Rozprawa doktorska

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
Wydział Leśny

Dawid Kupka

Wpływ symulowanego ocieplenia na właściwości chemiczne borówki czarnej oraz wierzchniej warstwy gleby w drzewostanach świerkowych

Effect of simulated warming on chemical properties of European blueberry and topsoil in Norway spruce stands

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Nauka to czary, które naprawdę działają.

Kurt Vonnegut, „Kocia kołyska”

Cała nasza nauka, w porównaniu z rzeczywistością, jest prymitywna i dziecinna – ale nadal jest to najcenniejsza rzecz, jaką posiadamy.

Albert Einstein

Praca ta nie powstałaby, gdyby nie wsparcie wielu Osób.
Pragnę podziękować przede wszystkim:

Promotorowi, Panu prof. dr. hab. inż. Piotrowi Gruba

za wieloletnią i owocną współpracę, rozwijające dyskusje,
poświęcony czas i nieocenioną pomoc w przygotowaniu tej rozprawy,

Pracownikom Katedry Ekologii i Hodowli Lasu wraz z Kierownikiem Katedry,
prof. dr. hab. inż. Stanisławem Małkiem

za profesjonalną pomoc, przyjazną atmosferę
i cenne wskazówki merytoryczne,

Przyjaciołom i Bliskim

za cierpliwość i wyrozumiałość,

Rodzicom i Rodzinie

za zaszczepienie we mnie ciekawości świata
i nieustające wsparcie.

Streszczenie

Globalne zmiany klimatyczne stanowią zagrożenie dla trwałości ekosystemów leśnych. Obserwowany wzrost temperatury atmosfery nie miał sobie równych w ciągu ostatnich dwóch tysięcy lat. Przewiduje się, że postępujące zmiany będą miały niebagatelny wpływ na wszystkie elementy ekosystemów leśnych. W Polsce gatunkiem szczególnie wrażliwym na zmieniające się warunki środowiskowe jest świerk pospolity (*Picea abies* (L.) H.Karst). Obserwowany od kilku dekad proces rozpadu świerczyn w terenach górskich jest przedmiotem licznych badań, jednak niewiele uwagi poświęcono relacji pomiędzy ociepleniem klimatu a właściwościami chemicznymi wierzchniej warstwy gleby oraz runa leśnego w drzewostanach świerkowych. W podjętych badaniach analizowano zmiany w chemizmie roślinności runa leśnego (na przykładzie borówki czarnej *Vaccinium myrtillus* L.), glebowej materii organicznej oraz wierzchniej warstwy gleby mineralnej w warunkach ocieplenia. W tym celu przeprowadzono trwające 656 dni doświadczenie symulujące ocieplenie gleby o 0,5°C na głębokości ok. 5 cm, wykorzystując otwarte klosze OTC (ang. *Open Top Chamber*). Badania, które przeprowadzono w dojrzałym drzewostanie świerkowym w Beskidzie Śląskim, wykazały istotny wpływ ocieplenia na chemizm wierzchniej warstwy gleby oraz borówki czarnej. Ocieplenie spowodowało spadek zawartości całkowitych i labilnych form węgla i azotu w glebie, a także całkowitego fosforu, dowodząc szybszej mineralizacji glebowej materii organicznej. Zaobserwowano znaczny wzrost zawartości azotu amonowego w ocieplanej glebie, co stanowiło praprzyczynę istotnego wzrostu pH ocieplanej gleby. Wzrost pH gleby pod wpływem ocieplenia miał związek ze zmniejszeniem się siły kwasów organicznych oraz wzrostem udziału glinu w kompleksie sorpcyjnym. Nadziemne części borówek poddanych ociepleniu cechowały się zwiększoną zawartością węgla w liściach oraz zmniejszonymi zawartościami magnezu, manganu, miedzi, sodu i żelaza. Ocieplenie spowodowało ponadto zmiany w relacjach stechiometrycznych węgiel:azot:fosfor w glebie oraz roślinach, wskazując na istotny związek między temperaturą a procesami biogeochemicznymi. Uzupełnieniem eksperymentu symulacyjnego było doświadczenie laboratoryjne, w którym wykazano, że zdolność gleby do sorpcji rozpuszczonej materii organicznej, pochodzącej ze ściółki świerkowej, jest zależna od pH i kompozycji jonowej kompleksu sorpcyjnego gleby. Stwierdzony wzrost pH pod wpływem ocieplenia może negatywnie wpłynąć na zdolność gleby do stabilizacji rozpuszczonego węgla organicznego. Uzyskane wyniki potwierdzają istotny wpływ wzrostu temperatury powietrza Ziemi na właściwości chemiczne badanego dna lasu. Zaobserwowane symptomy zmiany warunków siedliskowych wpłyną negatywnie na kondycję zdrowotną drzewostanów świerkowych. Uzyskane w niniejszej dysertacji wyniki mogą stanowić podstawę w planowaniu hodowlanym na terenach górskich, gdzie dominującym gatunkiem jest nadal świerk zwyczajny.

