to efficiently characterize the vertical and lateral spatial pattern of subsurface reactivity are urgently needed to determine sustainable limits of anthropogenic activity and predict recovery time frames of polluted ecosystems (18, 19).

Fig. 1. Stratification of reactivity determines nitrate removal in aquifers. a) Prefabricated reactor used to simulate depth and time scales in aquifers. b) Organic compound (methanol) was injected in the reactor, then anoxic conditions were created and NO3− was allowed to react with the microorganisms. c) The reactor was used to determine the apparent reaction rate of oxygen in aquifers, using NO3− and methanol as electron donors and acceptors. The apparent reaction rate of oxygen in aquifers is typically smaller than the apparent reaction rate of oxygen in aquatic ecosystems, which is energetically less favorable. This stratified reactivity framework is promising for mapping vertical reactivity trends in aquifers, generating new understanding of subsurface ecosystems and their capacity to remove contaminants.

Humans have exceeded the Earth’s capacity to receive and process nitrogen (1), triggering eutrophication in rivers, lakes, and coastal zones, which imposes billions of dollars of ecological and socioeconomic costs annually (2, 3). However, even if anthropogenic inputs of reactive nitrogen (e.g., NH4+, NO3−) were stopped today, elevated nitrate (NO3−) concentrations in aquifers could persist for decades to centuries, sustaining eutrophication in rivers, lakes, and estuaries (4–7). The capacity of aquifers to immobilize or remove reactive nitrogen is highly variable within and among aquifers, underlying their functional heterogeneity but complicating evaluation of sustainable limits at medium to large scales. Heterogeneity of reactivity is typical of all ecosystems, meaning that information about the biogeochemical transformation of nutrients (7) and the spatial organization of reactivity is necessary to determine ecosystem functioning (8, 9). There is substantial evidence from aquifers around the globe that NO3− is removed by denitrification or retained in the subsurface because of inorganic and organic electron donors (10–17); however, the spatial distribution of
Inferring Stratified Reactivity in Groundwater

In complex environments, such as aquifers, the investigation of reactivity requires the deconvolution of information about hydrological mixing, time-varying solute inputs, and reaction rates (7). Tracer concentrations in wells, C(t), can be modeled by weighting the input concentrations, C(p)(t), by the transit time distribution, p(u), using Eq. 1 (25, 32):

\[ C(t) = \int_0^\infty p(u) C_D(t-u) r(u) \, du. \]  

Degradation of O₂ and NO₃⁻ is generically expressed by a reaction term, r. The integration variable u represents the transit time of a water parcel. Assessing successive O₂ and NO₃⁻ depletion for each well is achieved by deconvoluting the effects of vertically separated flow paths (6, 33). For some of these flow paths, oxic conditions may prevent denitrification, while in others, conditions favorable to reaction times can inform about the apparent reaction times obtained from the multiwell sites (24). Published apparent reaction times of the individual wells were also determined by Eq. 1 and environmental tracers, whereas apparent reaction times at the multiwell sites resulted from the relation of measured concentrations to tracer-based groundwater ages based on the piston flow assumption. This modeling allowed independent verification of the stratified reactivity framework because the apparent reaction times of O₂ and NO₃⁻ could be estimated directly with data from multiple depths.

Results and Discussion

Observed Vertical Reactivity Patterns and Reaction Times. The relations of apparent O₂ and NO₃⁻ reaction times obtained from the unconfined crystalline aquifer located in an agricultural area in Brittany, France (6), show a predominance of the late start pattern with apparent O₂ reaction times larger than apparent NO₃⁻ reaction times (88% of the sampling locations; SI Appendix, section S1). The dominance of the late start pattern appears to be driven by biotically mediated sulfide minerals, which are found in borehole cuttings from deeper fractured zones (44), with reactions occurring between the weathered and fractured zones (44–48). The time to enter the reactive zone, τ_{zone}, is verified by comparing the depth when a water parcel reaches the reactive zone and the depth of the interface between the weathered and fractured zones (Fig. 2). The depths of the reactive zone were determined by the depth of a water parcel at the time it enters the reactive zone by using information about groundwater flow ages based on individual groundwater flow models (6). Alternatively, analytical solutions for the depth versus age relationship (42) could be used to determine the depth of the reactive zone. Existing NO₃⁻ stratum reaction times measured in Brittany show a similar range as determined here, ranging within a few hours and several years depending on the electron donor availability (47–51).

