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PAŃSTWOWY INSTYTUTU BADAWCZY**

Department Soil Science and Environmental Analyses

Discipline: AGRICULTURE AND HORTICULTURE

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**Factors Controlling Content and Transformations
of Dissolved Organic Matter Components in
Agricultural Soils
at Field and Global Scales**

Doctoral dissertation prepared under the scientific supervision of
dr hab. Bożena Smreczak, prof. of the Institute
Auxiliary supervisor: dr hab. Aleksandra Ukalska-Jaruga

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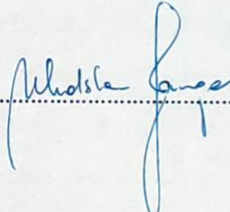
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List of Abbreviations

Abbreviation	Explanation of the abbreviation
ACT	Actinomycetes
AI	Aridity Index
AN	Available Nitrogen
ANOVA	Analysis of Variance
AP	Available Phosphorus
BD	Bulk Density
BRT	Boosted Regression Trees
CEC	Cation Exchange Capacity
CI	Confidence Interval (e.g., 95% CI)
CN	Carbon–Nitrogen (CN analyzer)
CT	Conservation Tillage
DOC	Dissolved Organic Carbon
DOC/SOC	Ratio of Dissolved Organic Carbon to Soil Organic Carbon
DOCX	DOC-efficiency metric (per-unit DOC effect on CO ₂ or yield)
DON	Dissolved Organic Nitrogen
DOP	Dissolved Organic Phosphorus
DOM	Dissolved Organic Matter
EC	Electrical Conductivity
EOC	Extractable Organic Carbon
E4/E6	Ratio of SUVA ₄₆₅ to SUVA ₆₆₅ (DOM molecular size and humification)
FEM	Fixed-Effects Model
REM	Random-Effects Model

F:B	Fungi-to-Bacteria ratio
GN	Gram-Negative bacteria
GP	Gram-Positive bacteria
ICP-MS	Inductively Coupled Plasma–Mass Spectrometry
ln(RR)	Natural log Response Ratio (meta-analysis effect size)
MAOC	Mineral-Associated Organic Carbon
MAP	Mean Annual Precipitation
MAT	Mean Annual Temperature
MBC	Microbial Biomass Carbon
MBN	Microbial Biomass Nitrogen
NIR	Near-Infrared spectroscopy
VIS–NIR	Visible–Near–Infrared spectroscopy
NH₄⁺-N	Ammonium-N
NO₃⁻-N	Nitrate-N
NM	Nitrogen fertilizer + Manure
NS	Nitrogen fertilizer + Straw
PLFA	Phospholipid Fatty Acids
POC	Particulate Organic Carbon
RF	Random Forest
SD	Standard Deviation
SE	Standard Error
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TOC	Total Organic Carbon
TC	Total Carbon

TN	Total Nitrogen
SUVA	Specific UV Absorbance
SUVA280	Specific UV Absorbance at 280 nm (aromaticity)
SUVA465	Specific UV Absorbance at 465 nm (humic substances)
SUVA665	Specific UV Absorbance at 665 nm (condensed aromatic structures)
SVP	Seasonal Variability of Precipitation
UV–VIS	Ultraviolet–Visible spectroscopy
βG	β-1,4-Glucosidase

Abstract (English)

Dissolved organic matter (DOM)—operationally comprising dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP)—is the most mobile and bioactive fraction of soil organic matter (SOM). Yet its controls and responses across climates, managements, and depths remain insufficiently resolved. This thesis evaluates how nitrogen (N) addition, climate warming, and agricultural practices regulate DOM quantity and quality, and how cropping system and soil depth modify these effects. Analyses span global syntheses and a long-term field experiment to better capture SOM/DOM dynamics. A global database (>6,700 observations) supported three meta-analyses: (i) 1,132 paired DOC observations from 103 studies on N addition; (ii) 321 DOC and 187 DON observations from 50 studies on warming; and (iii) 3,539 DOC, 196 CO₂, and 1,424 yield observations from 286, 15, and 92 studies on cropland management. The field component compared crop rotation versus monoculture, sampling 0–30, 30–60, and 60–90 cm, quantifying DOC, DON, DOP, optical indices (specific ultraviolet absorbance (SUVA), absorbance ratio at 465 and 665 nm (E4/E6)), and their drivers.

N addition increased SOC by 8.16% (humid) and 2.05% (non-humid). DOC and microbial biomass C (MBC) declined in humid regions (–2.49%, –2.60%) but increased in non-humid regions (+7.30%, +8.74%). Precipitation seasonality emerged as a universal driver; baseline SOC predicted DOC in humid regions, while N rate did so in non-humid regions. Soil pH reversed DOC associations between humidity regimes, underscoring the need for region-specific parameterization. Warming decoupled C–N dynamics: mean DOC and MBC were unchanged, whereas SOC (–3.36%) and DOC:DON (–10.79%) decreased while DON (+8.84%) and NO₃[–] (+13.12%) increased. Soil moisture governed DOC responses; nitrate availability predicted DON responses, indicating moisture–N interactions as the central mechanism of warming-induced C loss and nitrate accumulation. Across croplands, all evaluated practices increased DOC, with the largest gains under mineral N + manure (NM, +78%) > manure (M, +50%) > N + straw (NS, +41%). The DOC/SOC ratio generally rose under NM, NS, M, straw (S), and mineral N; it was unchanged under conservation tillage and declined under biochar (–25%), consistent with biochar’s stabilization bias. A new “DOC-efficiency” metric showed that DOC associated

with mineral N, straw, and NM more effectively increased yields and lowered CO₂ intensity than DOC under conservation tillage or biochar, emphasizing management rate and duration as key design factors.

In the long-term field study, depth strongly structured DOM: DOC, DON, and DOP decreased with depth, while SUVA increased and E4/E6 decreased, indicating more aromatic, humified, high-molecular-weight DOM in subsoils. Cropping effects were modest and depth-limited: monoculture retained higher DOC at 60–90 cm; DON and DOP did not differ between systems. Random-forest analyses identified element-specific drivers (DOC: total N, total C, DOP; DON: DOC, depth, available P; DOP: humus, total P, available P), reflecting C–N–P coupling with distinct constraints.

Collectively, the findings establish a regime-specific, moisture-sensitive, and quality-aware framework for DOM dynamics: (i) N effects on DOC invert across humidity regimes; (ii) warming mobilizes reactive N while promoting net C loss via moisture-mediated DOC turnover; (iii) synchronizing C supply with N maximizes DOC's agronomic benefits while minimizing CO₂ intensity; and (iv) subsoils harbor more humified DOM shaped by depth and mineral interactions. The work delivers actionable parameters and diagnostics (soil pH, initial SOC, N rate, soil moisture, DOC-efficiency, E4/E6) to improve ecosystem models and guide climate-smart, depth-aware soil management.

Keywords: dissolved organic matter components, nitrogen addition, global climate warming, agricultural management, crop rotation vs. monoculture, spectroscopy, meta-analysis

Streszczenie (Polish)

Rozpuszczona materia organiczna (RMO)—operacyjnie: rozpuszczony węgiel (RWO), azot (RAO) i fosfor (RFO)—jest najbardziej mobilną i bioaktywną frakcją materii organicznej gleby (MOG). Jej uwarunkowania i reakcje wzdłuż gradientów klimatu, praktyk rolniczych i głębokości są jednak słabo rozpoznane. W pracy oceniono, jak dodatek N, ocieplenie klimatu i praktyki rolnicze kształtują ilość i jakość RMO oraz jak modyfikują je system uprawy i głębokość gleby. Analizy przeprowadzono w skali globalnej (synteza literatury) i w skali pola (doświadczenie długoterminowe).

W skali globalnej zbudowano bazę >6,700 obserwacji dla gleb rolniczych i wykonano trzy metaanalizy: (i) 1132 wyników RWO z 103 badań — efekt dodatku N; (ii) 321 RWO i 187 RAO z 50 badań — efekt ocieplenia; (iii) 3539 RWO, 196 emisji CO₂ i 1424 plonów z odpowiednio 286, 15 i 92 badań — wpływ praktyk rolniczych. W skali pola porównano płodozmian z monokulturą (warstwy 0–30, 30–60, 60–90 cm), oznaczając RWO, RAO, RFO, wskaźniki optyczne (SUVA, E4/E6) i główne czynniki sterujące.

Dodatek N zwiększał węgiel organiczny gleby (WOG) o 8,16% w regionach wilgotnych i o 2,05% w suchych. RWO i węgiel w biomasie mikroorganizmów (MBC) spadały w klimacie wilgotnym (–2,49%, –2,60%), a rosły w suchym (+7,30%, +8,74%). Sezonowość opadów była uniwersalnym predyktorem; wyjściowy WOG determinował RWO w regionach wilgotnych, a dawka N — w suchych. Odczyn (pH) odwracał powiązania RWO między reżimami, wskazując na potrzebę regionalnej parametryzacji. Ocieplenie rozpręgało dynamikę C–N: średnie RWO i MBC nie zmieniały się, natomiast WOG (–3,36%) i stosunek RWO:RAO (–10,79%) malały, a RAO (+8,84%) i NO₃[–] (+13,12%) rosły. Reakcje RWO kontrolowała głównie wilgotność gleby, a RAO — dostępność azotanów, co dowodzi, że interakcje wilgotność–N napędzają utratę C i akumulację reaktywnego N w cieplejszym klimacie.

Na gruntach ornych wszystkie oceniane praktyki zwiększały RWO, najsilniej: N mineralny + obornik (NM, +78%) > obornik (M, +50%) > N + słoma (NS, +41%). Stosunek RWO/WOG rósł pod NM, NS, M, słomą (S) i N mineralnym, pozostawał bez zmian przy uprawie konserwującej, a spadał pod biowęgłem (–25%), zgodnie z jego tendencją do stabilizacji C. Nowy wskaźnik „efektywności RWO” wykazał, że RWO

związany z N mineralnym, S i NM skuteczniej zwiększa plony i obniża intensywność emisji CO₂ niż przy uprawie konserwującej lub biowęgla, podkreślając znaczenie dawki i czasu trwania praktyk.

W doświadczeniu polowym głębokość silnie różnicowała RMO: RWO, RAO i RFO malały z głębokością; SUVA rosła, a E4/E6 spadało, co wskazuje na bardziej aromatyczne, ushumifikowane, wysoko-cząsteczkowe frakcje w podglebiu. Efekty systemu uprawy były niewielkie i zależne od głębokości: w monokulturze wyższe RWO występowało na 60–90 cm; RAO i RFO nie różniły się między systemami. Analiza lasem losowym ujawniła zróżnicowane sterowniki (RWO: N całkowity, C całkowity, RFO; RAO: RWO, głębokość, P przyswajalny; RFO: próchnica, P całkowity, P przyswajalny), odzwierciedlając specyficzne ograniczenia pierwiastkowe w ramach sprzężeń C–N–P.

Łącznie wyniki definiują reżimowo specyficzne, wrażliwe na wilgotność i „świadome jakości” ramy dynamiki RMO oraz dostarczają parametrów diagnostycznych (pH, wyjściowy WOG, dawka N, wilgotność, efektywność RWO, E4/E6) do ulepszania modeli ekosystemowych i projektowania klimatointeligentnego, głębokościowo świadomego zarządzania glebą.

Słowa kluczowe: gleby rolnicze, rozpuszczalna materia organiczna, dodatek azotu, globalne ocieplenie klimatu, uprawy rolnicze, płodozmian a monokultura, spektroskopia, głębokości gleby, analiza metadanych

The structure of the Doctoral thesis

The presented Doctoral thesis is a thematically coherent series of four articles published in scientific journals:

Scientific publications comprising the series:

Paper I:

Tianjing Ren, Bożena Smreczak, Aleksandra Ukalska-Jaruga, Xiaojie Li, Waseem Hassan, and Andong Cai. "Differential impacts of nitrogen addition on soil dissolved organic carbon in humid and non-humid regions: A global meta-analysis." *Journal of Environmental Management* 377 (2025): 124744. <https://doi.org/10.1016/j.jenvman.2025.124744>

IF (Year of Publication) = 8.4; 5-Year IF=8.6; MNiSW Points (2024) = 200;
PhD Student Contribution= 55%

Paper II:

Tianjing Ren, Bożena Smreczak, Aleksandra Ukalska-Jaruga, Waseem Hassan, and Andong Cai. "Asymmetric responses of soil dissolved organic carbon and dissolved organic nitrogen to warming: A meta-analysis." *Catena* 252 (2025): 108871. <https://doi.org/10.1016/j.catena.2025.108871>

IF (Year of Publication) = 5.7; 5-Year IF=6.3; MNiSW Points (2024) = 140;
PhD Student Contribution= 60%

Paper III:

Tianjing Ren, Aleksandra Ukalska-Jaruga, Bożena Smreczak, and Andong Cai. "Dissolved organic carbon in cropland soils: A global meta-analysis of management effects." *Agriculture, Ecosystems & Environment* 371 (2024): 109080. <https://doi.org/10.1016/j.agee.2024.109080>

IF (Year of Publication) = 6; 5-Year IF=6.8; MNiSW Points (2024) = 200;
PhD Student Contribution= 55%

Paper IV:

Tianjing Ren, Guillaume Debaene, Bożena Smreczak, Aleksandra Ukalska-Jaruga. "Depth-dependent effects of crop rotation and monoculture on dissolved organic matter quantity and quality." *Frontiers in Plant Science* (2025). <https://doi.org/10.3389/fpls.2025.1668092>

IF (Year of Publication) = 5.8; 5-Year IF=5.7; MNiSW Points (2024) = 100;
PhD Student Contribution= 60%

1 INTRODUCTION

1.1 Why dissolved organic matter matters?

Dissolved organic matter (DOM), operationally including dissolved organic carbon (DOC), dissolved organic nitrogen (DON), and dissolved organic phosphorus (DOP). They are collectively named dissolved organic matter components (DOMC). DOM is a critical constituent of terrestrial and aquatic ecosystems, consisting of soluble organic compounds derived from the decomposition of plant litter, root exudates, microbial metabolites, and soil organic matter (Nanthi S Bolan et al., 2011; Kalbitz et al., 2000). DOM serves as the most dynamic and bioavailable fraction of soil organic matter, despite typically representing less than 5% of total soil organic carbon and nitrogen pools (Guo et al., 2020; Smreczak & Ukalska-Jaruga, 2021). DOM plays multiple ecological roles: it functions as an immediate source of carbon and energy for soil microorganisms, thereby sustaining microbial growth and metabolic processes (Gajda et al., 2020). DOM regulates nutrient cycling through mobilization and transformation of N, P, and S, and it contributes to C stabilization via interactions with minerals and aggregates (Jones et al., 2004; Kalbitz et al., 2000). Additionally, it contributes to the stabilization and sequestration of organic carbon through interactions with soil minerals and aggregates (Kaiser & Guggenberger, 2000; Kaiser & Zech, 2000; Lehmann & Kleber, 2015). In agricultural systems, DOM is a sensitive indicator of fertility and productivity, directly responding to practices like fertilization, tillage, and residue incorporation, and regulating nutrient availability and greenhouse gas emissions (J. Liu et al., 2023; W. S. Liu et al., 2023; Ren et al., 2024). Crucially, global change drivers and agricultural management practices jointly modulate DOMC dynamics: warming and nitrogen inputs can differentially alter DOC and DON through shifts in plant productivity, root exudation, microbial enzyme allocation, and sorption–desorption equilibria, while practices such as tillage and fertilization restructure soil physical and chemical environments that govern production, transport, and retention of dissolved pools (Ren, Smreczak, et al., 2025a, 2025b; Xu et al., 2023). These changes propagate to ecosystem functioning by reshaping microbial community composition, modifying biogeochemical connectivity between soils and waters, and influencing resilience and stability under disturbance. Despite continued progress in DOM research,

the patterns of DOMC and their controlling factors under global change remain poorly resolved both at the global (and field scales. Therefore, clarifying how factors such as nitrogen addition, climate warming, agricultural management practices and soil depth is essential for improving ecosystem predictions and guiding sustainable soil–carbon management in agricultural soils.

1.2 Factors influencing DOMC pattern in soils

1.2.1 Nitrogen addition

Atmospheric nitrogen (N) deposition has risen sharply since the mid-20th century due to fossil-fuel combustion, ammonia volatilization from agriculture, and industrial emissions. The use of mineral fertilizers in agriculture has also increased substantially. Nitrogen global footprint is heterogeneous, with hotspots over intensively farmed and rapidly industrializing regions and comparatively lower inputs in remote drylands (Galloway et al., 2008; Liu et al., 2013; Peñuelas et al., 2013). Because C and N cycles are tightly coupled, enhanced N inputs can reverberate through DOM dynamics, thereby influencing nutrient transport, microbial metabolism, and land–water C fluxes (Kalbitz et al., 2000).