Słowa kluczowe: glebowa materia organiczna, globalne ocieplenie, świerk zwyczajny, borówka czarna, gleby leśne, zmiany klimatu

Summary

Global climate change is threatening the sustainability of forest ecosystems. The observed increase in atmospheric temperature has been unparalleled in the past two thousand years. The ongoing changes are expected to have a significant impact on all elements of forest ecosystems. In Poland, the Norway spruce (*Picea abies* (L.) H.Karst) is a species particularly sensitive to changing environmental conditions. The process of spruce decay in mountain areas has been the subject of numerous studies and observed for several decades, but little attention has been paid to the relationship between climate warming and the chemical properties of the topsoil and ground vegetation in spruce stands. The undertaken study analyzed changes in the chemistry of ground vegetation (using European blueberry *Vaccinium myrtillus* L. as an example), soil organic matter and mineral topsoil under warming conditions. To achieve this, a 656-day experiment simulating soil warming of 0.5°C at a depth of about 5 cm was conducted using open top chambers (OTC). The study, which was conducted in a mature spruce stand in the Silesian Beskids, showed a significant effect of warming on the chemistry of the topsoil and blueberry. Warming caused a decrease in the content of total and labile forms of carbon and nitrogen in the soil, as well as total phosphorus, proving faster mineralization of soil organic matter. A significant increase in the content of ammonium in the warming soil was observed, which was the prime reason for the significant increase in the pH of the warming soil. The increase in soil pH under warming was related to a decrease in the strength of organic acids and an increase in the proportion of aluminum in the sorption complex. The aboveground parts of blueberries subjected to warming were characterized by increased leaf carbon content and decreased magnesium, manganese, copper, sodium and iron contents. In addition, the warming caused changes in the stoichiometric relationships of carbon:nitrogen:phosphorus in the soil and plants, indicating a significant relationship between temperature and biogeochemical processes. Simulated warming was complemented by a laboratory experiment, in which it was shown that the ability of soil to sorb dissolved organic matter from spruce litter is dependent on the pH and ionic composition of the soil sorption complex. The observed increase in pH under warming may negatively affect the soil's ability to stabilize dissolved organic carbon. The results confirm the significant impact of the increase in the Earth's air temperature on the chemical properties of the studied forest floor. The observed symptoms of changing habitat conditions will negatively affect the health of spruce stands. The results obtained in this dissertation can serve as a basis for silvicultural planning in mountainous areas where Norway spruce is still the dominant species.

Keywords: soil organic matter, global warming, Norway spruce, blueberry, forest soils, climate change

Spis treści

1. Struktura pracy

Przygotowana rozprawa doktorska stanowi spójny tematycznie cykl czterech publikacji w czasopismach naukowych:

Publikacja nr 1:

Kupka D., Pan K., Gruba P. 2023. Initial responses of soil chemical properties to simulated warming in Norway spruce (*Picea abies* (L.) H.Karst.) stands in Western Carpathians. *Geoderma*, 432, 116400. DOI: 10.1016/j.geoderma.2023.116400, **200 pkt, IF(6,1).**

Publikacja nr 2:

Kupka D., Pan K., Pietrzykowski M., Gruba P. 2023. Simulated warming decreases acidity of organic horizons of forest soils under Norway spruce (*Picea abies* (L.) H.Karst.) stands in the Western Carpathians. *CATENA*, 235, 107669. DOI: 10.1016/j.catena.2023.107669, **140 pkt, IF(6,2).**

Publikacja nr 3:

Kupka D., Pan K., Pietrzykowski M., Kraj W., Gruba P. 2023. Effect of warming on ground vegetation in Carpathian Norway spruce stands, exemplified by European blueberry (*Vaccinium myrtillus* L.) nutrient stoichiometry. *Science of the Total Environment*, 896, 166396. DOI: 10.1016/j.scitotenv.2023.166396, **200 pkt, IF(9,8).**

Publikacja nr 4:

Kupka D., Gruba P. 2022. Effect of pH on the sorption of dissolved organic carbon derived from six tree species in forest soils. *Ecological Indicators*, 140, 108975. DOI:10.1016/j.ecolind.2022.108975, **140 pkt, IF(6,9)**.