The synthesis of new and published data (44 sampling locations in total) shows that only 5% of the observed data points indicated a uniform reactivity pattern (data points along the 1:1 line ± 1 y) independent of aquifer type (e.g., crystalline, glacial, sedimentary). Approximately 79% of the data points had lower apparent O₂ reaction times for O₂ than for NO₃⁻, indicating a late start pattern. This suggests that relatively deep electron donor sources from geological deposits of organic matter or sulfide minerals exert a strong control on the reduction of O₂ and NO₃⁻ independent of the water table and well depth (SI Appendix, section S1.3). This is supported by studies of the alluvial aquifer in the Central Valley of California and the glacial sediment aquifers in Minnesota. In these aquifer systems, the solid phase electron donors are the main energy sources for groundwater denitrification compared with surface-derived dissolved organic carbon (DOC) that is not sufficiently available based on electron and mass balance calculations (42, 52). In the alluvial aquifer in California, pyrite and reduced iron minerals (along with organic matter) have been identified as electron donors for reduction reactions. The analysis of apparent reaction times obtained at the multiwell sites (24) allows one to sample the vertical profile of the aquifer and to independently verify the stratified reactivity framework. Apparent reaction times indicate a late start pattern that is supported by detailed vertical profiles (substrata scale) (23, 42) showing the electron donor availability and degradation of O₂ and NO₃⁻ with depth. NO₃⁻ can be quickly reduced when it encounters reactive strata shown for aquifers worldwide (47, 53, 54), providing additional evidence on the prevalence of deeper reactivity.
Sixteen percent of the data points showed the early stop pattern, with shorter apparent reaction times for $O_2$ than for $NO_3^-$. For these sites, the predominant electron donor is likely surface-derived DOC that has not been mineralized in the unsaturated zone before reaching the aquifer (23, 24, 29, 55, 56). The low occurrence of the early stop pattern in our sample could be partially due to human activity, because increased nitrogen application and disruption of soil aggregates during cultivation decrease organic carbon in agricultural soils, decreasing DOC, the primary electron donor in surface and near-surface waters (57).

Apparent $O_2$ and $NO_3^-$ reaction times at the studied sites varied widely, indicating both uniform and hot spot dynamics in reactive strata. This diversity of sites with different subsurface characteristics and nutrient loads demonstrates the general utility of the proposed stratified reactivity framework for extracting information about the location and intensity of $O_2$ and $NO_3^-$ reactions from apparent reaction times. Atmospheric tracers and dissolved gases are preferable for deriving apparent reaction times at the stratum scale, because $NO_3^-$ rate estimates are scale-dependent (25) and approaches such as in-situ mesocosms do not sample the full stratum (52).

One of the central advantages of the stratified reactivity framework is that it relies on widely measured analytical parameters (e.g., solute concentrations, dissolved gas concentrations) and allows identifying the dominant reactivity pattern by analyzing apparent reactivity and not actual rate measurements with an individual sampling location. A combination of reactivity patterns is conceivable and would need further investigations. Nevertheless, the stratified reactivity framework could allow regional and interbiome comparison of subsurface reactivity to quantify the relative influence of climate, geology, and surficial processes (e.g., human disturbance, ecosystem development) in determining initial groundwater chemistry and removal capacity. From the perspective of the receiving surface waters, the framework could also analyze downstream river quality data.

