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Contribution of wetlands to nitrate removal at the watershed scale

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Intensively managed row crop agriculture has fundamentally changed Earth surface processes within the Mississippi River basin through large-scale alterations of land cover, hydrology and reactive nitrogen availability. These changes have created leaky landscapes where excess agriculturally derived nitrate degrades riverine water quality at local, regional and continental scales. Individually, wetlands are known to remove nitrate but the conditions under which multiple wetlands meaningfully reduce riverine nitrate concentration have not been established. Only one region of the Mississippi River basin—the 44,000 km² Minnesota River basin—still contains enough wetland cover within its intensively agriculturally managed watersheds to empirically address this question. Here we combine high-resolution land cover data for the Minnesota River basin with spatially extensive repeat water sampling data. By clearly isolating the effect of wetlands from crop cover, we show that, under moderate–high streamflow, wetlands are five times more efficient per unit area at reducing riverine nitrate concentration than the most effective land-based nitrogen mitigation strategies, which include cover crops and land retirement. Our results suggest that wetland restorations that account for the effects of spatial position in stream networks could provide a much greater benefit to water quality then previously assumed.

Large-scale changes in land use and land cover and human amplification of the availability of fixed nitrogen have fundamentally changed nitrogen processing within the agriculturally dominated Mississippi River basin[1]. High fertilizer use on corn crops and in soybean production have increased nitrate inputs while hydraulic modifications to the landscape, such as subsurface drainage systems and the reduction of wetland cover, have reduced the nitrate removal capacity of the landscape. Both of these changes have been dramatic—nitrogen fertilizer inputs tripled between 1950 and 2000 and 60–90% of historic wetlands in the region have been drained since European settlement[2,3]. Elevated levels of nitrate in streams and rivers have serious human and environmental consequences, including degraded regional drinking water, harmful algal blooms and the formation of hypoxic zones[4,5]. In response to annually occurring hypoxia in the northern Gulf of Mexico, multiple states in the Mississippi River Basin have committed to reducing their nitrate exports by 40% or more; however, a recent meta-analysis of field-based nitrogen management strategies concluded this is not possible without removing large areas of land from agricultural production[6,7].

Mass balance studies across individual wetlands consistently show that wetlands remove nitrate[8,9]. In contrast, empirical studies at the watershed scale have found little to no influence of wetland cover on riverine nitrate[10,11]. The lack of response at the watershed scale could be due to interactions between terrestrial land cover and the wetland complex that mask the effect of a single land-cover variable[10], the large variability in the capacity of individual wetlands to remove nitrate[12] or simply insufficient range in the extent of wetland cover to detect a response. Given that multiple wetlands and wetland restorations within watersheds will be necessary to achieve water quality goals, better understanding of the parameters that influence the capacity of a wetland complex to reduce nitrate concentration at a watershed scale is needed. Without this knowledge, a comparative accounting of the water quality benefits and trade-offs of wetland protection and restoration relative to other management options in agricultural landscapes is not possible.

In this study, we investigated the interactive influences of multiple wetlands on riverine nitrate by isolating the effect of wetlands, crop cover and flow conditions. This was accomplished by land-use analysis and simultaneous observations of water chemistry for over 200 watersheds ranging in size from 1 to 6,000 km² and containing up to 2,000 wetlands. All observations occurred within a prototypical high-intensity agricultural basin, the Minnesota River Basin (MRB). The MRB is a 44,000 km² sub-basin of the Mississippi River Basin and, like much of the Midwestern USA, is heavily cultivated for corn and soybean production (Fig. 1a). Unlike the rest of the agricultural Midwest, however, the MRB retains a wide range in remnant wetland and shallow lake cover (Fig. 1b), providing an ideal natural laboratory for systematic and multidimensional examination of wetland effects on nitrate across a range of spatial scales. Riverine water samples were collected at an average of 53 locations per sample event (>200 sites in total) over 4 years and 7 sample events that spanned a wide range of seasonal and streamflow conditions (Fig. 1). For the drainage basin of each sampling site, we used 0.5-m-resolution land-use and wetland classification data to determine the extent of crop production, drainage area and wetland coverage, type and configuration as described more fully in the Methods. Wetland types included both permanent and ephemeral wetlands, isolated and flow-through wetlands, which could be vegetated marshes, lakes (primarily shallow) and riparian floodplains. Together, the suite of potentially interacting wetlands within a watershed forms a wetland complex, with a given topologic and dynamic connectivity structure that includes hydrologic exchange with the fluvial network, location, size and upstream intercepting area. Pairing spatially extensive water chemistry with high-resolution land-use information enabled robust analyses of the multiscale