Mechanistically, N addition and fertilization can alter DOM via four interacting pathways. First, source-side effects: N application alleviates N limitation, often increasing plant productivity and shifting root exudation patterns; these changes elevate the quantity and alter the composition of rhizosphere DOM entering soil solution (Jones et al., 2004; Peñuelas et al., 2013). Second, microbial metabolic reprogramming: N availability alters enzyme allocation and stoichiometry, influencing DOM solubilization, mineralization, and assimilation. DOC may decline when microbes prioritize resource acquisition and stress tolerance, or DON may rise via necromass and N-rich metabolite release (Hagedorn et al., 2011; Malik et al., 2020; Xu et al., 2023). Third, physicochemical controls: ammonium and nitrate modify soil pH and ionic strength, mobilize Al/Fe elements, and thus change mineral surface charges. These processes shift adsorption–desorption equilibria and metal–organic complexation, selectively retaining or releasing DOM fractions and altering optical/structural indices such as SUVA and aromaticity (Cheng et al., 2020; Kaiser & Kalbitz, 2012; Kaiser & Zech, 2000). Fourth, hydrological mediation: by influencing plant

cover, rooting, and soil structure, chronic N inputs can affect infiltration, soil moisture, and leaching pathways, thereby regulating DOM residence time and export to groundwater and streams (Nakhavali et al., 2021). The magnitude and direction of DOM responses to N addition are context dependent. Climate, especially water availability, constrains biogeochemical processes: in non-humid regions, water limits activity and solute transport, so modest N inputs can enhance productivity and root C flow, increasing DOC. In humid climates, higher leaching and sustained microbial activity can depress surface DOC or shift it toward more processed signatures (Berdugo et al., 2020; Delgado-Baquerizo et al., 2013). N form, rate, and duration matter at high or chronic dosing can acidify soils, suppress parts of the microbial community, and change colloidal behavior, lowering DOC or changing pathways of DON. Moderate inputs may increase labile DOM via plant and microbial sources (Boot et al., 2016; Wu et al., 2023; Yuan et al., 2022). Soil properties, initial SOC/TN ratio, texture and clay mineralogy, pH, Fe/Al oxide content govern DOM sorption and soil solution chemistry. Soil textures and low reactive mineral surfaces favor DOM mobility, whereas fine, Fe/Al-rich soils promote retention and compositional filtering (Kaiser & Zech, 2000; Lehmann & Kleber, 2015). Vegetation and land use may significantly modulate organic matter inputs and turnover. Croplands often show faster DOM responses to fertilization and residue management than natural systems (Chantigny, 2003; Ren, Smreczak, Ukalska-Jaruga, Li, et al., 2025). Finally, depth is crucial: DOM quantity typically declines with depth while mineral interactions intensify, so N effects may attenuate or invert in subsoils compared with topsoil (Kaiser & Kalbitz, 2012). Despite substantial progress in understanding the impact of N in agricultural soils, important research gaps still remain: (1) inconsistent global synthesis: meta-analyses report increases, decreases, or negligible DOC responses to N, reflecting limited coverage in drylands and lack of standardized research methods; (2) Multiple driver interactions remain understudied, as few experiments combine N addition with warming, altered precipitation, or elevated CO₂ emission, yet climate–N co-controls likely govern future DOM dynamics. Therefore, for predicting C–N feedback and managing agricultural soils under global change is essential to clarify how N application modifies DOM, and which climatic, edaphic, and biological factors control these responses.

1.2.2 Climate warming

Soil DOM is a rapidly cycled, mobile pool that links plant inputs, microbial metabolism, mineral interactions, and hydrology. Although it constitutes a small share of the total soil C and N, DOM disproportionately regulates nutrient availability, pollutant transport, and land–water carbon exchanges, making DOM a sensitive indicator of ecosystem change (Nakhavali et al., 2021; Neff & Hooper, 2002). Warming affecting DOM through coupled biological, physicochemical, and hydrological pathways (Moona et al., 2019). Elevated temperature accelerates enzyme activity and microbial respiration, enhancing substrate mineralization and turnover. This can simultaneously increase DOM production and consumption, affecting CO₂ and N₂O emissions (Ding et al., 2022; Georgiou et al., 2022). Plant responses also matter, shifting in allocation and root activity often enhance exudation, supplying fresh, labile compounds to pore water and potentially increasing DOC and DON concentrations (Sun et al., 2022; Zhou et al., 2022). Climate warming can alter soil moisture regimes, redox conditions, and aggregate structure, thereby changing adsorption–desorption equilibria on clays and Fe/Al oxides and differentiate DOM composition (German & Allison, 2015; Lehmann & Kleber, 2015). Hydrologically, warming-induced drying or altered precipitation patterns regulate infiltration and leaching, which set residence time and vertical export of DOM to deeper horizons and aquatic systems (Nakhavali et al., 2021).

Observed responses of DOC and DON to warming are mixed: positive, negative, or neutral, because several modulators control direction and magnitude of them. Water availability is first-order control. In drylands, modest warming can enhance productivity and root exudation when moisture is sufficient. In humid systems, it accelerates decomposition and leaching, lowering surface DOC or shifting it toward more processed, aromatic forms (Berdugo et al., 2020; Delgado-Baquerizo et al., 2013). Initial SOC and TN, texture, mineralogy, and pH determine sorptive protection and microbial strategies. Coarse soils favor mobility, while fine, oxide-rich matrices enhance retention (Kaiser & Zech, 2000). Inorganic N pools further shape DON pathways and the DOC:DON ratio, which integrates C–N coupling under thermal stress (Chapin et al., 2002; Malik et al., 2020).

Evidence for climate warming effects on DOC and DON remains contradictory but signaling limited consensus on its direction and magnitude. This variability reflects limited understanding of how temperature interacts with moisture, soil structure, aeration, and microbial strategies to regulate DOM dissolution, transport, and turnover.

1.2.3 Agricultural management practices

Agricultural management, including mineral N fertilization (N), straw return (S), organic amendments (manure, M), N + straw (NS), N + manure (NM), biochar applications, conservation tillage (CT), and crop rotation, modifies DOC dynamics by directly supplying carbon and by indirectly reshaping nutrient regimes and edaphic conditions. Because these practices alter both carbon inputs and the soil milieu, DOM concentrations in croplands can change over short timescales compared with natural systems (Li et al., 2019; Williams et al., 2016). This rapid response reflects the strong coupling between DOM and microbial processes in cultivated soils. DOC serves as an immediate energy and substrate source for microbial communities and thus tracks disturbance and input pulses closely (Gajda et al., 2020; Smreczak & Ukalska-Jaruga, 2021). Organic amendments are key regulators on DOM (Cai et al., 2019; Omari et al., 2020). Straw and manure release soluble compounds that elevate DOC, with manure causing stronger increases due to its larger labile fraction and faster decomposition solid phase of organic residues (Cai et al., 2019; Omari et al., 2020). Biochar, while promoted for SOC accrual, shows mixed DOC outcomes across studies—enhancements, depressions, or no change, reflecting differences in adsorption, pore structure, and stabilization processes (Sánchez-García et al., 2016; Wu et al., 2022; H. Xu et al., 2021b). Mineral nitrogen can stimulate SOC transformation to DOC through microbial activation, whereas excessive N inputs may counteract DOC formation by acidifying soils and disturbing nutrient balances (Laurent et al., 2020; Yang et al., 2022). Conservation tillage reduces mechanical disturbance, improves pore continuity and infiltration, and can facilitate DOC formation and movement compared with conventional tillage (Bu et al., 2020; Liu et al., 2014). However, current understanding of the direction and magnitude of their effects on DOC is limited, constraining evidence-based optimization for sustainable agriculture.

The direction and size of DOC responses emerge from the interaction of practice intensity and duration, microbial biomass and diversity, and edaphic conditions. No-till can

slow DOC decomposition relative to conventional tillage, while short-term trials frequently report DOC increases whose persistence over longer periods remains uncertain (Acharya et al., 2019; Li et al., 2019). Microbes both generate and consume DOC, decomposition releases dissolved substrates, but growth and respiration remove them, linking DOC to community size and activity (Li et al., 2019). Nutrient supply, soil moisture, and texture further regulate production, diffusion, and retention. For example, clays provide sorption sites that immobilize dissolved organic compounds, whereas moisture governs mass flow and diffusion (Dong et al., 2021; Gmach et al., 2019; Singh et al., 2017; Wang et al., 2021). Because DOC plays a dual role in stimulating microbial respiration and supporting plant nutrient availability, selecting management strategies that increase DOC while limiting CO₂ emissions is critical. Organic amendments can increase DOC, enhance yields, and intensify CO₂ release (Chen et al., 2021; He et al., 2020; Qaswar et al., 2020), while excessive N fertilization may cause microbial DOC overconsumption, raising CO₂ emissions and reducing yields (Guo et al., 2022). In contrast, conservation tillage has been shown to maintain higher DOC levels while mitigating carbon losses (Liu et al., 2013; Powlson et al., 2014).

DOM quality, its aromaticity, degree of humification, records sources and processing along the soil profile and is shaped by cropping system (Kalbitz et al., 2000; Smreczak & Ukalska-Jaruga, 2021). Rotation diversifies residue chemistry and rhizosphere inputs, potentially enriching DOM complexity relative to monoculture, which tends to provide more uniform inputs and may favor accumulation of more aromatic or recalcitrant fractions (Chen et al., 2022; Saadi et al., 2006; Undurraga et al., 2010). These contrasts can be tracked with optical proxies. Higher specific UV absorbance at 280 nm (SUVA₂₈₀) and lower E4/E6 ratios indicate more aromatic, humified, and larger-molecule DOM; shifts in these indices capture management-driven changes in composition (Marschner & Kalbitz, 2003; Ukalska-Jaruga et al., 2021; Wilson & Xenopoulos, 2008).

Surface soil (0–30 cm) where fresh inputs are concentrated, yet subsoils (30–90 cm) host distinct processes and can act as important reservoirs or filters for dissolved organic compounds (Rumpel & Kögel-Knabner, 2011). Reduced microbial activity and slower turnover at various depths may promote stabilization or selective transport of more recalcitrant molecules, altering DOM signatures compared with topsoil (Spaccini et al.,

2002). Rotation likely transmits more diverse molecular signals downward via varied inputs and root exudates. Monoculture, in contrast, may leave more uniform and potentially more aromatic profiles with depth (Saadi et al., 2006; Zhang et al., 2021; Zhang et al., 2025). Agricultural management practices may effect on DOM but the direction and magnitude of DOC, DON and DOP changes remain uncertain. There are also limited understanding of how other edaphic factors like depth within soil profile co-influence DOMC quantity and quality, especially since most studies concern the upper soil layer (0-20 cm or 0-30 cm)

1.3 Conceptual flow chart of Doctoral thesis

By integrating global meta-analyses with depth-explicit field data on rotation and monoculture, this thesis reveals the regime-specific, quality-dependent, and management-sensitive dynamics of DOMC. It motivates the Doctoral thesis' two-tier design: i) global analyses implementing N addition, climate warming, cropland management and ii) a long-term field experiment testing rotation vs. monoculture and various depths: 0-30 cm, 30-60 cm and 60-90 cm within soil profile. It specifies four specific objectives and four testable hypotheses and model-relevant parameters emerging from literature. The chart highlights the main objectives, hypotheses, and analytical approaches that integrate global-scale synthesis with depth-explicit field validation.

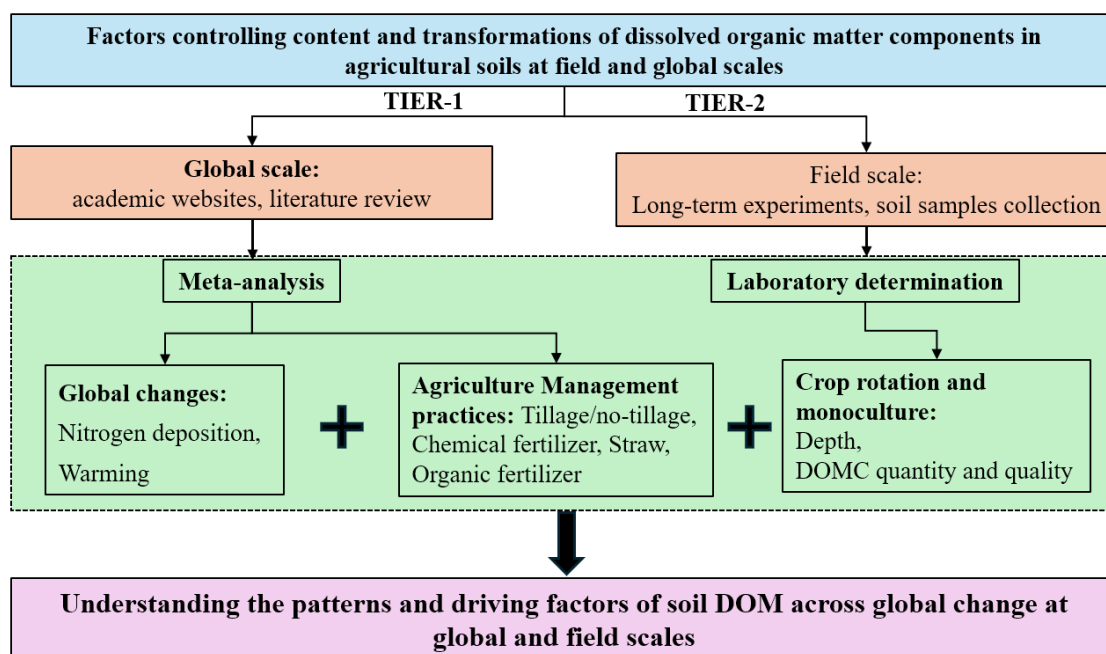


Figure 1. Conceptual flow chart of this research illustrates the two-tier design: (i) global meta-analyses assessing nitrogen addition, climate warming, and agricultural management practices and (ii) a long-term field experiment comparing crop rotation and monoculture across soil depths 0-30 cm, 30-60 cm and 60-90 cm) as affecting DOMC.

The literature review revealed a lack of information on the factors influencing the quantity and quality of DOMC on a global and field scale. Therefore, the aim of this doctoral thesis was to identify the drivers by determining the influence of nitrogen (N) addition, climate warming and agricultural management practices as well as the impact of cropping systems and soil depth on the quantity and quality of DOMC. This doctoral thesis builds a cohesive line of inquiry from boundary conditions, through mechanisms, to management leverage, and finally to depth-explicit field validation.

Meta-analysis on global scale begins by establishing how added nitrogen alters DOC across contrasting moisture regimes, because humidity sets first-order constraints on microbial activity, leaching, and sorption; this provides the climatic envelope within which other drivers operate (Paper I). Then climate warming influence on DOMC was examined to test the predicted decoupling of C and N in dissolved pools (DOC vs. DON), thereby identifying the proximal biophysical controls most sensitive to temperature and moisture (Paper II). Next evaluations concerned comparison of cropland practices to locate levers that raise DOC while improving yield and lowering CO₂ intensity (Paper III). Finally, because global syntheses are surface-biased and DOP-limited, the field study was designed to fill these gaps by measuring the DOMC (DOC, DON, DOP) at three soil depths (0–30, 30–60, 60–90 cm) under rotation vs. monoculture, thereby validating how cropping system and depth co-structure DOMC quantity and quality (Paper IV). Table 1 summarizes the specific objectives, hypotheses, candidate predictors, and analytical approaches corresponding to Papers I-IV.

Table 1. Specific objectives and hypothesis as related to the individual papers.

Paper I Global patterning of N addition effects
Specific objective 1
Quantification of how nitrogen addition influences DOC and identify the dominant moderators that bound DOC responses at the global scale
Hypothesis 1

DOC responses to N addition are climate-dependent
Predictors included (grouped): Climate: mean annual temperature (MAT), aridity index (AI), seasonal variability of precipitation (SVP), month/season. N inputs: N addition rate (and form/method categories). Soils: soil pH, soil organic carbon (SOC), texture, soil order, parent material. Microbial: microbial biomass C (MBC), fungi: bacteria ratio (F:B). Top predictors (model importance): Humid: month/season, SVP, MBC, MAT, SOC. Non-humid: SOC, F:B, N input rate, MAT, (secondary climatic factors).
Paper II Warming mechanism and moisture control
Specific objective 2 To quantify the responses of soil DOC, DON, and DOC:DON to experimental warming and to identify the climatic, edaphic, and microbial factors associated with variation in these responses.
Hypothesis 2 Warming lowers DOC:DON by accelerating DOC turnover
Predictors included (BRT for DOC and DON): soil moisture, initial SOC, microbial biomass N (MBN), microbial biomass C (MBC), soil pH, nitrate-N (NO_3^-), ammonium-N (NH_4^+), total N (TN), MAT, AI. Top predictors (model importance): DOC: soil moisture (strongest), SOC, MBN, MBC, pH/NO_3^- (similar tier). DON: NO_3^- (dominant), NH_4^+, TN, moisture, MBN/MBC.
Paper III Management leverage and DOC-efficiency
Specific objective 3 to compare cropland practices (CT, mineral N, straw, manure, NS, NM, biochar) by their capacity to increase DOC,
Hypothesis 3 Combined organic and mineral nutrient inputs (particularly N plus manure or straw) will produce larger increases in DOC than single-input practices, whereas conservation tillage and biochar will show smaller changes in DOC concentration or DOC/SOC because they primarily modify physical disturbance and carbon retention rather than directly supplying readily soluble carbon..
Predictors included (model-selection/RF set):

phospholipid fatty acids (PLFA), cellulase, clay fraction content, NH_4^+, AI, available N (AN), soil moisture, MBC, experimental duration, total bacteria, Gram-negative (GN) & Gram-positive (GP) bacteria, practice intensity, NO_3^-, β-glucosidase (βG), invertase, soil pH response ratio (pH-RR), TN. Top predictors (by practice; model-averaged importance): Manure / NM / Biochar: practice intensity (primary); clay fraction content, microbial traits. CT / N / Straw / NS: experimental duration (primary); moisture/AN and microbial traits.
Paper IV Soil-depth, DOMC-complete field validation
Specific objective 4 to determine how soil depth and cropping system co-structure DOM quantity and quality
Hypothesis 4 Soil depth is a first-order organizer of DOMC, yielding more aromatic, humified dissolved pools with depth.
Predictors included in RF: soil total C (TC), total N (TN), available P (AP), total P (TP), humus content, soil depth, and cross-component terms (e.g., DOC predicting DON, DOP predicting DOC). Top predictors (RF %IncMSE): <i>DOC</i>: TN, TC, DOP, (others lower); <i>DON</i>: DOC, depth, AP, (others lower); <i>DOP</i>: humus, TP, AP, (others lower).