**Fig. 1.** Schematic representation of the stratified reactivity framework. Potential vertical profiles of reactivity in an aquifer (Left) and resulting apparent reaction times (Right) are shown. The framework assumes similar stratum reaction times, $\tau_{\text{stratum}}$, (within a given layer) for $O_2$ and $NO_3^-$. A late start or an early stop of reactions along the flow paths results in differences in apparent $O_2$ and $NO_3^-$ reaction times. (A) Late start of reactivity creates the late start pattern, where the subsequent $O_2$ and $NO_3^-$ reduction only starts after a time, $\tau_{\text{enter}}$, when the water reaches the reactive layer. The late start increases the apparent $O_2$ reaction time compared with the stratum $O_2$ reaction time. The subsequent apparent $NO_3^-$ reduction is only marginally affected, because the time for $NO_3^-$ reduction starts only after $O_2$ is depleted, resulting in longer observed apparent $O_2$ reaction times compared with $NO_3^-$. (B) Early stop of reactivity results in $NO_3^-$ degradation first being limited by $O_2$ and then by the absence of electron donors. In the early stop scenario, the apparent reaction time for $O_2$ is smaller than for $NO_3^-$, and the difference between the two informs the characteristic time, $\tau_{\text{leave}}$, when reactive elements leave the reactive stratum. Evenly distributed electron donors throughout the aquifer correspond to a small $\tau_{\text{enter}}$ and large $\tau_{\text{leave}}$ and result in a uniform reactive stratum with a sequential reduction of $O_2$ and $NO_3^-$ starting at the water table. The relation of apparent $O_2$ and $NO_3^-$ reaction times in the case of a uniform reactive stratum is represented by the dashed line in the plot of apparent reaction times (also SI Appendix, section S2). The hot spot pattern is compatible with both stratified reactivity patterns (a*b*).
reaction times reveal important information about the subsurface, but solving the equations that govern these processes is extremely challenging. Existing estimates and predictions of subsurface reactions that affect groundwater as well as surface water quality. Because of the complexity of the system, it is clear that this protective capacity can be exceeded (61).

In the case of the early stop pattern observed in 16% of the samples, which occurs when surface-derived electron donors decrease with depth, NO$_3^-$ could pass through the reactive layer if electron donors are insufficient to substantially decrease O$_2$ and NO$_3^-$ concentrations. Remaining NO$_3^-$ below the reactive stratum would then persist for long time scales in the deeper aquifer, significantly affecting the quality of water resources and fueling the long-term eutrophication of surface waters as a function of the aquifer contribution to the overall streamflow.

Numerous studies deal with denitrification in aquifers and show that NO$_3^-$ is potentially removed in the subsurface, but solving the issue of heterogeneity of reactions and locating them has remained extremely challenging. Existing estimates and predictions of denitrification in aquifers often neglect stratified reactivity (55, 62), but accounting for stratified reactivity is central to understanding subsurface denitrification and global nitrogen cycling. Global estimates of groundwater denitrification of ~44 Tg of N per year derive from models of NO$_3^-$ reduction in shallow groundwater (0–5 m) with the assumption that DOC serves as an electron donor and that denitrification is negligible in deeper zones (55, 63, 64). Reconsidering this estimate by accounting for prevalent denitrification activity in deeper strata, the actual nitrate reduction in groundwater might be greater, toward the estimated upper limit of 138 Tg of N per year (55).

By adding information about apparent O$_2$ reaction times, previously estimated NO$_3^-$ reduction times could be reanalyzed with the stratified reactivity framework to quantify the reactive front (depth of electron donors) and related NO$_3^-$ stocks in reactive and nonreactive subsurface zones. The reactive patterns inferred from aquifers in different hydrogeological settings with this framework demonstrate the general applicability and capacity to improve predictions of subsurface reactions that affect groundwater as well as surface water quality. Because of the complexity of the system, it is clear that this protective capacity can be exceeded (61).

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could be used to infer reactivity patterns in “black box” situations using apparent reaction times and various chemical compounds, such as silica and carbon. For example, our proposed method could generate understanding about broader climatic, geological, and socioecological controls on weathering, freshwater chemistry, and transport of solutes from land to sea.