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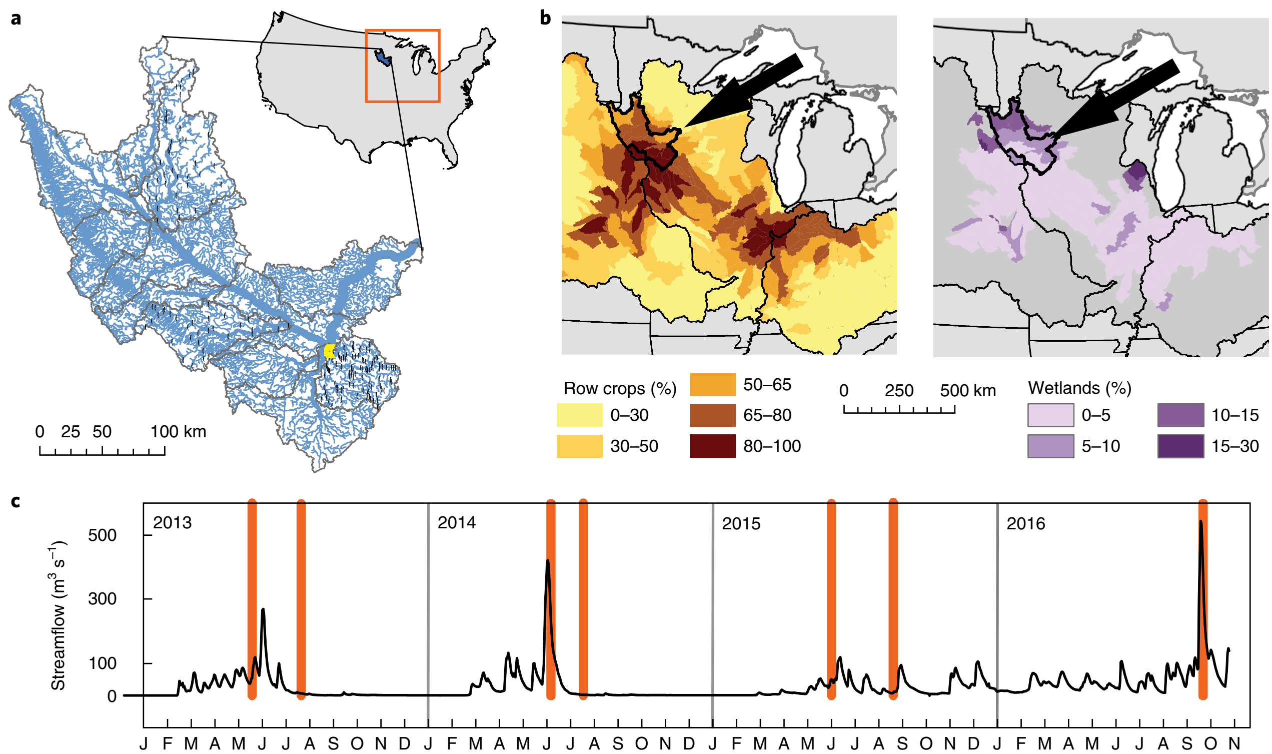

Fig. 1 | Nitrate observatory in the MRB. a, Nitrate concentration was observed at >200 sample sites (black markers) within the MRB. **b**, Row crop cover for all medium-sized river sub-basins (left) and wetland cover (wetlands + lakes) in sub-basins with > 50% corn or soybean crop cover (right) of the Ohio, Upper Mississippi and Missouri River basins. The MRB is indicated by the black arrows. **c**, Seven sampling events, shown as orange vertical lines, captured the range of hydrologic conditions within the four-year study period (daily hydrograph data from USGS station 05320500 located at the Le Sueur River outlet, highlighted in yellow in **a**).

interactions of hydrologic and biogeochemical processes that determine the influence of existing and potential expansions of wetland cover on water quality within an intensively cultivated landscape. It also allowed us to quantify landscape-scale controls (limiting or amplifying) on nitrate concentration while exploring the effect of wetland placement and hydrologic connectivity, inferred from streamflow conditions, on nitrate removal.

For the stream and river sites we examined, nitrate concentration was significantly related to land cover (composed of different percentages of crop and wetland cover) and mediated by streamflow and season. Under moderate- to high-streamflow conditions in spring, we found that riverine nitrate concentration decreased exponentially with increasing wetland cover (Fig. 2a, Supplementary Table 3, $n = 178$). Riverine nitrate also increased exponentially with crop cover under moderate- to high-streamflow conditions (Fig. 2b, Supplementary Table 3). Both results were independent of watershed size. Three of the four sampling events classified as moderate–high streamflow occurred in late spring when nitrate concentration and loading in the Upper Mississippi River Basin typically peak due to fertilizer applications and spring storms[13,14]. The significant relationship between riverine nitrate and land cover during higher-streamflow conditions in late spring is particularly important as nitrate loads during this period are predictive of the peak annual extent of the hypoxic zone in the Northern Gulf of Mexico[15,16]. The fourth sampling event used to capture moderate or high-streamflow conditions was in the fall of 2016. Although nitrate concentrations during this event were on average lower than during high-flow conditions in the spring when fertilizer application occurs, the response of nitrate to land cover was functionally the same as that of the late spring high-flow events and nitrate here also decreased exponentially with wetland cover at a similar rate (Supplementary Table 3). This result suggests that there are large terrestrial or groundwater stores of nitrogen that can be mobilized during a large event in any season[17].

Low nitrate concentration was consistently observed under low streamflow conditions and under all streamflow conditions at sites with either <50% crop cover or >15% wetland cover. Under low streamflow conditions ($n = 166$), the upper envelope of nitrate concentration showed a similar relationship to that seen at moderate and high streamflows (Supplementary Fig. 5). However, under these conditions many sites with low wetland cover also had low nitrate, probably due to a combination of low terrestrial nitrate input and high in-channel residence time[1,18]. At sites with <50% crop cover, nitrate was far lower than standards for human consumption under all streamflow conditions, and was often low enough to support sensitive aquatic taxa[19] (median NO_3^-–N = 0.21 mg l^{-1}, $n = 21$). This result suggests a potential threshold in intensive agricultural activity above which terrestrial processes for nitrogen removal or storage are saturated, similar to that previously reported for phosphorus[20].