2 Materials and methods

2.1 Global meta-analysis

2.1.1 Data sources and search strategy

To quantify the effects of nitrogen addition, climate warming, and cropland management on DOMC and related variables, a global database was assembled covering studies published from 1995 to 31 December 2022. Literature searches were conducted in Google Scholar, Web of Science, and CNKI using topic-specific keyword combinations, for example:

- Nitrogen addition: “dissolved organic carbon” OR “DOC” OR “DOM” AND “soil” AND (“nitrogen addition” OR “nitrogen enrichment” OR “N deposition”)
- Warming: “dissolved organic carbon” OR “DOC” OR “DOM” OR “dissolved organic nitrogen” OR “DON” AND “soil” AND (“warming” OR “temperature increase”)
- Management practices: “dissolved organic carbon” OR “DOC” OR “DOM” AND “soil” AND (“cropland” OR “agricultural land” OR “management practices”)

2.1.2 Inclusion criteria

Studies were included when they satisfied all of the following:

- terrestrial ecosystems (croplands, grasslands, forests, etc.; management analyses restricted to croplands);
- paired control–treatment design with a single focal factor (no multifactor interactions);
- ≥ 3 replicates per group;
- means with standard deviation (SD) or convertible standard error (SE; $SD = SE \times \sqrt{n}$);
- extractable information on treatment duration, intensity, and site;
- consistent DOC/DON determination, with physically extracted values converted to chemical-extraction equivalents;
- for N-addition and warming, analyses restricted to 0–30 cm; for management, emphasis on 0–20 cm due to limited deep-soil evidence.

Flow diagram demonstrating study selection for meta-analysis (PRISMA [Preferred Reporting Items for Systematic Reviews and Meta-Analyses] diagram) (Figure 2). PRISMA is an evidence-based minimum set of items for reporting in systematic reviews and meta-analyses. The flow diagram depicts the flow of information through the different phases of a systematic review. Please refer to the published papers (Papers *I, II, III*) for the specific numbers of records identified, included, and excluded, as well as the reasons for exclusion. Briefly, no-full text articles as well as full-text articles with no target data were excluded. Only publications with target data were collected in the database.

Although DOMC comprises DOC, DON, and DOP, DOP was excluded from the meta-analyses because the initial screening returned too few usable records across treatments and climates to support a robust synthesis. In addition, the database was restricted to surface soils (0–30 cm for N addition and warming; 0–20 cm for management) because comparable deep-soil observations were scarce and heterogeneous, limiting inference to topsoil processes and precluding a rigorous assessment of subsoil DOMC dynamics.

Final sample sizes were: N addition 1132 paired DOC observations from 103 studies; warming: 321 DOC and 187 DON observations from 50 studies; management practices: 3539 DOC, 196 CO₂ emission, and 1424 crop-yield observations (from 286, 15, and 92 studies, respectively).

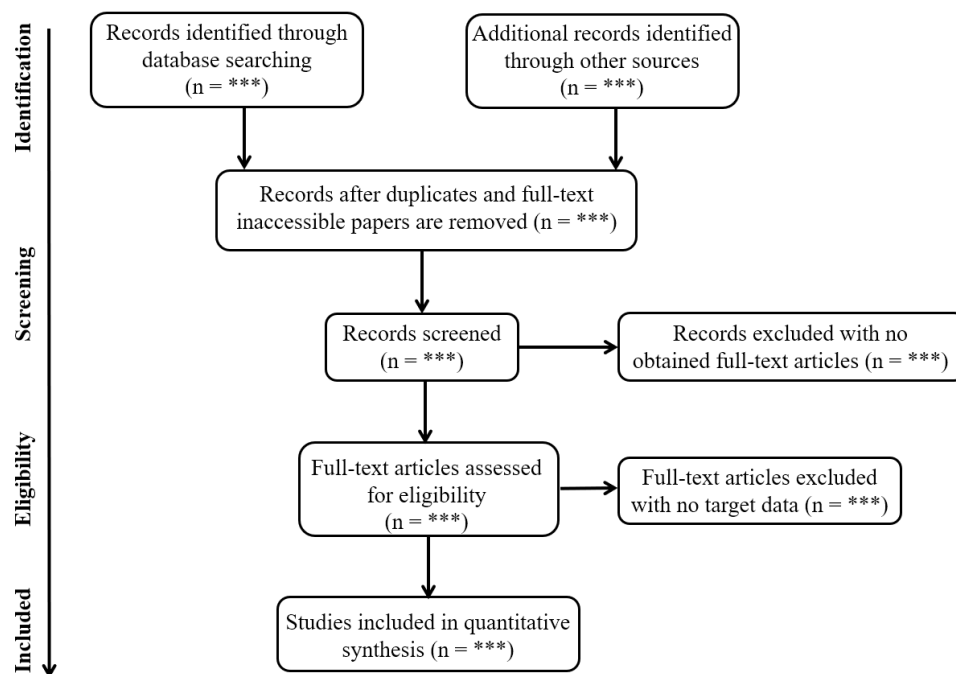


Figure 2. Meta-Analysis Flow Chart. Flow diagram demonstrating study selection for meta-analysis (PRISMA [Preferred Reporting Items for Systematic Reviews and Meta-Analyses] diagram). *Source: Papers I, II, III forming parts of this Doctoral thesis.*

2.1.3 Data extraction and grouping

For each observation we recorded location (latitude, longitude, elevation); climate (mean annual temperature, MAT; mean annual precipitation, MAP; aridity index, AI; precipitation seasonality, SVP); soil properties (pH, SOC, total N, available P, NO_3^- -N, NH_4^+ -N, bulk density, moisture); microbial attributes (MBC, MBN, community metrics, enzyme activities); crop yield; and CO_2 fluxes. Grouping rules were:

- N addition: humid ($\text{AI} > 1$) vs non-humid ($\text{AI} < 1$); N rate ≤ 50 , 50–100, $> 100 \text{ kg N ha}^{-1} \text{ yr}^{-1}$; climate zones defined by MAT ($< 10^\circ \text{C}$ vs $\geq 10^\circ \text{C}$).
- Warming: climate zones by MAT; warming magnitude ≤ 1 , 1–2, 2–4, $> 4^\circ \text{C}$.
- Management: conservation tillage (CT), mineral N (N), straw (S), manure (M), N+straw (NS), N+manure (NM), biochar (BC); intensity classified as low/medium/high; duration as short ($\leq 5 \text{ yr}$), medium (5–15 yr), long ($> 15 \text{ yr}$).

2.1.4 Standardization

Soil dissolvable organic carbon was classified into two operationally defined categories according to the sampling and determination procedures. The first category was extractable organic carbon, which was obtained by extracting soil with distilled or deionized water or salt solutions, such as KCl, K_2SO_4 , or CaCl_2 . The second category was DOC present in soil pore water, which was collected using physical methods, including centrifugation, ceramic suction cups, tension samplers, and tension-free lysimeters as described by Li et al. (2018). These two approaches measure related but not identical fractions of soil organic carbon. Soil-solution DOC represents carbon dissolved in pore water under the hydrological conditions prevailing at the time of sampling, whereas water or salt extraction may additionally release weakly adsorbed or readily desorbable organic carbon. The amount recovered by chemical extraction may also depend on the extractant, soil-to-solution ratio, extraction temperature, and extraction duration.

Over 95% of the DOC in our database were determined using chemical techniques reflecting their prevalence and reliability. The method comparison presented in Tables 2

and 3 showed that the standardized DOC concentration obtained using physical sampling was significantly lower than that obtained using salt or water extraction. In contrast, the mean DOC effect size was significantly greater for physical sampling than for the two chemical-extraction categories ($p < 0.05$). No significant difference was observed between salt and water extraction. These results indicate that sampling method was a potential source of systematic heterogeneity, particularly when absolute DOC concentrations were compared. To ensure consistency across the dataset, the DOC concentrations derived from physical methods were converted to equivalent values soil following Guo et al. (2020):

$$\text{DOC}_{\text{soil}} = (\text{DOC}_{\text{solution}} \times V \times 1000) / W \times [1 / (V \times (1 - W) \times \text{BD} \times 1000000)] \quad (1)$$

where, DOC_{soil} is the DOC concentration determined by chemical methods (mg g^{-1}); $\text{DOC}_{\text{solution}}$ is the DOC concentration measured by physical methods (mg L^{-1}); W is the volumetric soil moisture (m^3/m^3); V is the unit volume for the soil column used for extracting soil solution (m^3); BD is the soil bulk density (g cm^{-3}); 1000 is used to convert m^3 to L; and 1000000 is used to convert m^3 to cm^3 (Guo et al., 2020).

Table 2. The concentration and effect size of DOC under different extraction methods. Values are means (standard error). Different lowercase letters indicate significant ($p < 0.05$) differences. One-way analysis of variance (ANOVA) and least-significant difference tests at the 5% level of significance were performed to examine significant differences in extraction solvents and temperature. Chemical extraction solvents included K_2SO_4 , KCl , and CaCl_2 . Physical means the soil DOC concentration by physical methods via lysimeter collection or ceramic suction, etc. *Source: Paper III comprising part of Doctoral thesis.*

Extraction method	No.	Effect size of DOC	concentration of DOC
Chemical	1515	106.01(3.55) b	43.17(2.01) a
Extraction solvents	Water	148(5.81) b	44.8(2.37) a
Physical	90	334.2(39.49) a	13.03(3.78) b

Table 3. The concentration and effect size of DOC under different ratio of soil to extraction solvents. Values are means (standard error). Different lowercase letters indicate significant ($p<0.05$) differences. One-way analysis of variance (ANOVA) and least-significant difference tests at the 5% level of significance were performed to examine significant differences ratio of soil to extraction solvents. *Source: Paper III comprising part of Doctoral thesis.*

Extraction solvents	Ratio	No.	Effect size of DOC	Concentration of DOC
K ₂ SO ₄	1: 2	14	62.19(18.09) a	49.77(7.63) a
	1: 3	10	9.95(3.6) a	99.43(8.02) a
	1: 4	244	61.18(8.72) a	62.86(3.44) a
	1: 5	365	42.95(3.57) a	83.15(5.36) a
KCl	1: 4	9	94.59(44.56) a	26.65(0.91) b
	1: 5	54	18.12(5.79) b	73.65(8.6) b
	1: 10	49	36.18(5.96) b	157.33(12.51) a
CaCl ₂	1: 2	8	13.39(5.2) a	384.51(19.41) a
	1: 5	23	34.26(20.63) a	285.89(34.12) ab
	1: 10	8	17.59(10.93) a	163.78(10.07) b
Water	1: 2	200	29.18(4.08) a	134.69(17.04) b
	1: 3	100	11.42(3.32) a	717.85(38.57) a
	1: 4	26	22.23(8.52) a	105.46(10.11) b
	1: 5	763	54.4(3.75) a	116.55(4.97) b
	1: 10	225	31.18(5.67) a	94.23(8.07) b
	1: 20	13	61.26(22.99) a	137.21(23.36) b

2.1.5 Meta-analysis

Meta-analysis is a statistical method used for the systematic and quantitative analysis of multiple independent experimental results with a specific research purpose (Cai et al., 2021; Ren et al., 2023). MetaWin2.1 software was utilized for data processing for this meta-analysis (<https://www.metawinsoft.com/>). The indicators of the natural log-transformed response ratio ($\text{Ln}(\text{RR})$) and 95% confidence intervals ($\text{Ln}(95\%CI)$) were applied. The specific formula was as following:

$$\text{Ln}(\text{RR}) = \text{Ln}\left(\frac{M_t}{M_c}\right) \quad (2)$$

where M_t and M_c are mean values for treatment and control groups, respectively.

The variances (V) of $\text{Ln}(\text{RR})$ were calculated as the following equation:

$$V = \frac{SD_t^2}{N_t M_t^2} + \frac{SD_c^2}{N_c M_c^2} \quad (3)$$

where SD_t and SD_c represent standard deviation (SD) of treatment and control groups, respectively. The N_t and N_c represent the sample size of treatment and control, respectively. For our database, the missing SD was re-calculated based on recorded the mean and variance. The weighting factor (W_{ij}) and $\text{Ln}(95\%CI)$ were calculated as equations 4 and 5, respectively:

$$W_{ij} = \frac{1}{V_{ij}} \quad (4)$$

$$\text{Ln}(95\%CI) = \text{Ln}(\text{RR}) \pm 1.96 \sqrt{\frac{1}{\sum_{i=1}^m \sum_{j=1}^{k_i} W_{ij}}} \quad (5)$$

where W_{ij} ($i = 1, 2, \dots, m; j = 1, 2, \dots, k_i$), here, m is the number of groups, k_i is the number of comparisons in the group. The fixed effect model (FEM) and random effect model (REM) were used to deal with no heterogeneity ($p > 0.1$ and $I^2 < 50\%$) and heterogeneity among studies, respectively. The effect size (%) and 95%CI (%) were transformed by equations 6 and 7, respectively:

$$\text{Effect size} = (e^{\text{Ln(RR)}} - 1) \times 100\% \quad (6)$$

$$95\%CI = (e^{\text{Ln}(95\%CI)} - 1) \times 100\% \quad (7)$$

A significant effect is considered if the corresponding 95%CI does not overlap with zero.

Publication bias was assessed with Egger's and Begg's tests together with funnel-plot inspection; no significant bias was detected in the nitrogen-addition or warming syntheses (e.g. Paper I: Egger's $z = 1.00$, $P = 0.317$), and for the cropland synthesis Begg's rank-correlation test likewise indicated no significant small-study effect (DOC $\tau = -0.01$, $P = 0.63$; CO₂ $\tau = +0.01$, $P = 0.81$). Because several treatment–control contrasts can originate from one publication, the robustness of the pooled effects to study-level non-independence was additionally verified with three-level (study-random-effect) and cluster-robust models; the point estimates were essentially unchanged, while confidence intervals widened, so smaller effects are reported with corresponding caution (Table 4).

Outcome	k (obs) / m (studies)	ICC	Standard RE (obs. independent)	Multilevel (study random effect)	Cluster-robust RVE	Study-level aggregated
Paper I N addition DOC humid	544 / 50	0.24	−2.3 [−5.4, 1.0] ns	+0.3 [−6.1, 7.1] ns	−2.3 [−11.3, 7.6] ns	+1.2 [−5.4, 8.1] ns
Paper N addition DOC non-humid	574 / 56	0.53	+7.3 [4.4, 10.2] ***	+7.5 [−0.4, 15.9] ns	+7.3 [−1.7, 17.1] ns	+6.6 [−1.3, 15.2] ns
Paper II Warming DOC	328 / 46	0.34	+0.2 [−2.8, 3.2] ns	−1.8 [−7.2, 3.9] ns	+0.2 [−6.8, 7.7] ns	−4.0 [−10.5, 3.0] ns
Paper II Warming DON	187 / 24	0.10	+8.7 [2.6, 15.2] **	+7.5 [−1.2, 16.9] ns	+8.7 [−0.3, 18.5] ns	+11.4 [0.0, 24.2] ns
Paper III Management DOC	3539 / 308	0.46	+29.4 [27.6, 31.3] ***	+23.9 [19.5, 28.4] ***	+29.4 [22.5, 36.7] ***	+24.1 [19.5, 28.8] ***
Paper III Management CO ₂	196 / 15	0.28	+26.0 [20.2, 32.2] ***	+18.2 [5.8, 32.1] **	+26.0 [12.0, 41.8] ***	+14.1 [3.1, 26.3] *

2.1.6 Efficiency of DOC

Changes in the DOC can influence CO₂ emissions and crop yields. Thus, the efficacy of unit changes in the DOC to corresponding changes in CO₂ emissions and crop yields was calculated using the following equation:

$$\text{DOC}_X = (\text{effect size of X} / \text{effect size of DOC} - 1) \times 100\% \quad (8)$$

where, the efficiency of DOC_X is %, and X refers to CO₂ emissions and crop yields. The effect sizes of the DOC, CO₂ emissions, and crop yields were calculated via a meta-analysis. When DOC_X was positive, this indicated that each unit increase in DOC resulted in more than one unit of CO₂ emission or more than one unit of crop yield **increase**. The index is a dimensionless, relative measure: a positive value means X (CO₂ or yield) responds proportionally more than DOC, and a negative value means X rose proportionally *less* than DOC, not that it declined in absolute terms. It describes co-variation, not causation: a yield increase need not be caused by the DOC change (improved nitrogen availability is often the predominant driver), and the DOC-CO₂ association may arise because both respond to organic-matter input. Because it is a ratio, the index is unstable where the DOC effect size is small; its uncertainty was therefore quantified with a study-level bootstrap (3,000 resamples), and the CO₂-based values, resting on few studies, are interpreted qualitatively. This relationship is instrumental in understanding the broader impact of changes in DOC on crucial environmental and agricultural metrics.

2.2 Long-term field experiment

2.2.1 Site and experimental design

To address the two main limitations identified in the global syntheses—namely the scarcity of DOP observations and the surface-soil bias—the field campaign was explicitly designed to quantify the full DOMC suite (DOC, DON, and DOP) and to resolve vertical patterns along the profile. Accordingly, both cropping systems were sampled at three depths (0–30, 30–60, and 60–90 cm), allowing us to (i) capture depth-dependent changes in DOM concentration and quality beyond the top 30 cm, (ii) evaluate subsoil contributions to dissolved C–N–P dynamics under rotation versus monoculture, and (iii) link surface processes to subsoil retention and potential leaching pathways. This depth-explicit,

DOMC-complete design directly complements the meta-analyses and enables a more comprehensive assessment of management effects from the surface to 90 cm.