W badaniach wykonywanych podczas przygotowywania niniejszej rozprawy doktorskiej odgrywałem wiodącą rolę. Mój wkład obejmował założenie powierzchni badawczej, pobieranie materiału badawczego i jego obróbkę, a także część analiz laboratoryjnych. Miałem również wiodący wkład w analizę danych, koncepcyjne przygotowanie publikacji i redakcję tekstów. Procentowy udział w przygotowaniu publikacji zawiera dokument „Oświadczenia o udziale autorskim", załączony do niniejszej dysertacji. Publikacje stanowią część projektu badawczego „Zależności pomiędzy glebową siecią troficzną a aktywnością β-glukozydazy i ich reakcja na ocieplenie i suszę: badania porównawcze gleb zróżnicowanych drzewostanów świerkowych w Polsce i Chinach”, finansowanego przez Narodowe Centrum Nauki (kierownik projektu: prof. dr hab. inż. Piotr Gruba).

2. Wprowadzenie

W drugiej dekadzie XXI stulecia średnia globalna temperatura powierzchni Ziemi była o 0,99°C wyższa w porównaniu do epoki preindustrialnej (okres 1850–1990), a jej wzrost w ostatnich pięćdziesięciu latach jest szybszy niż w jakimkolwiek innym pięćdziesiątleciu na przestrzeni ostatnich dwóch tysięcy lat (Gulev i in. 2021). Przewiduje się, że o ile nie nastąpią radykalne ograniczenia w emisji gazów cieplarnianych, to na koniec obecnego stulecia średnia temperatura wzrośnie o 1,5–2°C w stosunku do epoki preindustrialnej (Gulev i in. 2021). Współczesne modele klimatyczne, zakładające stałe ocieplanie się powierzchni Ziemi, wskazują, że prowadzić będzie ono do większej częstotliwości ekstremalnych zjawisk atmosferycznych w ekosystemach lądowych, takich jak susze lub krótkotrwałe, lecz intensywne opady deszczu (Christensen i in. 2007; Ballester i in. 2010; Sheik i in. 2011; Gulev i in. 2021).

Postępujące zmiany klimatu negatywnie wpłyną na stabilność i kondycję ekosystemów leśnych. Za jedne z najbardziej wrażliwych na zmiany klimatyczne uznaje się drzewostany górskie strefy umiarkowanej (Hagedorn i in. 2010). W Polsce (jak i w pozostałych krajach Europy Centralnej) gatunkiem szczególnie wrażliwym na zmiany środowiskowe jest świerk pospolity (*Picea abies* (L.) H.Karst) (Małek i in. 2012; Čermák i in. 2019; Krejza i in. 2020). Gatunek ten należy do głównych gatunków lasotwórczych drzewostanów górskich w Europie (Yousefpour i in. 2010; Hanewinkel i in. 2013; Thiele i in. 2017). W Polsce zajmuje ok. 5% powierzchni leśnej, co czyni go drugim (po sośnie zwyczajnej) głównym gatunkiem iglastym (Biuro Urządzania Lasu i Geodezji Leśnej 2023). Na przestrzeni dwóch ostatnich stuleci udział świerka w górskich lasach Polski znacznie się zwiększył z uwagi na sztuczne wprowadzanie tego gatunku (Małek, Barszcz 2015), zwłaszcza w Sudetach i Karpatach Zachodnich. Wśród przyczyn zainteresowania środkowoeuropejskich leśników świerkiem można wymienić jego wysoki potencjał produkcyjny, cenioną jakość drewna oraz umiarkowane wymagania siedliskowe (Spiecker 2000; Hanewinkel i in. 2013; Thiele i in. 2017).