The primary nitrate-removal process in fluvial ecosystems, denitrification, can be limited by an insufficient supply of organic carbon from agricultural landscapes[21,22]. In our highly modified and intensively drained watersheds, dissolved organic carbon (DOC) was positively and linearly related to the percentage of wetland cover, and most strongly related to the percentage of emergent vegetative wetlands, as has been reported elsewhere[23,24] (Supplementary Fig. 4, Supplementary Table 3). DOC relationships with wetland cover were not dependent on streamflow, season, drainage area or crop cover. By producing DOC in excess of internal demand, wetlands could enhance downstream nitrate removal via denitrification by balancing organic carbon demand with nitrate supply in carbon-poor river reaches[21,23,24].

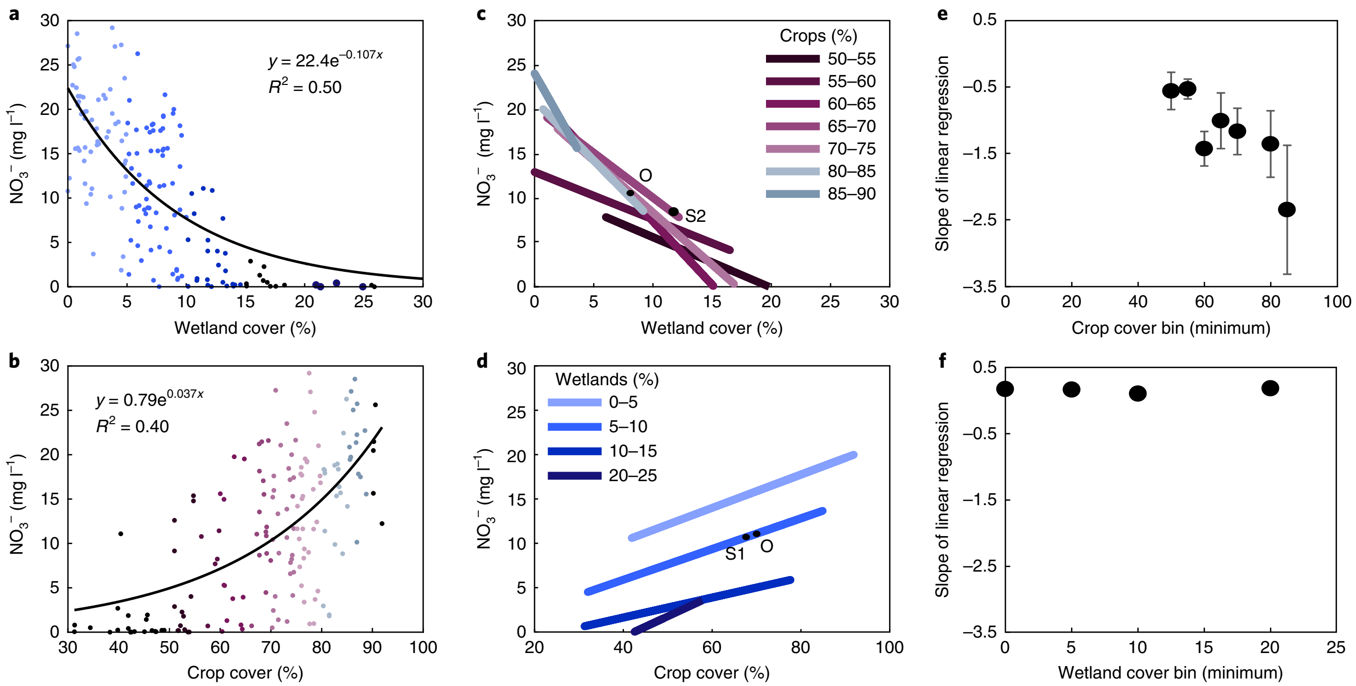

Fig. 2 | Effect of wetland cover/crop cover on riverine nitrate. a,**b**, Riverine nitrate decreases (increases) exponentially with wetland cover (crop cover) for moderate to high spring streamflows (**a**,**b**, respectively; where *y* is NO_3^-–N in the equation and *x* is land cover type; $n = 178$). **c**, Conditioning data on crop cover (in 5% bins as coloured) reveal statistically significant trends with wetland cover (**e**, and the slopes of the trends depend on crop cover; bars show the standard error). **d**, The same applies for the effect of wetland cover conditional on crop cover (**f**, but the slopes are independent of wetland cover). Nitrate management scenarios (S1, S2) and original (O) land use are indicated in **c**,**d**.