The agricultural long-term field experiment was located at the IUNG-PIB's Experimental Station in Osiny, situated in the eastern Poland (51°28'N, 22°02'E), on a Albic Luvisol (Loamic, Cutanic). Long-term experiment was established in 1994. It compared two cropping systems in a randomized block design (plot area 90 m²; three replicates):

- Crop rotation: oilseed rape → winter wheat → spring barley/wheat
- Monoculture: continuous winter wheat.

In September 2022, composite soil samples were collected from 0–30 cm, 30–60 cm, and 60–90 cm (six cores per layer per plot), air-dried, passed through a 2-mm sieve and kept dry before analysis. The detailed description of site and experimental site has been presented in Paper IV consisting of part of this Doctoral thesis.

2.2.2 Analysis of soil properties

Measured soil parameters included: pH (1 mol L⁻¹ KCl and H₂O; at ratio 1:2.5 m/v) and electrical conductivity (EC) by potentiometric method, particle-size distribution (sedimentation method and laser diffractometer, total C (TC) and total N (TN) (CN elemental analyzer). Organic C (by Tiurin's method with wet dichromate oxidation followed by titration with Mohr's reagent in the presence of phenolphthalein), humus (SOM) calculated from the equation $SOM = TOC \times 1.724$. Soil properties were analyzed as follows: available P by the Egner–Riehm method (Ca-lactate extraction), CEC by ammonium acetate extraction with ASA determination, total P by aqua regia digestion with ICP-MS, and mineral N (NO₂⁻, NH₄⁺, NO₃⁻) by 1% K₂SO₄ extraction with continuous-flow analysis (Seal Analytical). All details considering analytical methods used for soil properties determinations are in Paper IV consisting part of this Doctoral thesis.

2.2.3 DOM extraction and quality metrics

DOM was extracted with cold water (soil: water = 1:10, w/w; 25 °C; 200 rpm; 24 h; followed with 0.45-μm filtration). Concentrations of DOMC were determined for:

- DOC and DON (TOC/TN analyzer)
- DOP (ICP-MS)

Optical indices for DOM were derived from UV–VIS spectra, including SUVA₂₈₀ and E4/E6. Moreover, VIS–NIR was used to characterize DOM molecular features.

2.3 Statistical analyses

For inference beyond pooled effects, we used: (i) linear regression to relate DOC (or its effect size) to environmental, edaphic and microbial predictors; (ii) correlation screening to reduce multicollinearity before modeling; and (iii) multimodel selection in R (glmulti) with Akaike weights to identify influential drivers (variables with summed weights ≥ 0.8 were considered important). Group comparisons in the cropland meta-analysis also used two sample t-tests. Machine-learning models included random forests (up to 1000 trees; $mtry \approx p/3$) and boosted tree models (10–100 trees; depth 1–7; shrinkage 0.01–0.1) to predict DOC responses. Model interpretation used partial dependence analyses, and partial correlation analyses were applied to quantify factor effects while controlling for covariates. R packages included randomForest, gbm, pdp, caret, ppcor, metafor, and ggplot2.

For the field experiment on crop rotation vs. monoculture, assumptions of normality and homogeneity of variances were tested prior to analysis; differences among depths and systems were assessed with one-way ANOVA followed by Tukey's HSD ($\alpha = 0.05$). Random-forest modeling was also used to rank environmental drivers of DOM quantity and quality. Software details: meta-analyses were run in MetaWin 2.1. All other statistics and visualizations were conducted in R and OriginPro (OriginLab). All detailed descriptions of statistical methods used for evaluation results from the field experiment have been included in Paper IV, consisting part of this Doctoral thesis.

3 Results and Discussion

3.1 Impacts of nitrogen addition on soil dissolved organic carbon (DOC) across humid and non-humid regions - Paper I

Consistent with Objective 1, this section quantifies how nitrogen addition alters DOC across humid vs. non-humid regions and evaluates the key moderators constraining these responses (precipitation seasonality, soil pH, baseline SOC). In humid regions, nitrogen addition reduced DOC (−2.49%) and MBC (−2.60%), whereas in non-humid regions it increased DOC (+7.30%) and MBC (+8.74%). SOC responded more positively to nitrogen N addition in humid regions (8.16%) than in non-humid regions (2.05%). Additionally, the ratio of MBC to SOC showed opposite patterns, declining by 13.10% in humid regions but rising by 6.01% in non-humid regions (Figure 3). What confirms the Hypothesis 1.

Nitrogen addition exhibited contrasting impacts on DOC between humid and non-humid regions. In humid areas, DOC declined due to interacting mechanisms: (i) high baseline N and productivity limited further growth, reducing litter inputs and N-induced biomass contributions (Lu. et al., 2021); (ii) excess nitrogen disrupted microbial nutrient balance, suppressing respiration and decomposition activity while triggering negative priming effects that lowered DOC production (Lu. et al., 2021; Xu. et al., 2021); and (iii) acidic soils in these regions experienced further pH reductions under nitrogen enrichment, exacerbating cation desorption, increasing DOC leaching risk, and hindering microbial breakdown of organic matter (Wu et al., 2023). Enhanced macroaggregate formation also facilitated water movement, accelerating DOC loss (Kaiser et al., 2005; Kaiser & Zech, 2000). In contrast, non-humid regions, which are typically nitrogen-limited, displayed increases in DOC. Nitrogen enrichment stimulated carbon allocation to belowground biomass, root-associated microorganisms, and exudate production, directly elevating DOC levels (Yuan et al., 2022). Microbial stoichiometric demands further promoted the synthesis of carbon-acquiring enzymes, enhancing substrate decomposition and microbial growth, thereby boosting DOC release (Chen et al., 2018; Xu. et al., 2021). Higher baseline soil pH minimized acidification effects on DOC retention, while intermittent drying-wetting cycles physically disrupted aggregates and released protected organic carbon for microbial utilization (Ma et al., 2022; Wu et al., 2023). Collectively, these mechanisms

underscore strong regional heterogeneity in DOC responses to nitrogen inputs, highlighting the need for tailored nutrient management strategies to sustain ecosystem carbon dynamics under increasing nitrogen deposition (Dong et al., 2021).

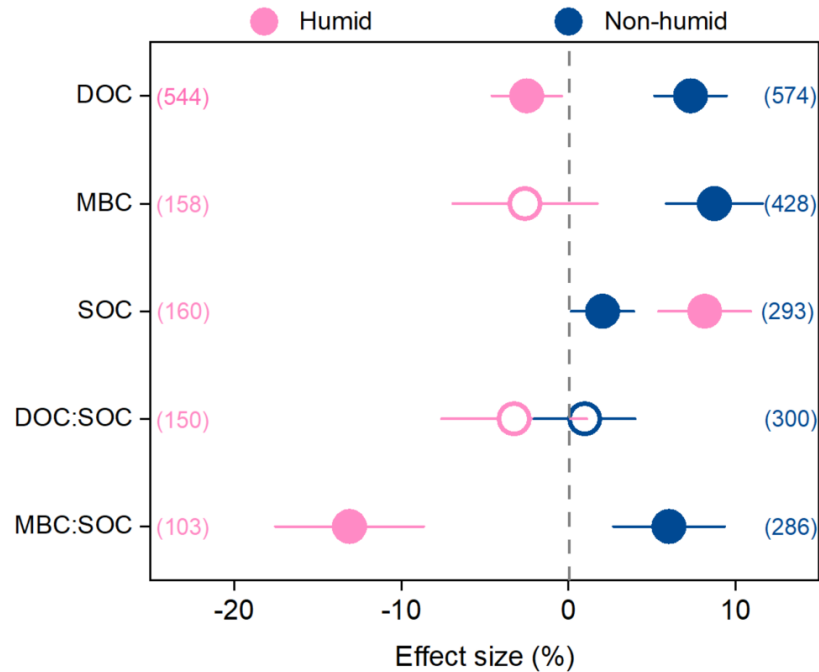


Figure 3. Effects of N addition on soil carbon in humid and non-humid regions. Each point and error bar illustrates the average effect size and its associated 95% confidence interval. Hollow circles positioned close to the dashed vertical line indicate that the corresponding effect is not statistically significant. An effect is considered significant when its confidence interval does not intersect the vertical dashed line. Numbers in parentheses denote the sample sizes for each comparison. Abbreviations: DOC – dissolved organic carbon; MBC – microbial biomass carbon; SOC – soil organic carbon. *Source: Paper I comprising part of Doctoral thesis.*

A significant linear relationship was observed between DOC effect size and the MBC to SOC ratio in both humid (decreasing) and non-humid (increasing) regions ($P < 0.01$) (Figure 4). In humid regions, DOC responses were associated with precipitation seasonality and increased across the observed SOC gradient (Figure 5). Nitrogen addition increased SOC but reduced DOC and MBC (-2.49% , -2.60% ; Figure 4), a pattern consistent with enhanced stabilization of mineral-associated organic carbon (MAOC) (Wu et al., 2023). This is consistent with preferential microbial consumption of labile DOC under N-rich conditions, as suggested by elevated enzyme activity and shifts toward heavier SOC

fractions, although these mechanisms were not measured directly here. Microbes preferentially consumed labile DOC under N-rich conditions, supported by elevated enzyme activity and shifts toward heavier SOC fractions (Chen et al., 2017; Gunina & Kuzyakov, 2022; Lu. et al., 2021). In non-humid soils, DOC and MBC increased (7.30% and 8.74%), driven by microbial SOC decomposition and root-derived carbon inputs (Deng et al., 2018). Higher enzymatic activity and CO₂ emissions (9.89–19.66%) accelerated carbon cycling and loss (Boot et al., 2016; Xu. et al., 2021), consistent with enhanced root biomass and nutrient acquisition under nitrogen limitation (Gunina & Kuzyakov, 2022; Malik et al., 2020; Silver et al., 2005). DOC responses were more pronounced in cooler climates (Figure 4), suggesting greater vulnerability of arid and semi-arid soils to nitrogen enrichment.

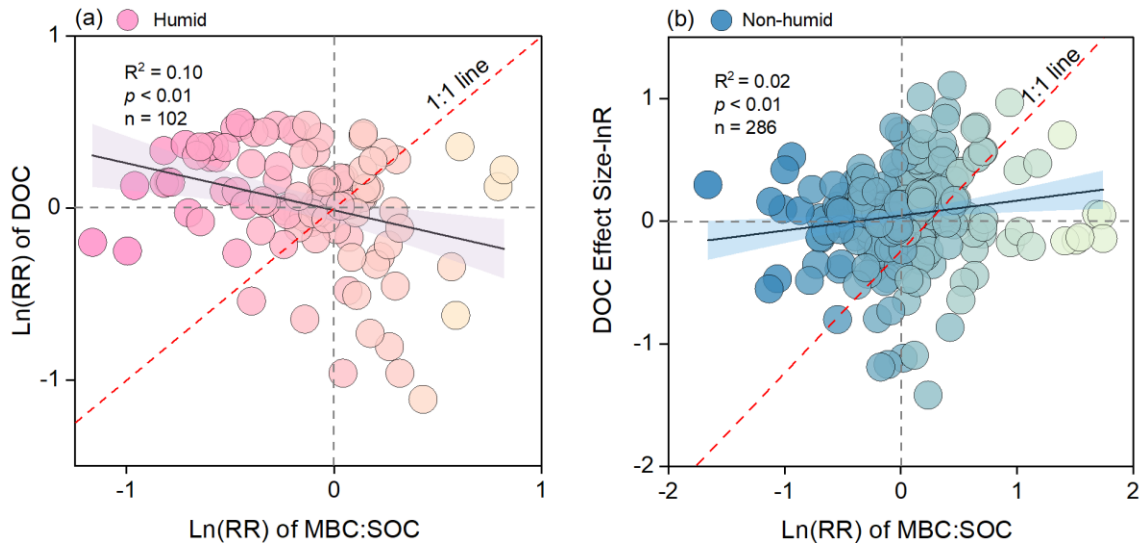


Figure 4. Co-variation patterns between soil dissolved organic carbon (DOC) and the ratio of microbial biomass carbon (MBC) to soil organic carbon (SOC). a, In humid environments, linear regression reveals a negative association between the natural log response ratios (Ln(RR)) of DOC and MBC:SOC (regression equation: $y = -0.27x - 0.01$). b, In contrast, a positive relationship is observed in non-humid regions ($y = 0.12x + 0.05$). Gray dashed lines indicate the zero-effect baseline, while solid black lines denote the fitted regression line. The red dashed line corresponds to the 1:1 reference. Sample sizes are indicated by n. *Source: Paper I comprising part of Doctoral thesis.*

In non-humid regions, the effect size of DOC decreased sharply with higher MBC and was notably impacted by both extremes of N addition rates (Figure 5). Overall, soil pH was

shown to be important for the N addition effect on DOC, regardless of AI. Partial correlation analysis highlighted the seasonal variability of precipitation as a critical factor affecting DOC in both regions (Figure 5). In humid regions, DOC responses to N addition correlated positively with MBC ($P < 0.001$) and negatively with pH ($P < 0.05$), contrasting with non-humid patterns. SOC was the main driver in humid regions, while N addition rate dominated in non-humid regions.

Partial correlation analysis identified seasonal precipitation variability as a key factor influencing DOC dynamics (Figure 5). In humid regions, abundant rainfall promoted microbial activity by supplying fresh organic inputs, enhancing MBC and DOC (Allison, 2023), whereas greater seasonal variability increased DOC leaching from litterfall (Nakhavali et al., 2021). These ecosystems exhibited muted seasonal DOC responses to N addition, reflecting high resilience. In contrast, DOC responses were stronger and more sensitive to seasonal fluctuations in non-humid areas. Initial SOC content also played a major role in humid regions. Low-carbon soils experienced microbial C limitation under N addition (Chen et al., 2014), reducing DOC production, while high-carbon soils promoted microbial decomposition and DOC release. Lower pH levels further decreased with N enrichment, facilitating DOC leaching via cation desorption yet also promoting co-precipitation with Al^{3+} and Fe^{3+} (Wu et al., 2023; Yuan et al., 2022), resulting in a negative DOC–pH relationship. Conversely, non-humid soils, typically more alkaline, exhibited a positive DOC–pH relationship as N-induced acidification enhanced cation desorption and DOC loss.

N application rates significantly influenced DOC responses in non-humid regions (Figure 5). Higher N rates reduced ecosystem buffering capacity and limited DOC increases, contrasting with humid regions where DOC responses initially declined then increased, likely due to environmental adaptation. This discrepancy highlights that experimental N levels ($>50 \text{ kg ha}^{-1} \text{ yr}^{-1}$) may underestimate DOC sensitivity in non-humid areas compared to atmospheric deposition rates ($<25 \text{ kg ha}^{-1} \text{ yr}^{-1}$) (Schwede et al., 2018). Variations in N form, application method, and frequency also shaped DOC responses. In non-humid regions, a persistent DOC–MBC correlation suggested direct microbial contributions to DOC under intensified metabolic demands. Differences in soil order and parent material further modulated these responses, underscoring the complex chemical and biological roles

of pH and other soil properties in regulating DOC dynamics across ecosystems.