Zwiększenie udziału świerka w lasach wiązało się z szeregiem nieprawidłowości z punktu widzenia współczesnej hodowli lasu, siedliskoznawstwa i szeroko rozumianej ochrony ekosystemów leśnych, do których zaliczyć należy przede wszystkim wprowadzanie monokultur w miejsce żyznych siedlisk i wielogatunkowych drzewostanów, niedostosowanie składów gatunkowych do lokalnych warunków siedliskowych czy też wykorzystywanie materiału sadzeniowego nieznanej proweniencji (Gorzelak 1995; Małek, Barszcz 2015). Efektem wyżej wymienionych zaniedbań były degradacja gleby oraz drastyczne obniżenie się odporności i stabilności lasów, które – w połączeniu z szeregiem czynników antropogenicznych (emisje przemysłowe, zanieczyszczenie środowiska), biotycznych (wzrost aktywności kambio- i ksylofagów, patogenów grzybowych) i abiotycznych (susze, gwałtowne zjawiska atmosferyczne) – spowodowały rozpad drzewostanów świerkowych i kurczenie się zasięgu występowania świerka. Choć apogeum zamierania świerczyn w Karpatach Zachodnich przypadło na lata 80. i 90. XX w. (Małek, Barszcz 2015), jeszcze dziś obserwowany jest postępujący rozpad istniejących monokultur. Jako że szkodliwe emisje przemysłowe (upatrywane jako główne źródło pogorszenia stabilności drzewostanów) oddziałujące na Karpaty Zachodnie w ostatnich latach znacznie się zmniejszyły (głównie poprzez ograniczenie działalności przemysłowej ośrodków Górnośląskiego Okręgu Przemysłowego od północy oraz Zagłębia Ostrawsko-Karwińskiego od południa), a drzewostany świerkowe nadal ulegały rozpadowi, przyczyn takiego stanu rzeczy zaczęto upatrywać w postępujących zmianach klimatycznych.

Modele klimatyczne opracowane dla Beskidu Śląskiego i Żywieckiego zakładają, że wzrost średniej temperatury powietrza o ok. 1°C może spowodować: (1) podniesienie się pionowych granic pięter klimatyczno-roślinnych o 100–150 m; (2) wzrost częstotliwości występowania okresów suszy, zwłaszcza jesienią; (3) kurczenie się zasięgu występowania świerka i zastępowanie go przez gatunki ciepłolubne, takie jak buk zwyczajny (*Fagus sylvatica* L.) (Durło 2012; Dyderski i in. 2018). Według raportu IPCC, średnia temperatura powietrza w lecie w Karpatach Zachodnich do końca XXI w. wzrośnie od 1,5–2°C (według scenariusza RCP4,5, zakładającego znaczny spadek stężenia gazów cieplarnianych w atmosferze do połowy wieku) do 4–4,5°C (według scenariusza RCP8,5, zakładającego niezmienione tempo wzrostu emisji gazów cieplarnianych) (Alberton i in. 2017). Wciąż niewiele wiadomo o tym, jak globalne ocieplenie wpłynie na glebę i zachodzące w niej procesy (Soong i in. 2020). Co więcej, ze względu na duże zróżnicowanie ekspozycji i wysokości nad poziomem morza w Karpatach brakuje dokładnych informacji na temat prognozowanego ocieplenia gleby. Przewiduje się jednak, że wzrost temperatury gleb lasów w strefie umiarkowanej na głębokości 1 cm wyniesie około 0,3°C, zakładając scenariusz RCP8,5 (Alberton i in. 2017; Soong i in. 2020).

Współczesne badania na temat wpływu zmian klimatu na drzewostany świerkowe w Europie Centralnej skupiają się głównie na reakcjach przyrostowych drzew bądź ich aktywności fizjologicznej w obliczu stresu wywołanego ociepleniem i suszą (np. Durło 2012; Małek i in. 2012; Cienciala i in. 2016; Altman i in. 2017). Względnie niewielka ilość prac poświęcona jest reakcji gleby tych drzewostanów na zmiany klimatyczne, co może dziwić, zważywszy, że gleba i procesy w niej zachodzące mają niebagatelny wpływ na produkcyjność ekosystemów leśnych i zapewnienie ich stabilności (Schoenholtz i in. 2000).

Oprócz gleby istotnym elementem ekosystemów leśnych jest warstwa runa leśnego, która uczestniczy w przepływie energii i obiegu materii (Landuyt i in. 2019). Mimo że nadziemna biomasa tej warstwy stanowi tylko około 1% biomasy leśnej, runo ma istotny wpływ na obieg kluczowych składników odżywczych (Muller 2003; Landuyt i in. 2019). Runo leśne dostarcza materii organicznej do dna lasu, zarówno z opadu organicznego, jak i rozkładu systemów korzeniowych. Jedną z najbardziej rozpowszechnionych roślin w górskich lasach świerkowych jest borówka czarna (*Vaccinium myrtillus* L.). Borówkę czarną nierzadko wykorzystuje się jako bioindykator w badaniach środowiskowych (Mikkonen, Huttunen 1981; Coudun, Gégout 2007; Kasiulienė i in. 2019). Ten cienioznośny gatunek rośnie na glebach kwaśnych, dobrze sobie radząc na płytkich, słabo rozwiniętych glebach górskich (Ritchie 1956). Globalne zmiany klimatyczne mogą wpłynąć na pobieranie składników odżywczych przez rośliny runa leśnego, a także na ich parametry wzrostowe. Dotychczas nie poznano reakcji tej warstwy roślinności leśnej na ocieplenie, jednak problem ten wydaje się być kluczowy, biorąc pod uwagę istotny udział roślinności runa leśnego w cyklach biogeochemicznych.