Disentangling nitrate sources and sinks

To isolate the effect of wetland presence (which is anticipated to reduce nitrate export) from crop absence (which by definition reduces nitrogen inputs) on riverine nitrate under moderate–high streamflow, we analysed the response of nitrate to wetland cover in the spring within eight subsets of the data for which crop cover was approximately constant. We observed statistically significant linear relationships between nitrate and wetland cover for seven of the eight data subsets (Fig. 2c). The slopes of the regression lines between nitrate and wetland cover increased with increasing crop cover, indicating that increases in wetland cover have a proportionally greater effect on nitrate removal in watersheds where crop cover is greater (Fig. 2e). For example, the reduction in nitrate concentration would be twice as great in a landscape with >80% cropland compared with a landscape with 65–80% cropland, and four times greater than in a landscape with 50–65% cropland for the same area converted to wetlands (Fig. 2e). We applied the same method to isolate the effect of crop cover under moderate–high streamflow, by evaluating relationships between nitrate and crop cover for five subsets of the data where wetland cover was approximately constant. We observed significant ($P<0.05$) linear relationships between nitrate and crop cover within four of the five wetland cover subsets (Fig. 2d) and little change in the slope of the regression lines (Fig. 2f). This result can be used to quantify the maximum crop cover for a given wetland cover based on an established threshold in nitrate concentration. For example, if the nitrate target is $10\,mg\,l^{-1}$ and the watershed contains 7% wetlands, then it can also support 70% crop cover without surpassing the target. A larger reduction in nitrate concentration is achieved by adding wetlands (Fig. 2e) than by removing the same area of crop cover (Fig. 2f).

To explore this areal efficiency further, we compare riverine nitrate concentration predicted for wetland restorations with those predicted from the most effective field-based nitrogen management practice possible: land retirement to pasture[6]. For this analysis we consider the maximum reduction in nitrate concentration that could be achieved from field nitrogen management. The base landscape for both scenarios is similar to current land use within the MRB, with 72% crop cover and 7% wetland cover (O in Fig. 2c,d). Predicted riverine nitrate for this landscape under high streamflow was $11.9\,mg\,l^{-1}$ (the *y*-coordinate for O, Fig. 2c,d). In the first scenario, S1, 5% of the cropped land in the base landscape is retired from crop production, effectively reducing crop cover by 5%. S1 resulted in a predicted nitrate of $11.1\,mg\,l^{-1}$, a 7% reduction (marker S1, Fig. 2d). In the second scenario, S2, 5% of cropped land in the base landscape is converted to wetlands; so wetland cover is increased by 5% and crop cover is decreased by 5%. Predicted nitrate under the wetland restoration scenario is $8.0\,mg\,l^{-1}$, a 33% reduction (marker S2, Fig. 2c). Comparing the two scenarios, wetland restoration is five times more effective at reducing nitrate concentration at the watershed outlet than field-based nitrogen management strategies applied to an equivalent land area. The high areal efficiency of wetlands can offset low voluntary adoption rates of field-based nitrogen reduction practices.

We used the exponential relationships between nitrate and land cover (wetlands and crop land) to quantify the loss of wetland water quality services that is incurred through the loss of the historic wetland complex to agricultural production in the Le Sueur River Basin, a sub-basin of the MRB. This sub-basin has lost an estimated 60% of wetland cover from pre-settlement cover yet still contains one of the more intact wetland complexes in the region[2,25]. If the Le Sueur River Basin had retained its entire historic wetland cover (20%) while maintaining the current agricultural land cover (72%), nitrate at the basin outlet in June would be 80% lower ($3.0\,mg\,l^{-1}$, compared with the current basin outlet nitrate of $14.1\,mg\,l^{-1}$, averaged for the June sample dates). This reduced nitrate concentration is well within established requirements for human consumption; however, full recovery of aquatic ecological function (that is, the recovery of sensitive aquatic species that require nitrate $< 1\,mg\,l^{-1}$)[19]

would necessitate wetland creation in excess of historical cover or reductions in nitrogen inputs.

Dynamic wetland connectivity

Both permanent wetlands and ephemeral wetlands, that is, areas that are periodically but not permanently wetted, have the potential to remove nitrate from surface water. However, our findings suggest that the influence of ephemeral wetlands on riverine nitrate concentration depends strongly on their hydrologic connectivity to the river network. Our analysis indicates that ephemeral wetlands contributed to watershed nitrate removal most substantially under high-streamflow conditions. Riverine nitrate was significantly related to ephemeral wetland cover under high-streamflow conditions in June 2014 and September 2016, with coefficients that are three times greater than the two significant relationships detected in moderate- to low-flow conditions (Fig. 3, Supplementary Table 3). As expected, the high-streamflow events had similar slopes but differed in magnitude (higher for June events), probably due to lower landscape nitrate inputs in the fall. We conclude that functionally effective wetland cover (that is, the fraction of wetland cover that is actively involved in nitrate removal) is a dynamic variable that changes with the same variables that affect streamflow: soil moisture, precipitation and evapotranspiration. Climate models for this region project an increased frequency and magnitude of precipitation that would increase ephemeral wetland hydrologic connectivity[26]. Under such conditions, we would expect ephemeral wetlands to play a larger role in reducing riverine nitrate[27]. Because of the dynamic nature of effective wetland cover, the contributions of wetlands to water quality can only be accurately assessed under conditions in which they are hydrologically connected to the fluvial network[28,29].