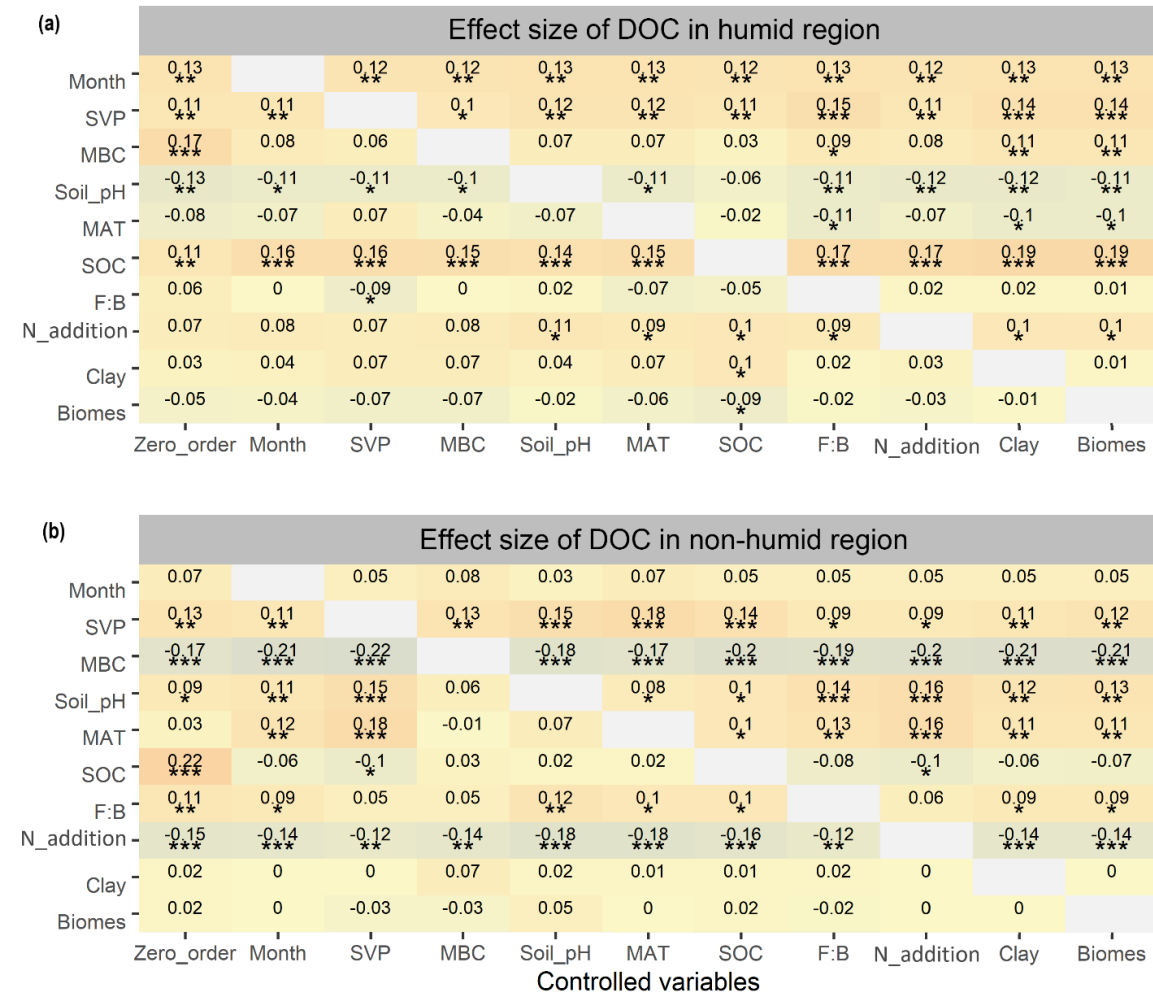


Figure 5 Partial correlation analyses between soil dissolved organic carbon (DOC) and key influencing variables in humid (a) and non-humid (b) regions. Along the x-axis are the zero-order correlations (i.e., unadjusted) and those adjusted by controlling specific variables. The y-axis lists the categories of potential drivers—climatic conditions, soil characteristics, microbial traits, nitrogen inputs, and biome types—whose associations with DOC effect sizes are evaluated. The color gradient and numerical values reflect the direction and magnitude of the correlations. Changes in values or shading intensity between zero-order and adjusted correlations indicate the extent to which the observed relationships depend on other variables (e.g., no change implies independence, whereas increases or decreases signal altered correlation strength). Abbreviations: SVP – seasonal variability of precipitation; MBC – microbial biomass carbon; MAT – mean annual temperature; SOC – soil organic carbon; F:B – fungi-to-bacteria ratio. Significance levels: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Source: Paper I comprising part of Doctoral thesis.

3.2 DOC–DON response to climate warming - Paper II

In line with Objective 2 and building on the moisture-bounded DOC responses identified in Objective 1, tests Hypothesis 2 that experimental warming decouples dissolved C and N pools (DOC vs. DON), with soil moisture as the principal statistical mediator of the DOC response (a mediation supported by our analysis but inferred from observational data rather than demonstrated experimentally). Experimental warming did not alter DOC or MBC levels, while it significantly reduced SOC by 3.36% ($P < 0.001$). In contrast, warming increased DON and nitrate-N concentrations by 8.84% and 13.12%, respectively ($P < 0.001$) (Figure 6). Neither microbial biomass nitrogen nor ammonium-N showed notable changes under warming conditions. Additionally, warming led to declines of 2.07% and 10.79% in the soil C:N and DOC:DON ratios, respectively ($P < 0.001$), whereas the DOC/SOC ratio remained stable. The DON/TN ratio, however, rose by 9.64% ($P < 0.001$). Warming also reduced soil moisture content by 8.04% ($P < 0.001$), while enhancing β -glucosidase activity and soil respiration by 16.64% and 48.16%, respectively ($P < 0.001$).

Consistent with hypothesis 2, warming decoupled DOC and DON dynamics, lowering the DOC:DON ratio (Figure 6). This pattern reflects a balance between DOC production, enhanced by organic matter decomposition and increased root carbon inputs (Liu et al., 2018; Sun et al., 2022; Zhou et al., 2022). DOC consumption, intensified as microbes shift toward resource acquisition strategies under warming (Malik et al., 2020). Elevated β -glucosidase activity and greater CO₂ emissions are consistent with accelerated microbial mineralization of DOC, which may create a positive feedback on carbon loss. These co-occurring changes indicate covariation rather than a directly measured causal pathway. DON increased under warming, further reducing the DOC:DON ratio. Rather than stemming from increased microbial biomass (Figure 6), this accumulation likely results from slower DON decomposition. Low decomposition rates of hydrophilic DON fractions (<3% of total DON; Jones et al., 2004) and carbon-driven nitrogen mineralization (Malik et al., 2020; Tucker et al., 2013), favor DOC over DON consumption. Combined warming and N addition experiments show that microbes preferentially metabolize DOC to maintain stability under stress (Kaiser & Zech, 2000; Malik et al., 2020), thereby decoupling C and N release and increasing soil C loss risk.

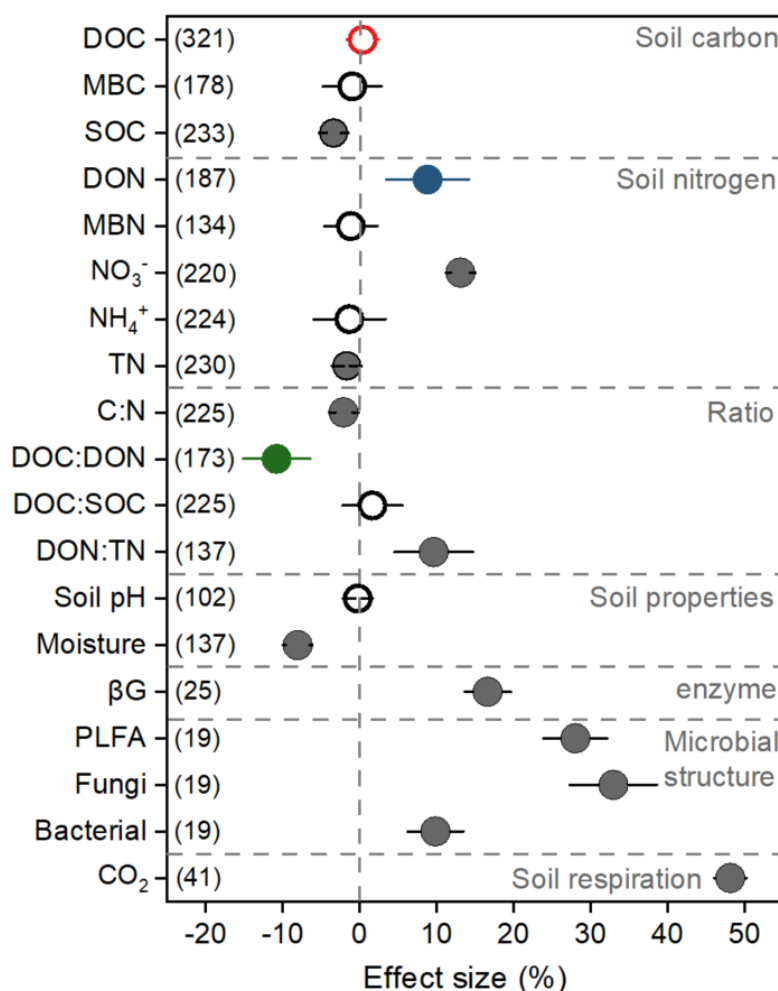


Figure 6. Percentage effect sizes across indicators of soil carbon, nitrogen, their stoichiometric ratios, physicochemical traits, enzyme activities, and respiration. Points indicate the average effect sizes, while the accompanying error bars represent their 95% confidence intervals. An effect is considered significant when the confidence interval does not intersect the vertical dashed reference line. Sample sizes are provided in parentheses; groups with fewer than five observations are excluded from plotting. Abbreviations: DOC – dissolved organic carbon; MBC – microbial biomass carbon; SOC – soil organic carbon; DON – dissolved organic nitrogen; MBN – microbial biomass nitrogen; NO₃⁻-N – nitrate nitrogen; NH₄⁺-N – ammonium nitrogen; TN – total nitrogen; βG – β-glucosidase. *Source: Paper II comprising part of Doctoral thesis.*

The boosted regression tree model analysis explained 64% of DOC variability, identifying soil moisture as the strongest predictor (19%). Initial SOC, MBN, MBC, pH, and N-related responses (NH₄⁺-N, TN, NO₃⁻-N) each contributed 8.8–12%. For DON, the BRT model explained 76% of variance, with nitrate-N as the dominant predictor (33.2%).

Ammonium-N, TN, soil moisture, MBN, and MBC each contributed 8.2–16.2%.

Soil moisture emerged as the dominant factor regulating DOC responses to warming (Figure 7). Reduced moisture under warming suppresses microbial activity, slows SOM decomposition, and lowers DOC levels, particularly under stronger warming scenarios (Allison & Treseder, 2008; Bai et al., 2013; Quan et al., 2019). Negative correlations between DOC and MAT/AI indicate that microbial DOC mineralization outpaces its production under warming (Sun et al., 2022), overshadowing seasonal variability.

DON responses were closely linked to nitrate availability. Warming-induced NO_3^- accumulation met plant nitrogen demands, reduced competition for DON, and promoted its role as a substrate for nitrogen mineralization. This fostered nitrification and a positive DON– NH_4^+ relationship (Jones et al., 2004). Abiotic processes also convert DON to nitrate, as nitrite formed during Fe^{2+} oxidation can bind with dissolved organic matter, forming DON (Dail et al., 2001; Davidson et al., 2003; Matus et al., 2019).

Other soil properties influenced DOC and DON responses through changes in substrate availability. Microbial depletion of labile carbon enhanced decomposition of recalcitrant compounds, decreasing SOC and TN (Frey et al., 2013; Gunina & Kuzyakov, 2022). Consequently, SOC and TN emerged as the strongest predictors of these responses (Kaiser & Zech, 2000; Smreczak & Ukalska-Jaruga, 2021). Responses of DOC and DON also increased with pH, reflecting greater cation exchange capacity, adsorption, and enhanced microbial activity in alkaline soils (Chantigny, 2003; DeForest, 2009; Evans et al., 2012; Kalbitz et al., 2000). These findings highlight the complex regulation of soil carbon and nitrogen cycles under warming, with implications for ecosystem management and climate modeling.

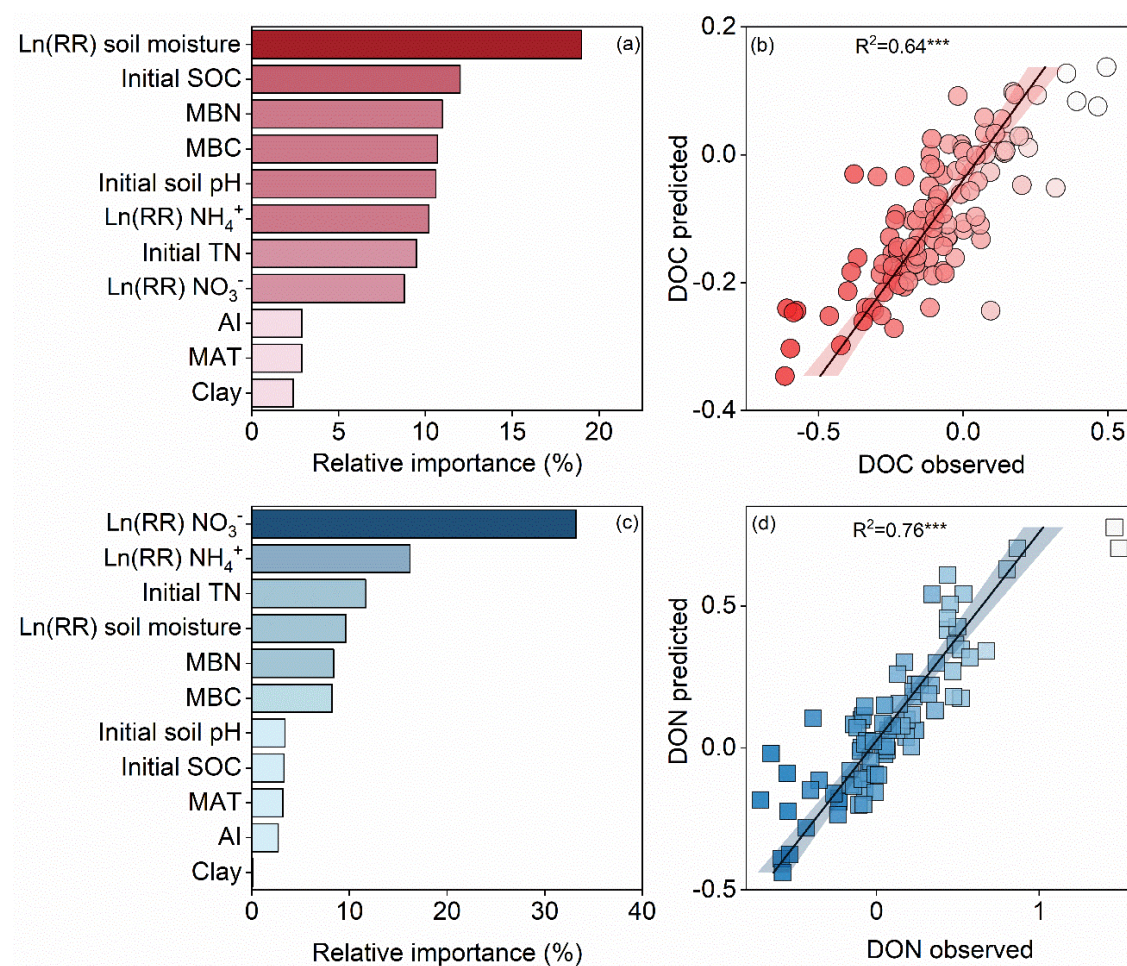


Figure 7. Boosted regression tree (BRT) analysis was used to determine the relative contributions of various predictors to the effect sizes of soil dissolved organic carbon (DOC) (panels a, b) and dissolved organic nitrogen (DON) (panels c, d). Panels b and c illustrate the correspondence between model-predicted and observed values of DOC and DON, respectively. Variables include: SOC – soil organic carbon (g kg^{-1}); MBC – microbial biomass carbon (mg kg^{-1}); MBN – microbial biomass nitrogen (mg kg^{-1}); AI – aridity index; MAT – mean annual temperature ($^{\circ}\text{C}$); TN – total nitrogen (g kg^{-1}); NO_3^- – nitrate nitrogen; NH_4^+ – ammonium nitrogen. Effect sizes are expressed as natural log response ratios (ln(RR)). *Source: Paper II comprising part of Doctoral thesis.*

Linear regression analyses revealed a strong, positive association between CO_2 emissions and warming-induced alterations in soil DOC. Additionally, warming enhanced soil ammonium nitrogen concentrations in proportion to increases in the DOC:DON ratio (Figure 8a), whereas nitrate nitrogen displayed an inverse relationship with the same ratio (Figure 8b). The slope estimates indicated that ammonium nitrogen exhibited roughly half

the sensitivity of nitrate nitrogen to warming effects on DOC:DON dynamics (Figures 8a and 8b). Reduced carbon availability under warming likely constrained microbial DON decomposition, thereby lowering net nitrogen mineralization rates and stabilizing $\text{NH}_4^+\text{-N}$ concentrations (Lu et al., 2011; Qualls & Haines., 1992; Schmidt et al., 2011; Wild et al., 2015). Concurrently, limited carbon availability suppressed heterotrophic microbial competition for $\text{NH}_4^+\text{-N}$, enhancing autotrophic nitrification and thus $\text{NO}_3^-\text{-N}$ accumulation (Cookson & Murphy, 2004; Dittman et al., 2007; Jones et al., 2004; Montañño et al., 2007; Schmidt et al., 2011). This increased nitrification could elevate nitrate leaching risks and enhance denitrification-related N_2O emissions, exacerbating soil nitrogen losses (Bai et al., 2013; Deng et al., 2023; Elrys et al., 2023).