Materials and Methods

Study Sites. We used new and published data from 44 well locations from six different aquifers in France and the United States to infer reactivity patterns in groundwater. We investigated 16 well locations of the Pleine-Fougères aquifer in Brittany, France. We used pointwise atmospheric and anthropogenic tracer data (chlorofluorocarbons (CFCs), as well as O\textsubscript{2}, NO\textsubscript{2}, and N\textsubscript{2}O excess) interpreted within adapted lumped parameter models to derive apparent reaction times (Modeling Approach and SI Appendix, sections S1.1 and S1.2). The unconfined crystalline aquifer is partly characterized by granite and schist. The 16 wells are distributed over the 76-km\textsuperscript{2} study area with depths ranging from 28 to 98 m below the surface. The water table remains shallow, from close to the surface down to a few meters. The tracer-based mean groundwater age is around 40 y, with mean travel distances around 350 m (6).

We supplemented the dataset with already published O\textsubscript{2} and NO\textsubscript{2} apparent reaction times that were derived using a similar method to the Pleine-Fougères study at 23 wells in another aquifer in the United States. Published data of 23 wells were obtained from the regional alluvial fan aquifer in the Central Valley of California in a 4,000-km\textsuperscript{2} study area (39, 60). The 24 wells’ depths ranged from 3 to 95 m below the water table, with a median of 33 m, and water table depth ranged from 2 to 106 m below ground surface, with a median of 11 m. Tracer-based groundwater ages range from 0.2 to >100 y, with a median of 25 y. The five other O\textsubscript{2} and NO\textsubscript{2} apparent reaction times were obtained from a multivwell analysis, each from a separate aquifer system located in the United States (24). The aquifer in Stevinson, California, is also located in the Central Valley of California containing alluvial sand, silt, and clay, with a saturated thickness of 24 m. The water table is 5–10 m below the ground surface, and tracer-based groundwater ages range from approximately 10 to 30 y (52). The aquifer in Brighton, Michigan, is characterized by glacial outwash and till, with a saturated thickness of 17 m. The unsaturated zone is between 3 and 6 m below the ground surface. The tracer-based groundwater age varies between approximately 1 and 30 y (24, 40). Another aquifer is located in the North Carolina coastal plain. The saturated subsurface contains marine deposits of medium to fine sand and is 13 m thick. Tracer-based groundwater ages are between approximately 3 and 40 y. The water table is shallow, with just a few meters below the ground surface (24, 43). The Perham and Princeton aquifers, located, respectively, in central Minnesota and the Anoka Sand Plain in Minnesota, are characterized by glacial outwash with a saturated thickness of 5 m and 14 m, respectively. The water table depth of the Perham aquifer ranges from close to the ground surface up to a depth of 10 m. Tracer-based groundwater ages range between 5 and 50 y. The Princeton aquifer shows tracer-based groundwater ages within <1 and 30 y, and the water table is between 0 and 4 m depth below the ground surface (24, 41, 42).

Mixing of different waters arriving at the well allows the determination of characteristic O\textsubscript{2} and NO\textsubscript{2} reaction times. Based on apparent reaction times, the time spent in the reactive stratum, the time needed to enter the reactive stratum (late start pattern) or to leave it (early stop pattern), and the characteristic denitrification time in the reactive stratum (reactive stratum reaction time) can be deduced (SI Appendix, section S2).

Definitions.

Apparent reaction time. The apparent reaction time, \(\tau_{app}\), derives from the degradation of an element from its inlet concentration in the aquifer, \(C(t)\), to its sampled concentration, \(C(t)\). Assuming first-order kinetics and reaction occurrence along the whole flow path in uniform or randomly distributed hot spots, the relative reduction of concentration can be expressed as a function of travel time, \(t\):

\[\tau_{app}(t) = \exp(-\frac{C(t)}{\tau_{app}}) \quad \tau_{app} = \frac{1}{k_{app}}\]  

Sampled concentrations \(C(t)\)s are obtained by using Eq. 1.