Effect of wetland position on riverine nitrate

Although riverine nitrate was strongly related to wetland cover at moderate to high streamflows, there was still considerable unexplained variability in nitrate after accounting for effects of crop cover. In an analysis of a subset of data for which crop cover, permanent wetland cover and contributing drainage area were similar, we found that spatial patterning (that is, the configuration of multiple wetlands relative to the river network and to each other) explained substantial additional variability in nitrate response that wetland cover alone did not capture (Fig. 4a–e). For this analysis, the effect of wetland location was parameterized as the fraction of a site's contributing area that was intercepted by a wetland (intercepted fraction). In sub-basin A, which had the highest nitrate concentration at its outlet, all of the wetlands were isolated from the fluvial network and thus do not impact nitrate inputs from much of the watershed. In sub-basins B and D, which had intermediate nitrate concentrations at their outlets, the primary wetlands were located on the network but the intercepted fractions were only approximately half of the watershed area. By contrast, in sub-basins C and E, which had the lowest nitrate concentration at their outlets, the entire contributing area was intercepted by wetlands that were large relative to the contributing drainage area. These results show that network position moderates the effectiveness of wetlands for reducing riverine nitrate. For example, a wetland complex that intercepts 100% of the drainage area is three times more effective than one that intercepts 50% of the drainage area (Fig. 4). Wetland spatial patterning thus plays a key role in determining the nitrate removal potential for a suite of wetlands within a watershed.

The effect of wetland spatial patterning may explain why our analysis of land conversion scenarios concluded that a considerably larger area of wetland cover (~5%) will be required to achieve a 30% reduction in nitrate than previously estimated (2%) from linearly upscaling individual wetland performance[30,31]. The complex interactions across multiple wetlands in the context of crop land

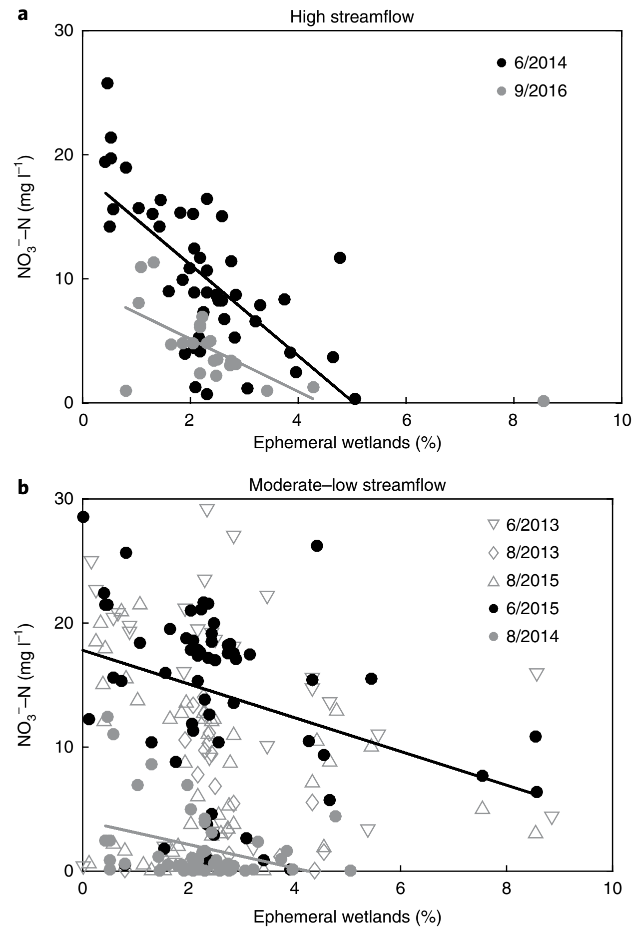

Fig. 3 | Effect of wetland connectivity and streamflow on riverine nitrate. **a**,**b**, Significant relationships between nitrate concentration and the percentage of ephemeral wetlands are detectable for a range of hydrologic and seasonal conditions and exhibit steeper slopes and higher predictive ability at high streamflow (**a**) than at low or moderate streamflow (**b**) (sampling dates in legend next to symbols are in the format month/year). Filled symbols represent data where significant relationships were detected (shown with lines, two excluded outliers from the 9/2016 linear regression are described in Methods). Open symbols represent data where no relationship was detected. Streamflow conditions are provided in Supplementary Table 1 and full statistics in Supplementary Table 3.

that we have shown suggest that upscaling from one wetland to a wetland complex within a watershed is nonlinear. Furthermore, the existing wetlands that informed our empirical analysis—the size and location of which were determined by a combination of long-term hydrogeological processes and past human land management—are almost certainly not optimally positioned, with respect to each other and to the river network, to remove nitrate, yet probably represent the locations where wetland restoration would occur, due to the physical and economic constraints on locating them elsewhere. Thus, our results also portray a more realistic estimation of the nitrate reduction potential that is achievable from wetland restoration.

With a growing global population, increasing affluence and development of biofuel as an energy source, agricultural expansion and intensification will continue to pressure riverine water quality in the Mississippi River basin and other predominately agricultural watersheds worldwide. Our detailed, large-scale data collection and observational analyses of nitrate in a range of agricultural watersheds show that a quantitative framework for assessing wetland