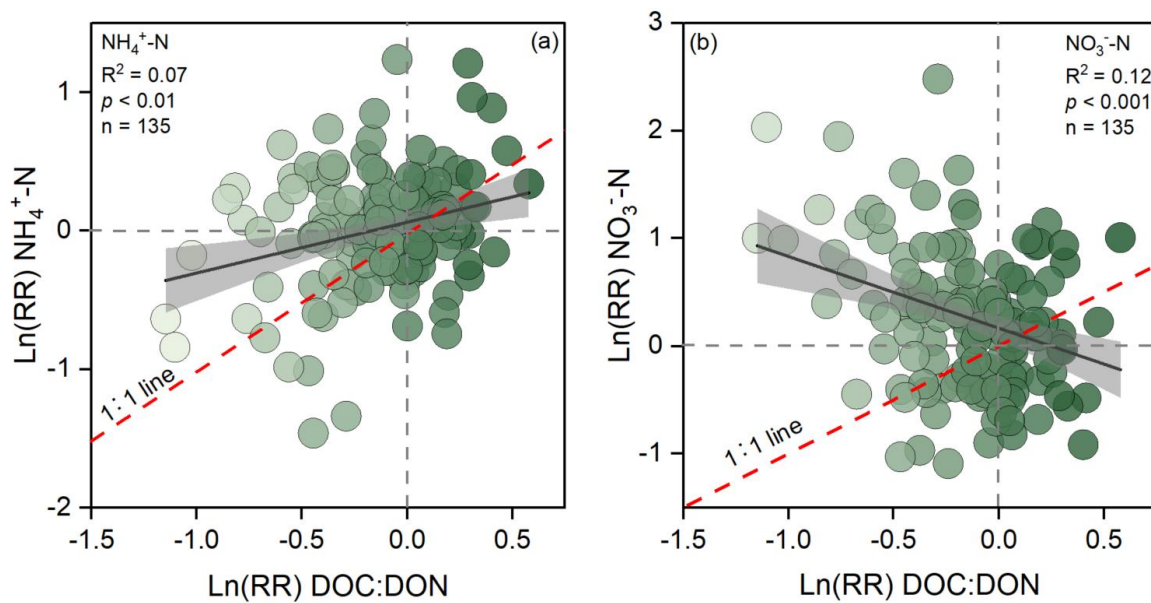


Figure 8. Associations between the DOC:DON ratio and concentrations of ammonium nitrogen (a) and nitrate nitrogen (b). $\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$ represent ammonium and nitrate nitrogen, respectively. The gray dashed line marks the zero-effect threshold, while shaded regions depict the 95% confidence intervals. Red lines indicate the 1:1 reference. Sample size is denoted by n . Effect sizes are expressed as natural log response ratios (Ln(RR)). *Source: Paper II comprising part of Doctoral thesis.*

3.3 Effects of agriculture management practices on soil DOC - Paper III

In line with Objective 3 this section evaluates agricultural practices as management levers to raise DOC while improving yield and lowering CO₂ intensity. We compare CT, mineral N, straw, manure, NS, NM, and biochar using both effect sizes and a per-unit DOC efficiency metric. Relative to the control treatment, DOC concentrations exhibited substantial increases under NM (78.21%), manure application (49.91%), NS (41.02%), straw incorporation (25.68%), nitrogen fertilization (18.4%), biochar amendment (14.89%), and CT (9.74%) (Figure 9a). Enhancements in the DOC/SOC ratio were most pronounced under NM (24.73%), with progressively smaller positive responses under NS (15.79%), manure (12.02%), straw (11.13%), and nitrogen addition (7.66%). Conversely, biochar application resulted in a significant reduction in the DOC/SOC ratio (−25.02%) (Figure 9b).

Management practices significantly influenced DOC concentrations what was consistent with hypothesis 3. CT and biochar showing distinct patterns compared to other treatments (Figure 9). While CT increased DOC and SOC similarly, it did not alter the DOC/SOC ratio, likely due to reduced soil disturbance enhancing aggregate stability and microbial activity, thus promoting carbon accumulation and decomposition (Khursheed et al., 2019; Li et al., 2021; Mondal & Chakraborty, 2022). In contrast, nitrogen fertilizer, manure, straw, NM, and NS increased both DOC and DOC/SOC ratios. These practices introduced exogenous organic compounds and nutrients, promoting root growth, biomass production, microbial activity, and DOC generation (Cai et al., 2019; Haynes & Naidu, 1998; Li et al., 2015; Luo et al., 2020; Zhang et al., 2023). Biochar increased DOC but reduced the DOC/SOC ratio by improving soil moisture, pH, and providing adsorption sites and stable carbon sources, thus disproportionately enhancing SOC relative to DOC (Liu et al., 2016; Weber & Quicker, 2018). These insights underline the potential for targeted management practices to optimize soil carbon sequestration.

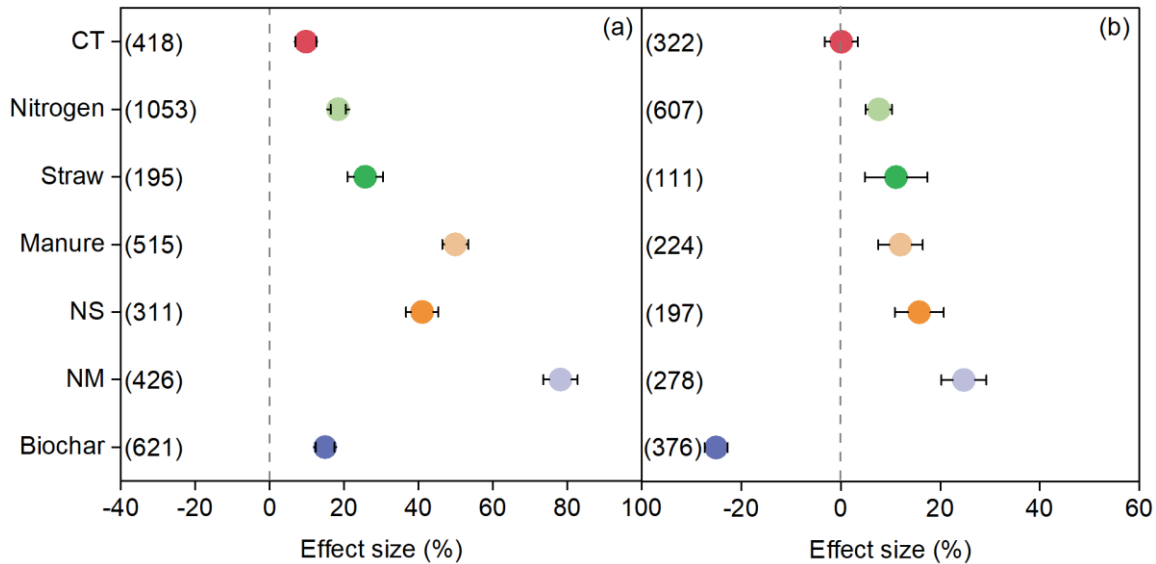


Figure 9. Impacts of different management strategies on soil dissolved organic carbon (DOC) levels (a) and the DOC-to-SOC ratio (b). Points indicate average effect sizes (%), with black error bars showing their 95% confidence intervals. The vertical dashed lines serve as reference thresholds for non-significant changes—significance is inferred when the confidence interval does not intersect these lines. Sample sizes are provided in parentheses. Evaluated management interventions include nitrogen fertilization, straw return, manure application, biochar addition, conservation tillage (CT), and combinations of nitrogen with straw (NS) or manure (NM). *Source: Paper III comprising part of Doctoral thesis.*

The magnitude of DOC responses was jointly regulated by management practice intensity, experimental duration, soil physicochemical conditions, and microbial community characteristics (Figure 10). Among these factors, the intensity of practices was the strongest predictor of DOC responses under manure application, NM, and biochar treatments. For manure and NM, DOC initially declined at low application rates due to insufficient carbon inputs, forcing microbes to mine existing SOC for DOC production (Mooshammer et al., 2014). Moderate inputs balanced carbon supply and microbial demand, stabilizing organic matter and limiting DOC increases (Schmidt et al., 2015). Excessive applications overwhelmed microbial processing capacity, leading to elevated DOC (Wu et al., 2021). Biochar acted as a stabilizing agent with high DOC adsorption capacity (Wang et al., 2017), where low doses mainly enhanced SOC retention (Hernandez - Soriano et al., 2016). At higher doses, biochar introduced labile carbon and

stimulated microbial activity, accelerating SOC transformation into DOC (Schmidt et al., 2015). These results highlight the importance of optimizing organic amendments to balance nutrient supply and promote sustainable carbon management.

In contrast, experimental duration was the strongest predictor of responses under CT, nitrogen fertilization, straw addition, and NS. For CT, DOC and DOC/SOC ratios decreased over time as improved water retention and reduced erosion reached equilibrium, leading to stabilized organic matter and reduced DOC release (Cai et al., 2022; Mondal & Chakraborty, 2022; Wardak et al., 2022). Long-term nitrogen fertilizer use enhanced plant growth and residues, stimulating microbial activity and DOC formation, but also gradually lowered soil pH, which may slow DOC decomposition (Wu et al., 2021). DOC responses were stronger under NS compared to nitrogen fertilizer or straw alone, likely because the high C/N ratio of straw limits microbial decomposition without sufficient nitrogen input (Cai et al., 2018). Adding nitrogen balances the C/N ratio, enabling microbes to more effectively process straw-derived carbon and increase DOC production. These findings emphasize the importance of long-term, balanced fertilization strategies to sustain soil carbon dynamics in agricultural systems.

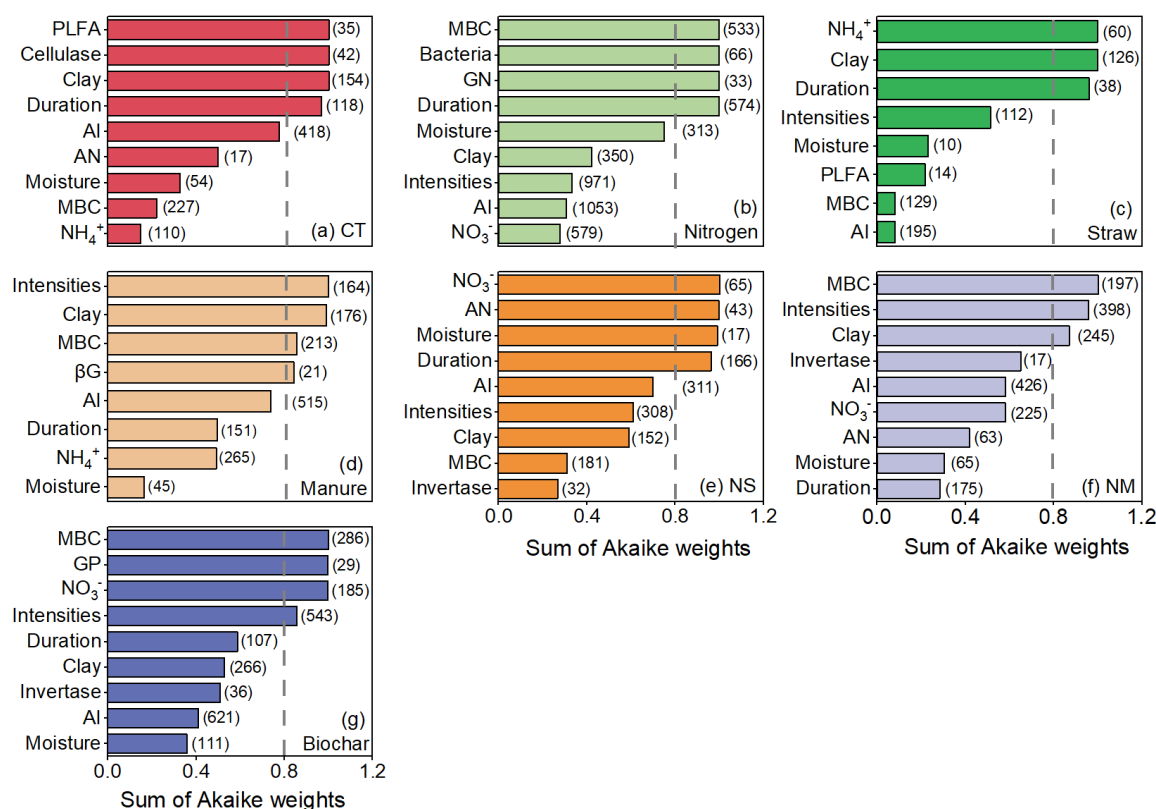


Figure 10. Model-averaged factor importance for predicting changes in soil dissolved organic carbon (DOC) under various management practices (panels a–g). Importance values are calculated as the cumulative Akaike weights from model selection procedures. A threshold of 0.8 (dashed line) is used to identify the most influential predictors. The management interventions examined include conservation tillage (CT, a), nitrogen fertilization (b), straw incorporation (c), manure application (d), combined nitrogen and straw (NS, e), combined nitrogen and manure (NM, f), and biochar addition (g). Considered predictors encompass a range of biotic and abiotic variables: phospholipid fatty acids (PLFA), cellulase activity, clay fraction content, soil ammonium (NH_4^+), aridity index (AI), available nitrogen (AN), soil moisture, microbial biomass carbon (MBC), experiment duration, total bacterial abundance, gram-negative (GN) and gram-positive (GP) bacteria, experimental intensity, soil nitrate (NO_3^-), β -1,4-glucosidase (βG), invertase, soil pH response ratio (pH-RR), and total nitrogen (TN). *Source: Paper III comprising part of Doctoral thesis.*

Nitrogen fertilization, manure application, NS, NM, and biochar treatments markedly elevated CO_2 emissions, with increases ranging from 10.00% to 43.20% (Figure 11). All management interventions significantly enhanced crop yields (Figure 11), with NM

producing the greatest yield improvement (145.07%) and CT showing the smallest gain (8.11%). The effect size of DOC demonstrated a robust positive linear association with both CO₂ emissions and crop yields. However, the efficiency with which DOC influenced these outcomes varied across management practices (Figure 11). Specifically, nitrogen fertilizer, straw incorporation, manure application, NS, and NM diminished the efficiency of DOC in driving CO₂ emissions, whereas nitrogen fertilizer, straw, NS, NM, and biochar enhanced the capacity of DOC to improve crop yields.

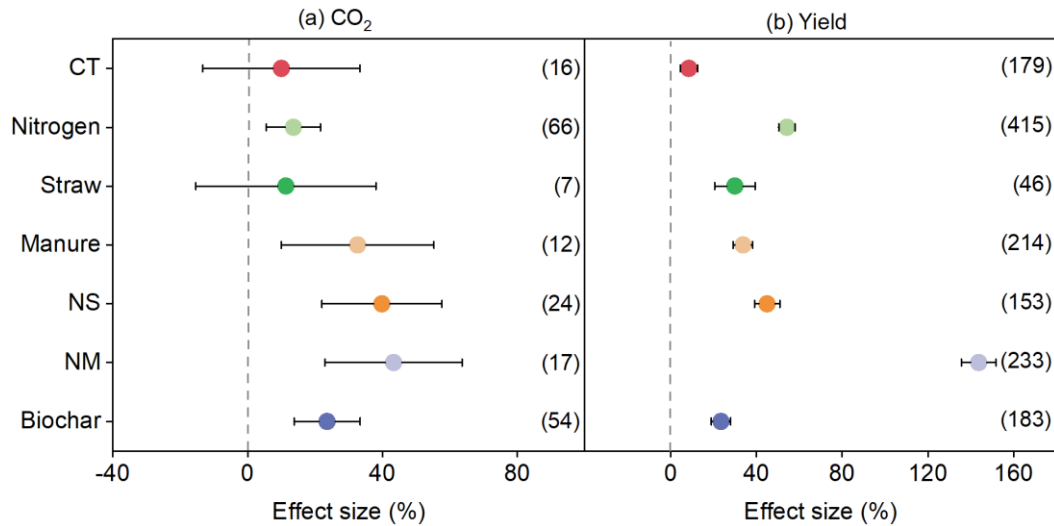


Figure 11. Effects of management practices on soil respiration (a) and crop yields (b). Dots and black bars represent mean and 95% confidence intervals of effect size (%). Vertical dashed lines indicate non-significant effects. The effect sizes are statistically significant if the 95% confidence intervals do not overlap with the vertical dashed lines. Values in parentheses represent sample sizes. Management practices include conservation tillage (CT), nitrogen fertilizer, straw, manure, nitrogen fertilizer combined with straw (NS), nitrogen fertilizer combined with manure (NM), and biochar. *Source: Paper III comprising part of Doctoral thesis.*

DOC co-varies with both CO₂ emissions and crop yields, increasing alongside each across the management practices examined. Under straw, NM, manure, nitrogen fertilizer, and NS, DOC accumulation outpaced CO₂ release, as reflected by negative DOC–CO₂ efficiencies (–55.81% to –2.94%) (Figure 12). These efficiencies describe **co-variation** between the DOC and CO₂ responses rather than a causal relationship; the pattern is **consistent with** microbial decomposition favouring DOC formation over immediate mineralization (Wu et al., 2021), and DOC was stabilized through adsorption to soil

particles (Kaiser & Zech, 2000) or transformed during nitrogen mineralization (Mooshammer et al., 2014). The positive DOC–CO₂ efficiencies under biochar and CT are consistent with enhanced microbial respiration and carbon oxidation, though both DOC and CO₂ may respond independently to organic-matter input (Jiang et al., 2016; Manzoni et al., 2012).

All management practices increased crop yields (Figure 11), and DOC positively correlated with yield responses. Nitrogen fertilizer showed the highest DOC–yield efficiency (201.23%) (Figure 12); this index reflects co-variation rather than causation, and the yield gain is likely driven predominantly by improved nitrogen availability rather than by the DOC change itself, alongside N-stimulated root exudation and biomass accumulation (Ren et al., 2022). Although NM led to high yield gains (145%), its lower DOC–yield efficiency indicates that the DOC increase exceeded yield gains, likely due to higher carbon input relative to nutrients. Biochar improved soil fertility and boosted yields through enhanced nutrient availability and stable carbon accumulation, though its contribution to DOC was limited due to the dominance of recalcitrant carbon forms (He et al., 2017; H. Xu et al., 2021a). Overall, DOC efficiencies highlight the trade-offs between carbon accumulation, CO₂ release, and productivity, informing sustainable input strategies.