Zdolność gleb leśnych do sorpcji rozpuszczonej materii organicznej jest istotną cechą w kontekście zmian w ekosystemach (takich jak zmiany klimatu) i wynikających z nich zmian w cyklu węglowym (C). Dotychczasowe badania skupiały się wokół relacji sorpcji rozpuszczonego węgla organicznego z uziarnieniem gleby (np. Asano i in. 2006), kompozycją mineralną (np. Yano i in. 2000) lub zawartością C (np. Lilienfein i in. 2004). Biorąc pod uwagę sorpcję rozpuszczonego węgla organicznego w świetle badań właściwości jonowymiennych gleby, badany był głównie wpływ proporcji pomiędzy kationami w kompleksie sorpcyjnym i kwasowości (np. Mulder i in. 2001; Ross i in. 2008; Mueller i in. 2012; Gruba i Mulder 2015). Wpływ pH i relacji jonowymiennych na sorpcję rozpuszczonej materii organicznej przez glebę pozostaje wciąż stosunkowo słabo poznany (Gmach i in. 2020). Implikuje to potrzebę rozwoju badań skupiających się na powiązaniach między sorpcją a właściwościami chemicznymi gleby (Yeasmin 2020).

Niedawne badania, których autorami są Gruba i Socha (2019), wykorzystując dane z Wielkoobszarowej Inwentaryzacji Stanu Lasów w Polsce, wykazały znaczny wzrost akumulacji C w wierzchniej warstwie gleby o $pH < 4,0$ oraz $pH > 6,0$. Stwierdzono natomiast istotny spadek akumulacji C w glebach o pH od 4 do 6. Jedną z hipotez, którą można wysnuć na podstawie przytoczonych wyników, jest to, że różnice w zawartości C w wierzchnich warstwach gleb o zróżnicowanym pH są spowodowane różnicami w sorpcji rozpuszczonego węgla organicznego. Przypuszczać można ponadto, że zmiany pH gleby pod wpływem czynników zewnętrznych, takich jak zmiany klimatu, mogą wpłynąć istotnie na jej zdolność sorpcyjną i potencjał sekwestracji C.

3. Uzasadnienie wyboru tematu badawczego

Istnieje szereg badań poświęconych odpowiedzi górskich gleb leśnych na zmiany klimatu (np. Spiecker, 2000; Egli i in. 2007; Bockheim, Munroe, 2014; Labaz i in. 2014; Bojko, Kabala, 2017), przy czym skonstruowanie spójnego wzorca zmian właściwości chemicznych gleb nie jest możliwe ze względu na zróżnicowanie ich morfologii i właściwości fizykochemicznych, lokalnych warunków klimatycznych, strefowości pionowej, podłoża geologicznego czy w końcu aktywności mikrobiologicznej i pokrywy roślinnej (Ghosh i in. 2014; Labaz i in. 2014; Józefowska, Miechówka 2015; Bojko, Kabala 2017). Ta niespójność w zakresie reakcji właściwości gleb na ocieplenie wymusza badania skoncentrowane na poszczególnych typach ekosystemów leśnych (Xu i in. 2010; Prietzel i in. 2016; Li i in. 2018).

Biorąc pod uwagę postępujący wzrost stężenia gazów cieplarnianych w atmosferze, a co za tym idzie wzrost średniej temperatury powierzchni Ziemi, istniejące strategie hodowlane dotyczące lasów świerkowych mogą stać się niewspółmierne do zmieniających się warunków siedliskowych (Lindner 2000; Thiele i in. 2017). Poleganie na praktykach wypracowanych przez wiele lat na stabilnych drzewostanach może stać się niebezpieczne w obliczu zmian środowiskowych (Lindner 2000; Spittlehouse, Stewart 2003). Dostosowanie składu gatunkowego drzewostanów do zmieniających się warunków siedliskowych, obok uwzględnienia indywidualnych wymagań gatunków, powinno oprzeć się na znajomości kierunku zmian właściwości fizykochemicznych gleby (Rehschuh i in. 2017) a także na znajomości wpływu ocieplenia na roślinność runa leśnego w drzewostanach świerkowych, która pomimo niewielkiego udziału w całkowitej biomasie drzewostanu stanowi istotny element ekosystemu.