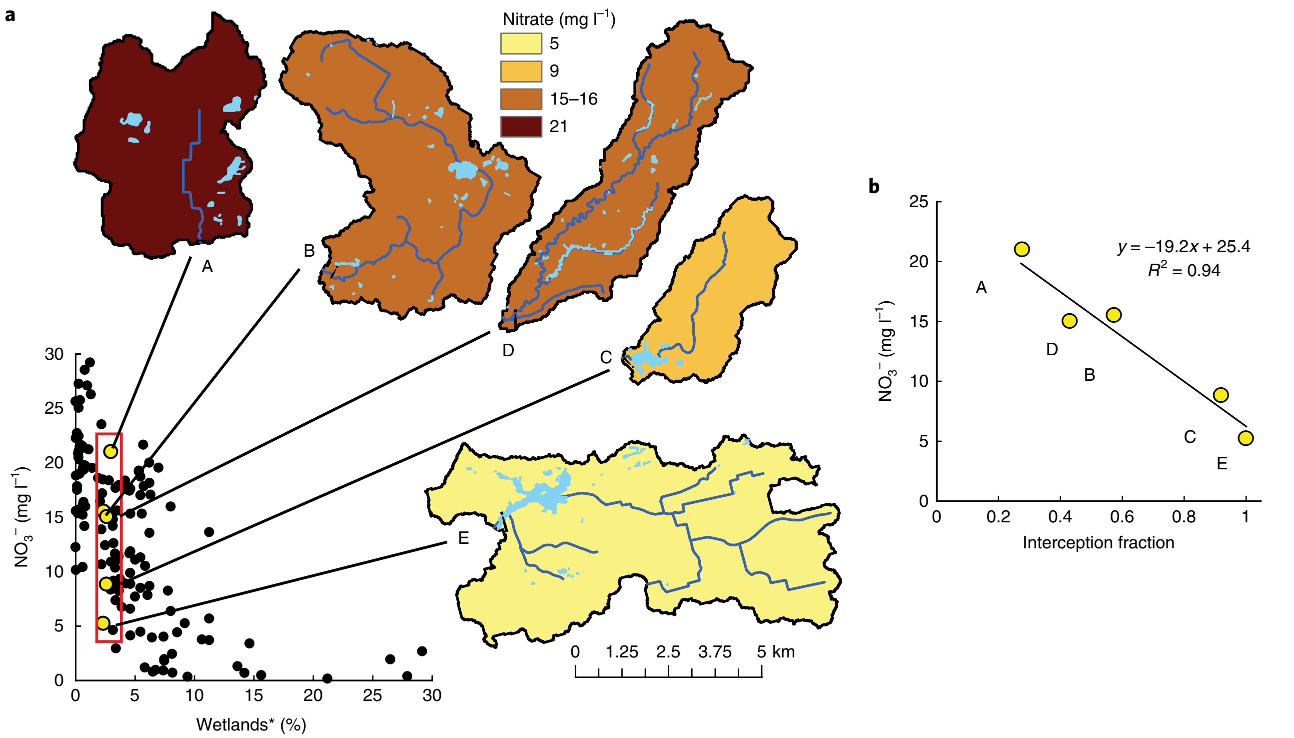

Fig. 4 | Effect of wetland spatial patterning on riverine nitrate. a, Five sites with similar crop cover (69–84% in increasing order B–E–D–A–C) and 2.32–2.97% non-ephemeral wetlands showed different nitrate concentrations. **b**, However, accounting for spatial wetland patterning (parameterized as the percentage of intercepted area = fraction of a site's watershed area intercepted by a wetland) significantly reduced variability, suggesting a reliable predictive relationship. Nitrate observations at A–C were measured in 2015 and at D–E in 2014.

functioning is imperative. Our analyses develop a framework that considers the value of multiple wetlands for nitrate export reduction at the watershed scale and includes interactions with land use, streamflow and spatial patterning that regulate the ability of wetland complexes to reduce riverine nitrate. Our findings provide the basis on which comparative analysis of the water quality benefits and trade-offs of wetland protection and restoration relative to other management options in agricultural landscapes can be performed. Specifically, we show that nitrate concentrations are significantly related to wetland cover under high-streamflow conditions, when much of the nitrate export occurs. Ultimately, we show that re-incorporating wetlands into intensively managed agricultural watersheds would reduce riverine nitrate and contribute to improving local water quality and reducing downstream environmental degradation.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41561-017-0056-6.

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Author contributions

A.T.H. and J.C.F. conceived of the project. A.T.H. and C.L.D. conducted field work and analysed laboratory data. E.F.-G., J.C.F. and A.T.H. interpreted results. A.T.H. wrote the original paper while C.L.D., J.C.F. and E.F.-G. contributed significantly to the final version.

Competing interests

The authors declare no competing financial interests.

Additional information

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Methods

Study area. Our analysis included more than 200 study sites in the MRB, mostly located within the river network of three major tributaries to the Minnesota River: the Cottonwood River, the Chippewa River and the Le Sueur River (Fig. 1). Current land use within the MRB is dominated by intensively managed row crop agriculture (~74% land use), primarily corn and soybean[32]. The annual incremental nitrate yield from the MRB is amongst the highest within the Mississippi River Basin[33]. MRB soils are predominantly poorly drained glacial tills similar to much of the central plains region. The majority of historic natural wetlands were drained during agricultural expansion, first through the construction of ditches and later with the installation of subsurface tile drainage as occurred throughout much of the Upper Mississippi River Basin[2].