Stratum reaction time. The stratum reaction time, \(\tau_{stratum}\), characterizes the degradation of a chemical compound within the reactive stratum of the aquifer, following either the early start or late stop pattern (Fig. 1). The reaction rate along a flow line solely depends on the time spent in the reactive stratum, \(\tau_{stratum}\). Assuming first-order kinetics, the relative stratum reaction rate along a flow line is expressed as

\[\tau_{stratum}(t) = \exp(-\frac{t}{\tau_{stratum}}) \quad \tau_{stratum} = \frac{1}{k_{stratum}}\]  

Because the reactive stratum may be determined by lithology, such as for autotrophic denitrification (24), or by surficial processes, such as for heterotrophic denitrification, the vertical stratification of reactivity indicates the type of electron donors supporting denitrification hot spots. The stratum reaction time would thus be substantially larger than the intrinsic reaction time, which can be measured in laboratory experiments with only the reactive minerals.

Modeling Approach. A time-modeling approach was used to infer apparent reaction times and stratum reaction times as well as the time to enter or leave the reactive stratum at the unconfined crystalline aquifer in Brittany, France. The modeled tracer concentrations at a well, \(C(t)\), were calculated from the convolution integral of Eq. 1 (32). CFC dating proxies interpreted within a formerly developed groundwater flow and transport model were used to generate the transit time distributions and CFC concentration distributions, from which we derived mean groundwater ages (6, 69). While site transmissivity and porosity were well constrained by the overall flux and CFC-12 concentrations, local sampling conditions in agricultural wells were calibrated using the detailed observed tracer concentrations. We evaluated the reliability of measured CFC-12 concentrations by comparing the agreement between all quantified CFCs sulfur hexafluoride concentrations (another anthropogenic gas used as a tracer of transit time), and dissolved silica concentrations (6, 7, 45, 70). This modeling approach let us include data from wells that were not drilled specifically for scientific monitoring and for which the depths of water arrivals were unknown (32). Additionally, transit time distributions at well locations could be derived by comparing parameter-estimation results. The solution term of Eq. 3 derives from the vertical distribution of electron donors (Fig. 1).

Calibration of NO\textsubscript{2} inputs to the aquifer. Land use information from 1991 to 2013 shows a general uniformity of agricultural practices over the catchment area with no significant evolution of specific land use types in any of the well capture zones, supporting the assumption of uniformly distributed NO\textsubscript{2} inputs to the aquifer. However, overall NO\textsubscript{2} input concentrations have strongly changed, with large increases from 1945 to 1980 and a maximum around 2000, followed by a gradual decline (73). Because of local differences in land management practices, we used the extensive dataset of NO\textsubscript{2} concentrations, N\textsubscript{2}O excess, and transit time distributions to reconstruct the NO\textsubscript{2} input chronicle \(\tau_{\text{input}}(t)\) with Eq. 1. This method also has the advantage of being applicable in areas where historical contaminant inputs are not known. Calibration of apparent and stratum reaction times. Apparent and stratum reaction times for O\textsubscript{2} and NO\textsubscript{2} reduction were calibrated to produce the best fit between simulated and measured O\textsubscript{2} and NO\textsubscript{2} concentrations using Eq. 1 for each location. Apparent concentrations are interpreted in terms of apparent O\textsubscript{2} and NO\textsubscript{2} reaction times, and stratum concentrations are interpreted in terms of stratum reaction times and the time to enter or leave the reactive stratum. The stratum reaction times are similar for O\textsubscript{2} and NO\textsubscript{2}, based on similar intrinsic reaction times (36) and similar limitations of the access to the reactive sites in the reactive stratum. We assumed that denitrification only started after O\textsubscript{2} was depleted below a threshold concentration, \(O_{2,\text{crit}}\), of 2 mg/L, which creates a time lag for denitrification, \(t\). Initial O\textsubscript{2} concentrations, \(O_{2,\text{init}}\), of 7 mg/L were considered to be constant over time, agreeing with measured concentrations in shallow piezometers.

ACKNOWLEDGMENTS. Financial support for this research was provided by the European Union’s Seventh Framework for research, technological development and demonstration under Grant 607150. This work was supported by the European Union Innovative Training Network: “INTERFACES: Ecosystemal interfaces as critical hot spots for transformations of ecosystem exchange fluxes and biogeochemical cycling.”