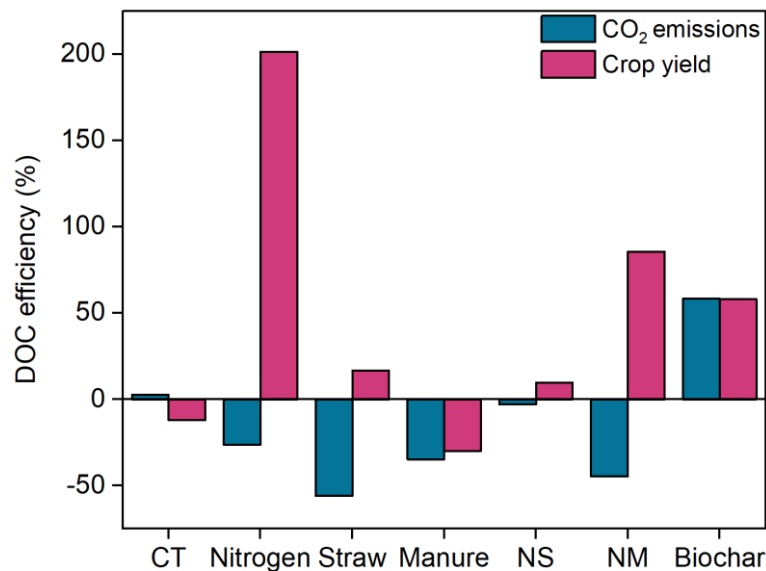


Figure 12. Soil dissolved organic carbon (DOC) use efficiency across various management interventions. The blue bars represent the efficiency of DOC in reducing soil CO₂ emissions per unit increase in DOC, while the red bars indicate the efficiency of DOC in enhancing crop yield

per unit increase. Evaluated management strategies include nitrogen fertilization, straw return, manure application, biochar amendment, conservation tillage (CT), and combinations of nitrogen with straw (NS) or with manure (NM). *Source: Paper III comprising part of Doctoral thesis.*

3.4 Effects of crop rotation vs monoculture and soil depth on DOMC - Paper IV

Addressing Objective 4, this section uses a depth-explicit, DOMC-complete field design (DOC, DON, DOP at 0–30, 30–60, 60–90 cm) to validate how soil depth and cropping system (rotation vs. monoculture) co-structure DOM quantity and quality. This field component directly remedies the global synthesis gaps in DOP coverage and subsoil evidence revealed in Objectives 1–3. The results showed that DOM concentrations (DOC, DON, and DOP) declined consistently with increasing soil depth (0–30, 30–60, 60–90 cm) under both monoculture and crop rotation systems what confirmed hypothesis 4 (Figure 13). Significant cropping system effects were observed only for DOC in the deepest layer (60–90 cm), where monoculture plots contained more DOC than rotation plots ($P < 0.05$). DON and DOP showed no significant differences between cropping systems at any depth (Figs. 13b, c). Vertical stratification was evident for DOC and DON across all layers ($P < 0.05$), while DOP varied only between surface and subsoil layers, suggesting a more stable vertical profile for phosphorus.

Overall, soil depth exerted a stronger influence on DOM distribution than cropping system. Reduced plant residue input, root exudation, and microbial activity in deeper soils likely explain the depth-dependent decrease (Kaiser & Kalbitz, 2012; Kalbitz et al., 2000). Minimal differences between cropping systems in surface layers may result from long-term homogenization due to similar fertilization and tillage practices. These findings will not allow to accept hypothesis 4. While crop rotation can enhance microbial diversity and activity (Chen et al., 2022), and monoculture can reduce diversity and favor specialized decomposers (Zhang et al., 2025), these effects did not produce significant DOM differences in topsoil. In deeper layers, higher DOC in monoculture soils may reflect slower decomposition of recalcitrant fractions (Findlay & Parr, 2017; Zhang et al., 2025), and impaired aggregate stability, facilitating downward transport and retention of DOC (Dou et al., 2025; Rumpel & Kögel-Knabner, 2011; P. Xu et al., 2021). The lack of

significant cropping system effects on DON and DOP suggests these fractions are more controlled by intrinsic microbial mineralization and adsorption processes (Helfenstein et al., 2018; Karl & Björkman, 2015). The vertical distribution of DOP aligns with the established surface enrichment and deep depletion pattern of phosphorus driven by mineral adsorption (Jobbagy & Jackson, 2001).

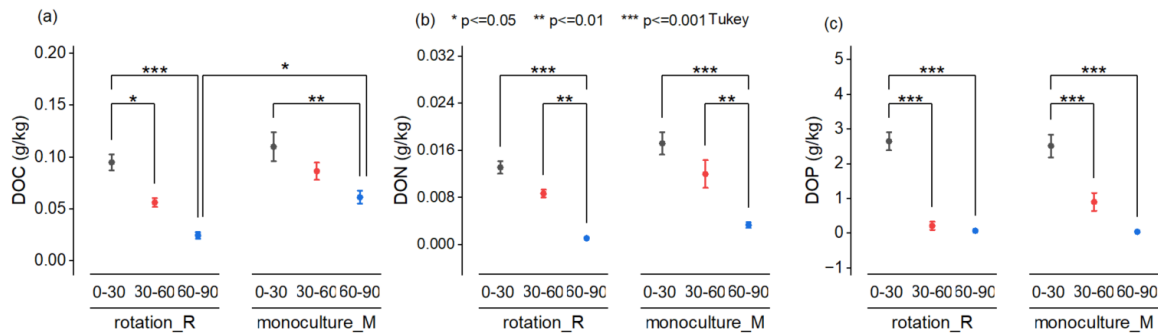


Figure 13. Profiles of soil dissolved organic carbon (DOC), nitrogen (DON), and phosphorus (DOP) under rotation and monoculture systems across depth gradients. Panels a–c display the concentrations of cold-water extractable DOC, DON, and DOP, respectively, measured at three depth intervals (0–30 cm, 30–60 cm, and 60–90 cm) under crop rotation (R) and monoculture (M) systems. Values are shown as means $\pm$ standard error ($n = 12$). Differences among soil layers within each system were assessed using Tukey’s HSD test, with significance levels denoted as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). *Source: Paper IV comprising part of Doctoral thesis.*

Spectroscopic analyses revealed that soil depth had a stronger influence on DOM molecular composition than cropping system. UV–Vis results showed increased DOM aromaticity with depth, indicated by higher SUVA₂₈₀ values ($P < 0.01$; Figure 13), while the E4/E6 ratio decreased, suggesting greater humification and molecular weight in deeper soils (Figure 13). SUVA₄₆₅ and SUVA₆₆₅ also rose in the deepest layers under both cropping systems but were not statistically significant (Figure 14). VIS–NIR spectra supported these findings, with deeper monoculture soils showing higher visible absorbance and rotation soils exhibiting greater NIR absorbance (Figure 15). These spectral patterns align with elevated DOC in monoculture subsoils and indicate that DOM becomes increasingly aromatic, humified, and stable with depth.

Although crop rotation can influence microbial activity and enzyme production, leading to faster DOM mineralization (Saadi et al., 2006), long-term homogenization of

soil properties under similar fertilization likely limited management-induced differences in DOM molecular features. Depth-driven changes are governed by microbial decomposition of labile compounds near the surface and the downward migration of recalcitrant aromatics. These processes lead to the accumulation of stabilized humic substances in deeper horizons (Sanderman et al., 2009; Weishaar et al., 2003). Oxygen-limited subsoil conditions further promote oxidative condensation reactions that enhance DOM complexity and carbon persistence (Bravo-Escobar et al., 2024; Khan et al., 2024; Rumpel & Kögel-Knabner, 2011). Future studies employing high-resolution molecular tools (e.g., FT-ICR-MS, ^{13}C -NMR) and microbial assays are needed to uncover subtle management effects on DOM chemistry and carbon sequestration processes (Ding et al., 2020).

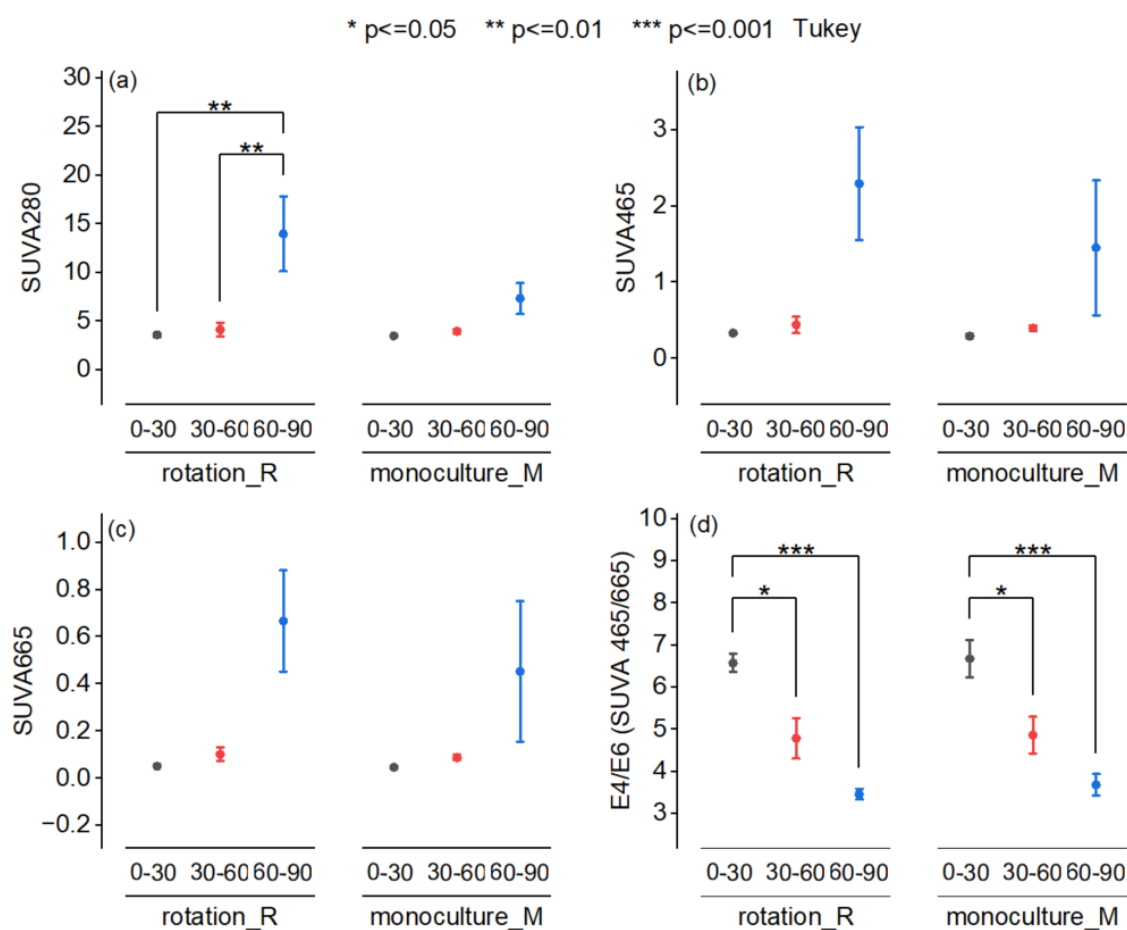


Figure 14. UV–Vis spectral properties of cold-water extractable dissolved organic matter (DOM) under rotation and monoculture across soil depths. Spectroscopic indices were calculated from UV–Vis absorbance measurements of DOM extracted from soils at 0–30 cm, 30–60 cm, and 60–90 cm depths under crop rotation (R) and monoculture (M) systems. (a) SUVA₂₈₀ represents specific UV

absorbance at 280 nm, indicative of aromatic and protein-like compounds. (b) SUVA₄₆₅ reflects absorbance at 465 nm, typically linked to humic substances. (c) SUVA₆₆₅ corresponds to absorbance at 665 nm, associated with highly aromatic, condensed organic structures. (d) The E₄/E₆ ratio (i.e., SUVA₄₆₅/SUVA₆₆₅) provides insight into DOM molecular weight and humification status. Data are reported as means $\pm$ standard error ($n = 12$). Depth-wise comparisons within each system were analyzed using Tukey's HSD test. Significance levels are denoted as $P < 0.05$ (*), $P < 0.01$ (**), and $P < 0.001$ (***). *Source: Paper IV comprising part of Doctoral thesis.*

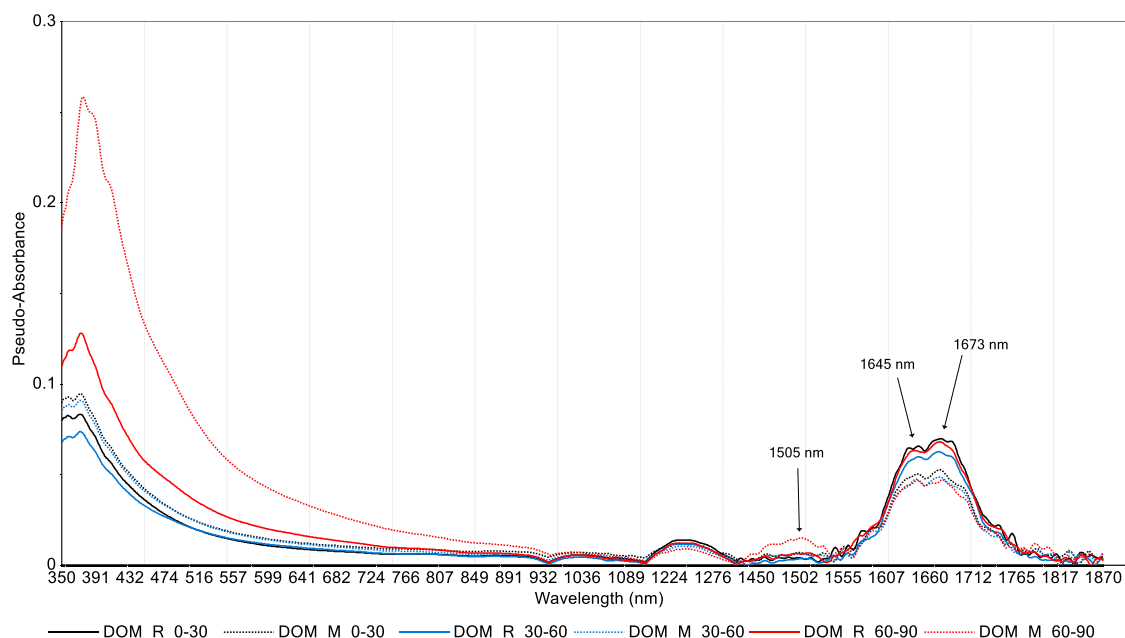


Figure 15. Average VIS–NIR spectral profiles of cold-water extractable dissolved organic matter (DOM) across cropping systems and soil depths. Mean pseudo-absorbance spectra in the 350–1850 nm range were obtained from soils under crop rotation (solid lines, DOM_R) and monoculture (dotted lines, DOM_M) at three depth intervals: 0–30 cm (black), 30–60 cm (blue), and 60–90 cm (red). Spectral curves represent average values derived from either triplicate or composite samples. *Source: Paper IV comprising part of Doctoral thesis.*

Correlation analysis and Random Forest modeling identified key soil properties regulating DOM fractions (Figure 16). DOC variability was mainly driven by total nitrogen (14.92%), total carbon (11.68%), and DOP (9.67%), highlighting the importance of soil organic matter mineralization as the primary source of dissolved carbon (Nanthi S. Bolan et al., 2011; Kaiser & Kalbitz, 2012; Karavanova, 2013). DON was mainly influenced by DOC (17.64%), soil depth (16.00%), and available P (15.59%). These patterns reflect the

structural linkage between organic N and C in DOM (Neff et al., 2003) and depth-related declines in biological turnover (Berman & Bronk, 2003; Sipler & Bronk, 2015). Available phosphorus effects suggest cross-regulation between nitrogen and phosphorus cycles, where microbial P demand accelerates N mineralization and elevates DOP (Karl & Björkman, 2015).

DOP dynamics were driven by humus content (15.56%), total phosphorus (15.45%), and available phosphorus (15.43%), indicating that humus-bound phosphorus acts as a long-term reservoir for DOP release via microbial decomposition and enzymatic hydrolysis (Kalbitz et al., 2000; Zsolnay, 2003). The asymmetric link between DOP and DOC—where DOP predicts DOC but not vice versa—suggests that phosphorus-rich compounds contribute carbon to DOM, while broader DOC pools lack phosphorus-specific controls (Ding et al., 2020; Richardson & Simpson, 2011). These findings underscore the need to enhance SOC and balance nutrient availability to regulate DOM cycling and improve carbon, nitrogen, and phosphorus retention in agricultural subsoils.

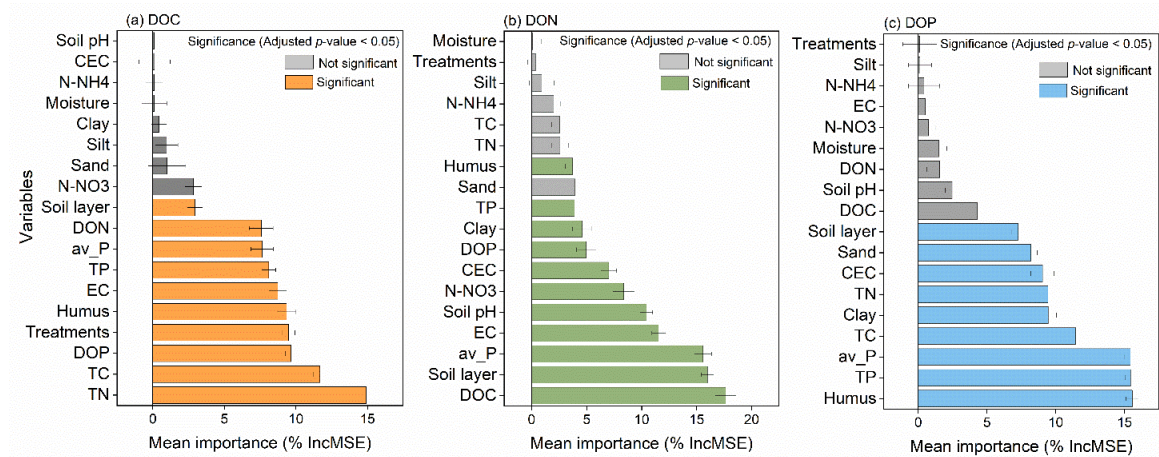


Figure 16. Key predictors of DOC, DON, and DOP concentrations identified through random forest analysis. The random forest algorithm was applied to assess the relative influence of individual variables on cold-water extractable dissolved organic carbon (DOC; panel a), nitrogen (DON; panel b), and phosphorus (DOP; panel c). Predictor importance is quantified as the percentage increase in mean squared error (%IncMSE) resulting from variable permutation. Bars display the average $\pm$ standard deviation of importance scores across decision trees. Statistically significant predictors (adjusted $P < 0.05$) are highlighted in color—orange for DOC, green for DON, and blue for DOP—while non-significant variables are shown in grey.