Sample collection and laboratory analysis. Water samples were collected in early summer (June) and late summer (August or September) each year for three years (2013 to 2015) with an additional sampling event in September 2016 when an unusually large event for this season occurred. On average, 53 sites were sampled per event (>200 sites in total) but not all sites were sampled for each event (Supplementary Table 1). Sampling events consisted of large-scale, labour-intensive efforts to collect water samples for chemical analysis from as many sites as possible over a short enough period of time that hydrology and nitrate concentrations were fairly constant, within a 3 day window (Supplementary Fig. 2). Note that during short and intense streamflow events, the underlying assumption of uniform flow conditions throughout the watershed would not be met due to transience in the hydrograph, especially in the headwaters. However, our results appear to be robust to this condition, perhaps due to hysteresis in the nitrate response to discharge[34]. To examine the hydrologic controls on nitrate removal, sampling occurred under a range of streamflow and soil moisture conditions. In 2013, 2015 and 2016 all samples were collected within the Le Sueur River Basin, a medium-sized river (USGS hydrologic unit code size 8; that is, HUC-8) sub-basin of the MRB, under typical high spring flow conditions (daily streamflow on sample dates had exceedance probability of 10% and 13%, respectively, based on a frequency analysis of all daily flows observed over the past 40 years[35]). In 2014, samples were collected within the Chippewa River, Cottonwood River and Le Sueur River sub-basins immediately following an extreme event (daily streamflow exceedance probability of 0.1%). Sites were chosen to span the full range in land use and drainage area that exists within these basins (Supplementary Fig. 1). On average, 73% of the land use within the contributing drainage areas for all sites was row crop agriculture, 10% was pasture or prairie (together referred to as grasslands), 6% was impervious surfaces and 5% was wetlands (where wetlands includes all landscape features that appear wetted in spring aerial photographs, including ephemeral wetlands that are not wetted under other conditions). Further classification of wetlands to specific types is available within the attribute table for the National Wetland Inventory (NWI) layer. The categorization within the NWI uses the Cowardin classification system[36].

Four sites were sampled but excluded from all analyses and plots because they were located downstream of a hypereutrophic lake where nitrate cycling was heavily influenced by algal fate and transport. Water samples were also collected from subsurface drainage outlets and analysed for water chemistry. For reference, the average April–June subsurface drainage (tile) outlet nitrate estimated from samples collected over the 3 years was 20.3 mg l^{-1} (standard deviation (s.d.) = 6.2 mg l^{-1}, n = 19).

Water samples were collected in acid-washed polypropylene bottles rinsed twice with site water before sample collection. All samples were transported back to the lab in coolers and filtered within 24 h of collection. Water samples were analysed for concentrations of nitrate plus nitrite (referred to as NO_3^-–N), ammonium–N (NH_4^+–N), total dissolved nitrogen (TDN) and DOC at the laboratories at the University of Minnesota. All concentrations are reported as mass of N (or C) atoms per unit volume. Samples were filtered through pre-ashed glass-microfiber filters with a nominal pore size of 0.7 µm (Whatman GF/F filters). Water samples for TDN and DOC were acidified with 2 N HCl to pH 2 and stored at 4 °C until analysis using a Shimadzu TOC V CHN analyser (Shimadzu Scientific Instruments). NO_3^-–N samples were frozen before analysis. NO_3^-–N was analysed using cadmium reduction to NO_2–N followed by colorimetric analysis with a Lachat Quickchem FIA (Hach Company). NH_4^+–N was also analysed by colorimetric analysis with a Lachat Quickchem FIA.

Hydrologic conditions. Streamflow data from the three larger sub-basin outlets was used to classify hydrologic conditions during each sampling event (Supplementary Table 1). Events with exceedance probabilities below 5% were classified as high streamflow, between 5 and 25% as moderate streamflow and above 25% as low streamflow. Although hydrology probably was not behaving as a unit across all sites during high-streamflow events, we used it primarily to classify event type and intentionally did not include it in detailed analyses. Actual discharge conditions were measured using the velocity–area method for a subset of events and sites and scaled linearly with drainage area for these events[37]. The probability of exceedance for daily streamflow on the seven sampling events, as reported in Supplementary Table 1, was calculated using all dates from the USGS streamflow monitoring records at the three sub-basin outlets over the 40 year period from 1976–2015. Streamflow records extend earlier than this range but streamflow is probably not stationary outside this window[38]. June 2013 and June 2015 sampling occurred under moderate but typical early summer streamflow conditions. In contrast, June 2014 sampling was on the falling limb of a large event and occurred under streamflows with less than 1% probability of exceedance for all the three basins.

Land cover and spatial analysis. Land cover was defined as the percentage of upstream drainage area contributing streamflow to the sampled location that was classified with a given land-use category. The contributing drainage area for each site was determined by applying Arc Hydro watershed delineation tools to 30-m-resolution digital elevation models in ArcGIS (ESRI ArcGIS Desktop release 10.3.1). Contributing drainage areas ranged from 0.25 to 5,239 km^2 across all sites. The percentage of land use for each drainage area was determined using intersection analysis between the delineated boundaries of the drainage areas for each site and the attribute data in the 2015 update to the NWI[39] and the 2013 Minnesota Land Cover Classification layer (MLCC)[40]. Land use was grouped into 11 categories based on data from the MLCC (Supplementary Table 2). Some land-use categories were consolidated when appropriate; all impervious surfaces were consolidated into one land use and the category perennial grass cover in our analysis includes grassland (prairie), hay and pasture from the MLCC. There are four primary land uses in the MRB; row crop agriculture (median land cover = 73.4%), wetlands (median land cover = 5.7%), impervious surfaces (median land cover = 2.4%) and perennial grasses (including pasture, hay, grasslands; median land cover = 11%). Land-use analysis of the Upper Mississippi River basin, the Ohio River basin and the Missouri River basin, used to demonstrate the extent of intensively managed agriculture, was derived from the National Land Cover Classification layer (2011 NLCD)[32]. The 2011 NLCD, derived from Landsat satellite data, has a spatial resolution of 30 m. As such, this dataset is too coarse to detect the many small depressional wetlands within the MRB landscape and therefore was not used for land-use classification for empirical analysis within this study, where higher resolution was required. The United States Department of Agriculture crop data layer (NASS 2014) was used to further classify HUC-8 watershed land use by crop[41]. In Fig. 1, wetlands are shown for HUC-8 watersheds with >50% corn or soybean land cover only (NASS 2014 crop data layer categories 1, 5, and 241).