Zawarte w niniejszej pracy badania uzupełniają obecny stan wiedzy o informacje na temat wpływu ocieplenia klimatu na właściwości chemiczne roślinności runa leśnego (na przykładzie borówki czarnej jako jednego z najpospolitszych gatunków runa w drzewostanach świerkowych) oraz wierzchniej warstwy gleby pod tymi drzewostanami.

4. Cel dysertacji i hipotezy badawcze

Głównym celem przeprowadzonych badań było określenie zmian właściwości chemicznych tkanek borówki czarnej oraz wierzchniej warstwy gleby pod wpływem symulowanego ocieplenia w drzewostanach świerkowych. Autorowi pracy przyświecało założenie, że scenariusz zmian można zarysować na postawie analizy chemicznej zmian parametrów wierzchnich warstw gleby pod wpływem ich symulowanego ocieplenia w funkcji czasu.

W ramach badań zweryfikowane zostały następujące hipotezy badawcze:

H_1: ocieplenie gleby wpływa na właściwości chemiczne glebowej materii organicznej w drzewostanach świerkowych;

H_2: ocieplenie gleby modyfikuje relacje jonowymienne w wierzchniej warstwie mineralnej gleb drzewostanów świerkowych;

H_3: ocieplenie gleby wpływa na stechiometrię węgiel:azot:fosfor (C:N:P) oraz zawartość makro- i mikropierwiastków nadziemnej biomasy roślinności runa leśnego w drzewostanach świerkowych na przykładzie borówki czarnej;

H_4: wywołane ociepleniem zmiany w relacjach jonowymiennych wpłyną na zdolność gleby do stabilizacji rozpuszczonej materii organicznej.

Weryfikację postawionych hipotez osiągnięto na podstawie dwóch doświadczeń – terenowego doświadczenia symulacyjnego (weryfikującego hipotezy nr 1, 2, 3) oraz laboratoryjnego (weryfikującego hipotezę nr 4). Rezultaty obu doświadczeń ogłoszono w postaci czterech publikacji naukowych, których cele kształtowały się w następujący sposób:

Publikacja nr 1. Celem pracy było określenie wpływu ocieplenia na zawartość: całkowitego węgla, całkowitego azotu, rozpuszczonego węgla organicznego, rozpuszczonego azotu organicznego, azotu amonowego, azotu azotanowego oraz pH w poziomie organicznym (O) oraz wierzchnim poziomie mineralnym (A) gleb drzewostanów świerkowych.

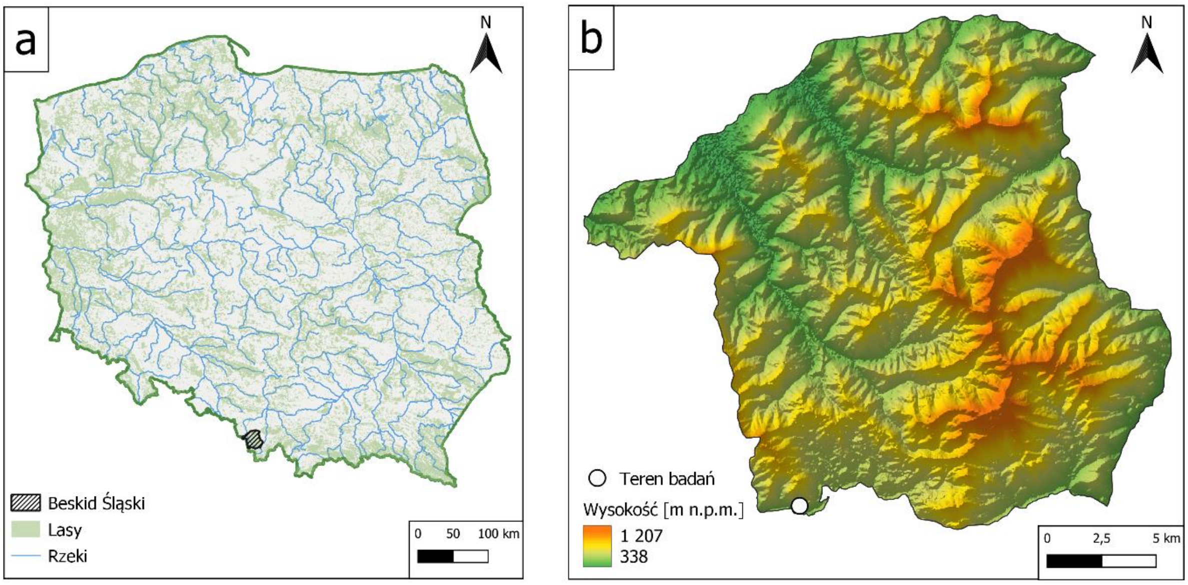
Publikacja nr 2. Celem pracy było określenie zmian relacji jonowymiennych wierzchniej warstwy gleby (poziom organiczny O i wierzchni mineralny A) drzewostanów świerkowych pod wpływem ocieplenia. W pracy podjęto próbę odniesienia zmian w pH gleby do relacji pomiędzy kationami zasadowymi (wapń, potas, magnez, sód) a wodorem oraz glinem, uwzględniając amfoteryczny charakter tego ostatniego. Do wyjaśnienia zaobserwowanych zmian użyto równania Hendersona-Hasselbalcha.