Source: Paper IV comprising part of Doctoral thesis.

4 Summary and Conclusions

4.1 Summary

4.1.1 Differential impacts of nitrogen addition on soil dissolved organic carbon in humid and non-humid regions

Synthesizing global evidence, nitrogen enrichment consistently increased SOC yet drove opposite DOC responses across humidity regimes, declined in humid systems but increases in non-humid ones. These contrasts aligned with inverse biotic–abiotic controls: precipitation seasonality independently shaped DOC outcomes everywhere, but initial SOC best explained variability in humid regions, whereas the applied N rate dominated in non-humid regions. Covariation with soil and microbial pools shifted across regimes. In humid sites, DOC responses aligned with higher MBC and lower pH, while the opposite held in non-humid sites. Likewise, the DOC effect scaled inversely with the MBC:SOC ratio between regimes. Mechanistically, humid systems appear closer to N saturation, where added N stabilizes or reduces plant C inputs and dampens respiration, shrinking labile C (lower DOC and MBC) while favoring accrual of more recalcitrant SOC. In non-humid systems, N alleviation of limitation boosts productivity and reconfigures microbial activity, elevating DOC and MBC, with SOC gains sourced largely from less stable pools. These regime-specific pathways highlight the need for regional parameterization of N effects in ecosystem models to better project SOC dynamics under global change. They also underscore the heightened vulnerability of non-humid landscapes to warming, intensifying drought, and shifting (including acid) deposition regimes (Figure 17).

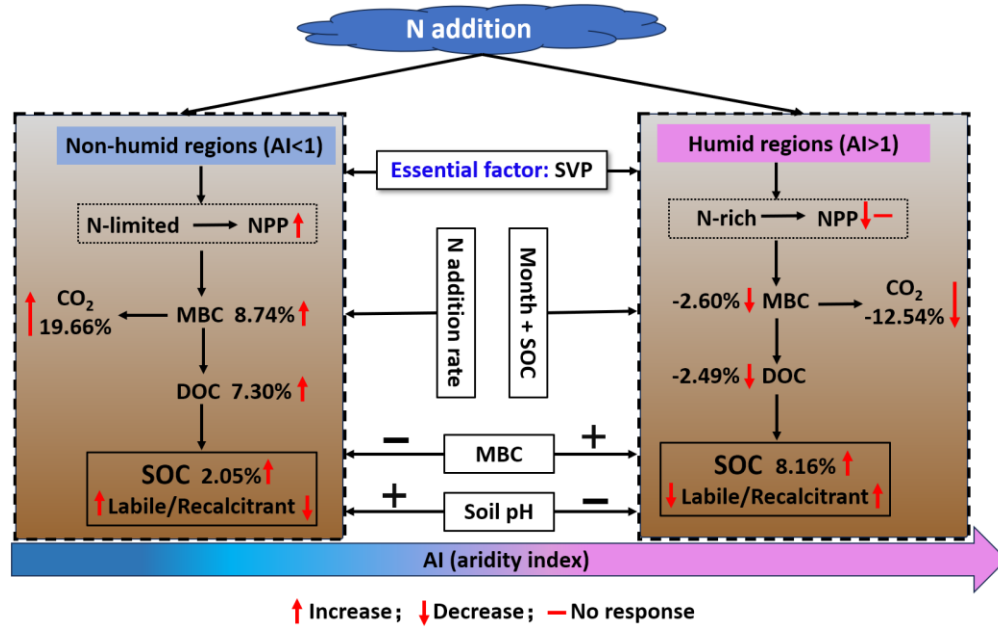


Figure 17. The conceptual model illustrates the main processes of nitrogen (N) addition effects on soil dissolved organic carbon (DOC) in humid and non-humid regions. *Source: Paper I comprising part of Doctoral thesis.*

4.1.2 Asymmetric responses of soil dissolved organic carbon and dissolved organic nitrogen to warming

Our global synthesis shows that warming decouples soil C–N cycling: DON increases while DOC remains, on average, unchanged, driving a lower DOC:DON. Mechanistically, higher temperature raises microbial maintenance costs and limits labile-C availability at the microscale. Microbes shift toward stress-adapted (S–A) strategies, accelerating turnover of available DOC without necessarily elevating its bulk concentration, and increasingly mining recalcitrant SOC. This elevates CO₂ emissions and reinforces positive climate feedback. Constrained carbon supply simultaneously reduces heterotrophic NH₄⁺ assimilation, leaving more NH₄⁺ available for autotrophic nitrification, which promotes NO₃[–]–N and DON accumulation. The resulting rise in mobile N elevates risks of leaching and N₂O production. In sum, warming pushes soils toward net C loss while mobilizing reactive N, producing an asymmetric DOC–DON response and partial C–N decoupling. Earth-system models should represent these regime shifts—stable bulk DOC with faster turnover, DON and NO₃[–] buildup, and strengthened CO₂/N₂O fluxes—to more realistically project biogeochemical feedback under continued warming (Figure 18).

carbon supply with nitrogen availability, (ii) sustaining them over multi-year horizons, and (iii) tuning rates to local soil–climate context. Future work should examine DOC turnover and composition, not only concentration, linking surface gains to deep-soil processes and hydrological losses. These management-sensitive DOC pathways should be embedded in models to improve forecasts of carbon sequestration and agroecosystem performance (Figure 19).

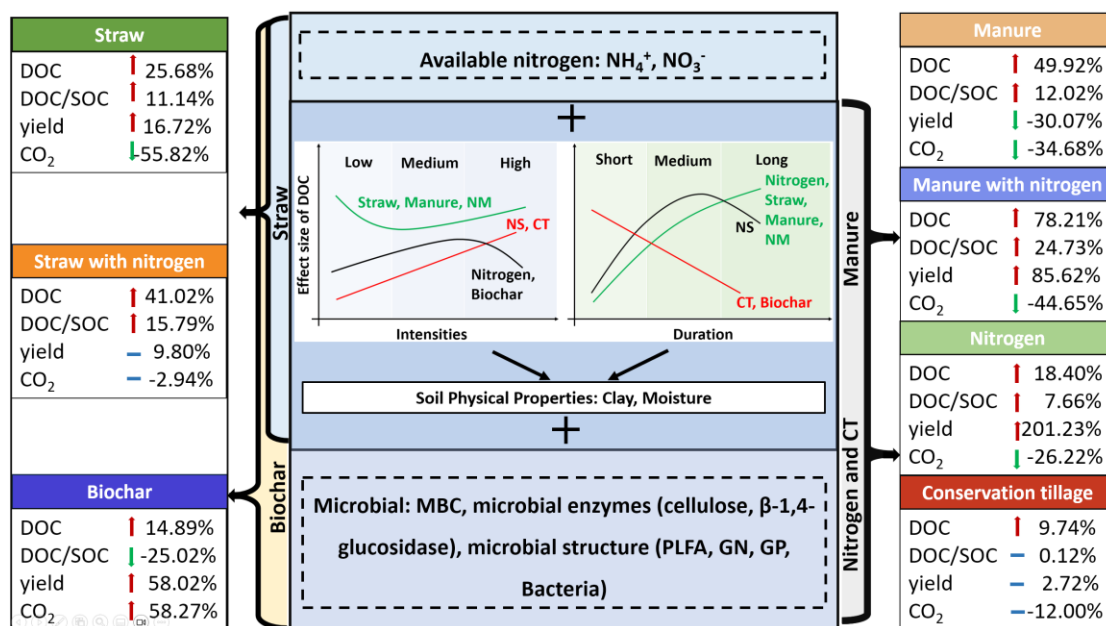


Figure 19. Graphic summary of the main processes of management practices effect on soil dissolved organic carbon. Management practices include nitrogen fertilizer, straw, manure, biochar, conservation tillage (CT), nitrogen fertilizer combined with straw (NS), and nitrogen fertilizer combined with manure (NM). *Source: Paper III comprising part of Doctoral thesis.*

4.1.4 Depth-dependent effects of crop rotation and monoculture on quantity and quality of dissolved organic matter components

In the long-term Osiny experiment, soil depth—not cropping system—sets the primary template for DOM: DOC, DON, and DOP decline down-profile while aromaticity and humification increase, signaling progressively condensed, stable subsoil DOM. The cropping signal emerges only at depth: in 60–90 cm soils, monoculture maintains higher DOC than rotation, whereas DON and DOP remain indistinguishable across systems—patterns corroborated by UV–Vis and VIS–NIR diagnostics. Distinct controls govern each

DOM fraction: DOC tracks total N, total C, and DOP; DON aligns with DOC, depth, and available P; DOP is shaped by humus, total P, and available P, underscoring differentiated C–N–P linkages along the profile. Practically, steering subsoil DOM requires depth-aware strategies. Multi-year crop choices that promote deep rooting and diverse exudation should be combined with profile-resolved monitoring using chemical and optical indices. Monoculture systems, in particular, need management to address potential deep DOC accumulation and nutrient mobility risks. To refine mechanism and modeling, pair future depth-explicit sampling with high-resolution molecular and microbial assays, integrating these pathways into agroecosystem models to capture subsoil contributions to carbon storage and nutrient cycling (Figure 20).

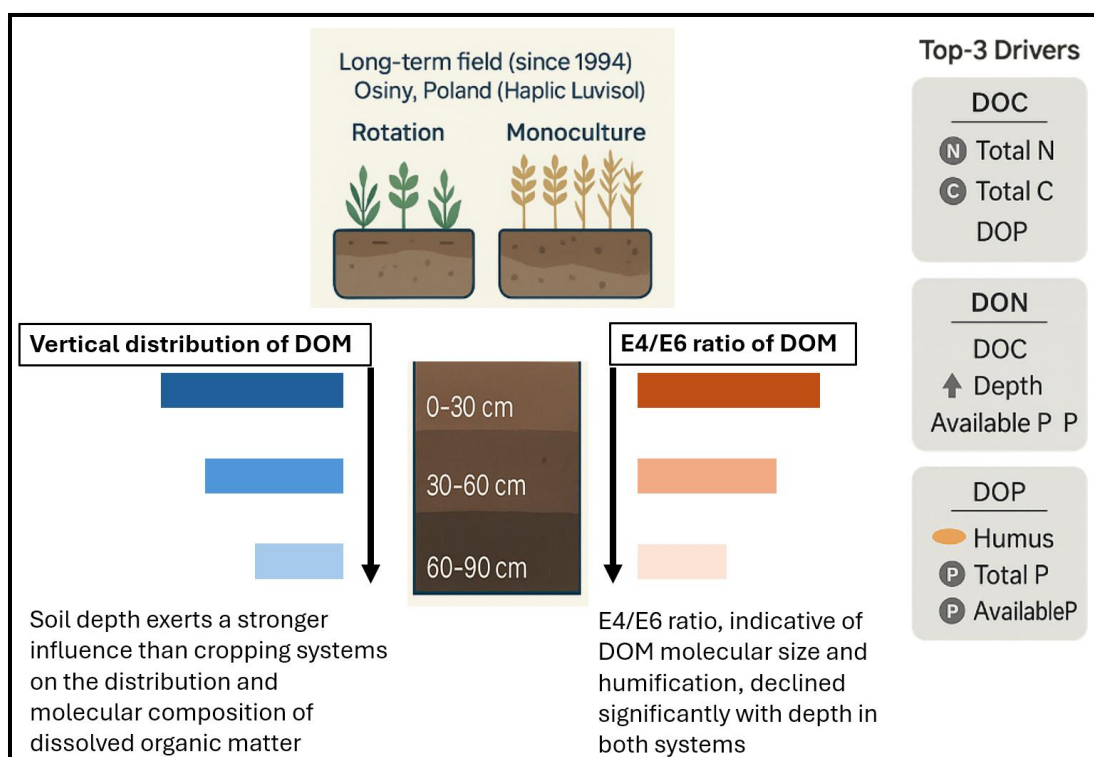


Figure 20. Graphic summary of depth-dependent effects of crop rotation and monoculture on dissolved organic matter quantity and quality. *Source: Paper IV comprising part of Doctoral thesis.*

4.2 Conclusions

1. N addition reduces DOC and MBC but increases SOC in humid regions (stabilization into MAOC), whereas it raises DOC and MBC in non-humid regions (enhanced cycling), with seasonality and pH setting sensitivity.
2. Warming lowers DOC:DON consistent with accelerated DOC turnover (without raising bulk DOC) and with increased DON and NO_3^- that may arise from reduced heterotrophic NH_4^+ uptake and enhanced nitrification with soil moisture the strongest predictor of the DOC response. These proposed mechanisms are consistent with the data but were not directly measured.
3. Practices that synchronize C supply with N (NM, NS, N + straw) are associated with the most favorable DOC-for-yield and CO_2 -intensity outcomes, while biochar uniquely increases SOC and lowers DOC/SOC, a pattern consistent with carbon stabilization.
4. Depth is the first-order organizer of DOM (declining DOC/DON/DOP; lower E4/E6, higher SUVA with depth). Management signals are subdued except at depth, where monoculture retains more DOC; DOC tracks TN/TC/DOP, DON follows DOC/depth/available P, and DOP aligns with humus/total P/available P.

5. Limitations and future directions

Although this thesis integrates global meta-analyses with a depth-resolved field study to reveal how global-change drivers and agricultural practices regulate soil DOMC across scales, several limitations remain.

- (i) Meta-analyses: DOP records were too scarce for robust synthesis, constraining global inference for the full DOMC stoichiometry. Evidence was also surface-biased (0–30 cm), limiting insight into subsoil processes. Methodological heterogeneity (extraction vs. pore water, differing extractants and reporting formats) introduced between-study variances that could not be fully harmonized, and many primary studies employed single-factor designs, restricting attribution of interactions among temperature, moisture, nutrients and disturbance.
- (ii) Field experiment: Results derive from a single site, which narrows representativeness across climates and soils; DOMC quality was assessed with optical proxies without molecular-level resolution; and sampling lacked seasonal/event coverage needed to capture wetting–drying pulses and hydrologic exports.

To address these gaps, future work should:

- (1) build a global DOP dataset alongside DOC and DON to enable tri-element stoichiometric analyses.
- (2) extend evidence below 30 cm using depth-explicit sampling (≥ 1 m) coupled with pore-water samplers and lysimeters to quantify vertical fluxes and residence times.
- (3) standardize methods through preregistered protocols and cross-walks between extraction and pore-water measurements, and apply hierarchical models that account for method effects.
- (4) prioritize factorial, long-term experiments (e.g., N $\times$ warming $\times$ moisture $\times$ management) to resolve main effects vs. interactions.
- (5) expand multi-site field networks across humidity regimes, textures and cropping systems to test generality.

(6) pair optical indices with high-resolution mass spectrometry (e.g., FT-ICR MS/Orbitrap) to resolve molecular composition and link it to bioavailability and stabilization.

(7) increase temporal resolution to track seasonal dynamics and event-scale pulses, integrating concentration with flux measurements and embedding these constraints in depth-aware models. Together, these steps will deliver a more representative, mechanistic and management-relevant understanding of DOMC under global change.

6. References

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7. Declarations of the Author and the Supervisor of the Doctoral Dissertation

Załącznik 6

*do Regulaminu przygotowywania rozpraw doktorskich
w formie zbioru artykułów naukowych*

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Oświadczenie autora rozprawy doktorskiej

Świadom odpowiedzialności prawnej oświadczam, że rozprawa doktorska przygotowana przeze mnie nie zawiera treści uzyskanych w sposób niezgodny z obowiązującymi przepisami.

Oświadczam również, że przedstawiona rozprawa nie była wcześniej przedmiotem procedur związanych z uzyskaniem stopnia naukowego w wyższej uczelni lub innej uprawnionej instytucji naukowej.

Oświadczam ponadto, że niniejsza wersja rozprawy jest identyczna z załączoną wersją elektroniczną.

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OŚWIADCZENIE PROMOTORA ROZPRAWY DOKTORSKIEJ

Oświadczam, że niniejsza rozprawa doktorska pt. : „Factors controlling content and transformations of dissolved organic matter components in agricultural soils at field and global scales.” autorstwa Pana/Pani mgr inż. ... Tianjing Ren, MSc, została przygotowana pod moim kierunkiem. Stwierdzam, że niniejsza praca doktorska spełnia warunki do przedstawienia jej w postępowaniu o nadanie stopnia doktora nauk rolniczych.

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28. 10. 2015

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OŚWIADCZENIE PROMOTORA POMOCNICZEGO ROZPRAWY DOKTORSKIEJ

Oświadczam, że niniejsza rozprawa doktorska pt. : „Factors controlling content and transformations of dissolved organic matter components in agricultural soils at field and global scales.” autorstwa Pana/Pani mgr inż. ... Tianjing Ren, MSc, została przygotowana pod moim kierunkiem. Stwierdzam, że niniejsza praca doktorska spełnia warunki do przedstawienia jej w postępowaniu o nadanie stopnia doktora nauk rolniczych.

Puławy, dn 28.10.2015

.....
(podpis promotora pomocniczego pracy)

8. Publications of the PhD candidate