The NWI further classifies wetlands and lakes by vegetation type, soil, and water regime based on the Cowardin classification system[36,39]. The updated NWI data layer was not available at the time of analysis for the Chippewa River basin so analysis of ephemeral wetland effects was restricted to the Cottonwood and Le Sueur River basin. The percentage of wetland cover from the MLCC layer agreed with the percentage of wetland cover from the NWI layer (root mean squared error = 0.40%) and for any given site the error was a maximum of 8%.

The primary wetland types in our study area are: emergent vegetative wetlands (referred to as marshes), shallow lakes (<2 m maximum depth) and riparian floodplains. Deep lakes (>2 m maximum depth) are present in this landscape but accounted for <0.5% of the Le Sueur River basin land cover and <0.1% of the Cottonwood River basin land cover (Supplementary Table 2). All wetlands and lakes of all sub-classes within the contributing drainage area were included in the calculation of the percentage of wetland cover (Supplementary Table 2). Ephemeral wetlands were defined as all wetlands in the NWI layer that were not classified as permanent.

Geospatial analysis was applied to the NWI wetland data and the 30-m-resolution digital elevation model to determine interception area for five sites using the NWI data as presented in Fig. 4. Two of these sites were located in the Cottonwood River basin and three were in the Le Sueur River basin. The area contributing drainage to each wetland within the watersheds for these sites was calculated using watershed delineation tools available within ArcGIS. Interception fraction was defined as the fraction of the area contributing drainage to the sample site that was first intercepted by a wetland. The inability to account for residence time is a limitation of this analysis. We excluded riparian floodplains with surface area less than 0.7% of their intercepting area on the grounds that they were probably not hydrologically accessed under the moderate flow conditions of June 2013 and 2015. For similar reasons and to simplify the calculations, we also excluded ephemeral isolated depressional wetlands from the analysis that had a surface area of less than 0.7% of their intercepting area. Due to these simplifications, actual interception area is slightly underrepresented in our analysis.

Statistical analysis. All statistical analysis was completed using the Statistics Toolbox in Matlab R2015b. Land-use effects on nitrate were determined using a regression analysis of water chemistry with percentage land cover type. Impervious cover was low (Supplementary Table 2) and not significantly related to nitrate (Supplementary Table 3). Grassland cover was moderate (Supplementary Table 2), and often significantly related to nitrate although not the focus of this study (Supplementary Table 3). Adjusted R^2 was used when comparisons were made across datasets of different sample size. Statistical significance was evaluated at P < 0.05 for all analyses.

The effect of wetland cover was disentangled from crop cover using conditional analysis. In conditional analysis the dependency of the response variable to one of the independent variables is evaluated within a subset of the data for which there is no response to the other independent variable. The two predictor variables in our analysis, crop cover and wetland cover, were highly correlated with each other ($R^2 = 0.49$, $P < 0.0001$, $n = 201$) and were part of the same type—that is, not independent of one another. These conditions restricted the application of other forms of analysis such as analysis of covariance (ANCOVA) or multiple regression analysis[42].

We evaluated the effect of ephemeral wetlands on nitrate concentration using linear regression (Fig. 3). Sites with ephemeral cover >6% were relatively rare in our dataset, and the greater range in ephemeral wetland cover under moderate flow conditions was a sampling artifact. For the September 2016 regression we excluded two points that we considered outliers in the regression (points not shown). This regression was significant with or without these points (see statistical results in Supplementary Table 3) but the sites were unrealistically effecting the regression. Both points had nitrate concentrations near zero. One had ~9% ephemeral wetland coverage, which was outside of the linear range, and the other was located at an outlet of a deep lake.

Data availability. Land use was determined using multiple available spatial data layers, two derived from 0.5-m-resolution aerial photography; the 2015 update to the National Wetland Inventory (NWI)[39] and the 2013 Minnesota Land Cover Classification layer (MLCC)[40]. The National Land Cover Classification layer (2011 NLCD)[32] was used for land-use analysis on the Mississippi, Ohio and Missouri river basins. The United States Department of Agriculture crop data layer (NASS 2014) was used to further classify HUC-8 watershed land use by crop[41].

Daily average streamflow data were obtained from USGS gauges 05320500 (Le Sueur River outlet), 05317000 (Cottonwood River outlet) and 05304500 (Chippewa River outlet). Nitrate concentration at sub-basin outlets were obtained from the Minnesota Pollution Control Agency (MPCA), which maintains a water chemistry monitoring program at these sites[43].

The MPCA typically samples every two weeks in addition to targeting high-flow events: reported nitrate concentrations at the tributary outlets are from the date nearest our sample event date (Supplementary Table 1). MPCA nitrate concentration data was included in our analysis of land-use effects only if water samples were collected within our sampling event window. New data generated from this study have been deposited in a persistent repository and can be accessed at https://doi.org/10.13020/D6FH44).

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