Publikacja nr 3. Celem pracy było określenie wpływu ocieplenia na zawartość węgla, azotu, fosforu, stechiometrię tych pierwiastków (C:N, C:P, N:P) oraz zawartość makro- i mikropierwiastków (wapnia, miedzi, żelaza, potasu, magnezu, manganu, sodu i cynku) w nadziemnych częściach (łodygi i liście) roślinności runa leśnego drzewostanów świerkowych na przykładzie borówki czarnej (*Vaccinium myrtillus* L.) oraz w wierzchnim poziomie mineralnym (A) gleby.

Publikacja nr 4. Celem pracy było określenie zależności pomiędzy pH gleby a zdolnością do sorpcji rozpuszczonej materii organicznej pochodzącej ze ściółek sześciu gatunków drzew leśnych: świerka zwyczajnego (*Picea abies* (L.) H.Karst), sosny zwyczajnej (*Pinus sylvestris* L.), jodły pospolitej (*Abies alba* Mill.), buka zwyczajnego (*Fagus sylvatica* L.), grabu zwyczajnego (*Carpinus betulus* L.) oraz dębu szypułkowego (*Quercus robur* L.).

5. Charakterystyka terenu badań

Teren badań, których rezultaty prezentują publikacje nr 1, 2 i 3, był zlokalizowany na obszarze Beskidu Śląskiego w Nadleśnictwie Wisła, w leśnictwie Bukowiec (49°33'47.7"N, 18°52'06.5"E) (Ryc. 1).



Rycina 1. Teren badań: (a) lokalizacja Beskidu Śląskiego; (b) lokalizacja powierzchni badawczej w Beskidzie Śląskim. Mapy stworzono z wykorzystaniem oprogramowania QGIS 3.26.3 (QGIS Association 2023).

Beskid Śląski jest najbardziej wysuniętą na zachód częścią Beskidów Zachodnich, położonych w Zachodnich Karpatach. Obszar ten należy do górskiego regionu klimatycznego, cechującego się znacznym zróżnicowaniem częstości występowania różnych typów pogody w ciągu roku. Wynika to z faktu, że obszar Beskidu Śląskiego znajduje się w strefie ścierania się wpływów oceanicznych oraz kontynentalnych. Średnia temperatura powietrza w Beskidzie Śląskim wynosi 6,5°C, suma opadów 1089 mm, a liczba dni z opadem 185. Najcieplejszym miesiącem jest lipiec (średnia temperatura 14,9°C), a najzimniejszym styczeń (średnia temperatura -3,7°C). Średnia liczba dni z pokrywą śnieżną w roku wynosi 105 (Januszek i in. 2015).

Pasma górskie Beskidu Śląskiego zbudowane są z utworów geologicznych fliszu karpackiego, na który składają się głównie piaskowce, łupki, zlepieńce oraz utwory trzeciorzędowe (Aleksandrowicz 1991; Gruba, Mulder 2008). Skały macierzyste zawierają domieszki muskowitu, skaleni i glaukonitu. Barwa wietrzejących skał jest zazwyczaj czerwonawa za sprawą wietrzenia minerałów zawierających żelazo (Gruba, Mulder 2008).

Do badań wyznaczono obszar o powierzchni 1 ha w osiemdziesięcioletnim, gospodarczym drzewostanie świerkowym na siedlisku boru mieszanego górskiego świeżego (BMGśw), z zespołem roślinnym *Abieti-Piceetum* (Fot. 1). Cała powierzchnia cechowała się wystawą południowowschodnią o jednorodnym spadku wynoszącym 20°. Drzewostan składał się z jednego, głównego piętra, bez rozwiniętego podszytu. Zadrzewienie zostało określone na 0,4, a zwarcie było umiarkowane, jednorodne na całej powierzchni badawczej (Bank Danych o Lasach, 2023). Runo leśne składało się z borówki czarnej (*Vaccinium myrtillus* L.) z niewielką domieszką mchów: piestrzenicy czerwonej (*Pleurozium schreberi* (Brid.) Mitt.) oraz bielistki siwej (*Leucobryum glaucum* (Hedw.) Ångstr.).

Fotografia 1. Drzewostan stanowiący powierzchnię badawczą. Autor: Dawid Kupka.

Zgodnie z „Klasyfikacją gleb leśnych Polski" (Biały i in. 2000) glebę na powierzchni badawczej zaklasyfikowano jako brunatną kwaśną, natomiast według klasyfikacji WRB jako *Skeletic Dystric Cambisol (Loamic)* (IUSS Working Group WRB 2022). Gleby brunatne kwaśne są dominującym typem gleb w Beskidzie Śląskim, czemu sprzyja obfitość glinokrzemianów w warstwach fliszu karpackiego uraz umiarkowany klimat (Gruba i in. 2011).

Doświadczenie, którego wyniki zostały zaprezentowane w publikacji nr 4, zostało przeprowadzone w warunkach laboratoryjnych. Materiał przeznaczony do jego przeprowadzenia został pobrany z kompleksu leśnego, zlokalizowanego pomiędzy Kielcami a Skarżyskiem-Kamienną (51°02′54.1″N 20°43′45.5″E). Teren ten położony jest na mezozoicznej tarczy masywu Gór Świętokrzyskich. Gleba na obszarze kompleksu leśnego została zaklasyfikowana jako brunatna kwaśna według „Klasyfikacji gleb leśnych Polski" (Biały i in. 2000), natomiast według klasyfikacji WRB jako *Dystric Cambisol* (IUSS Working Group WRB 2022). Gleby brunatne kwaśne na opisywanej powierzchni rozwijały się z materiału wietrzeniowego, wymytego z czwartorzędowych piaskowców i iłowców (Gruba i in. 2015).

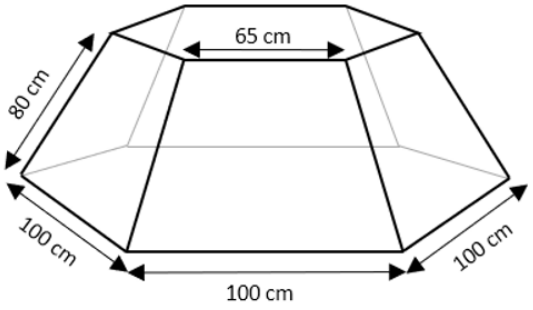
6. Metody badań

6.1. Doświadczenie symulacyjne (publikacje nr 1, 2, 3)

6.1.1. Ogólne założenia eksperymentu

Śledzenie zmian zachodzących w glebie pod wpływem zmian klimatu jest możliwe dzięki zastosowaniu aktywnych lub pasywnych eksperymentów symulacyjnych *in situ* (Sun i in. 2013). Pasywne doświadczenia symulacyjne nie wymagają zewnętrznego źródła zasilania i są przeprowadzane z powodzeniem od ponad trzech dekad. Najczęściej stosowaną metodą symulacji ocieplenia klimatu, którą wykorzystano w niniejszych badaniach, jest metoda otwartych kloszy OTC (ang. *Open Top Chamber*). Jest to metoda pasywnej symulacji ocieplenia, wykorzystująca konstrukcje z Pleksiglasu, mające za zadanie zmniejszać wypromieniowywanie ciepła z otaczanej powierzchni gleby (Aronson, McNulty 2009). Gleba otoczona kloszem jest chroniona przed wiatrem, co dodatkowo eliminuje potencjalne zakłócenia w efektywności ocieplania (de Frenne i in. 2010). Stosunkowo niski koszt, wygodny transport i instalacja oraz brak konieczności stosowania zewnętrznego źródła zasilania to zalety metody OTC, pozwalające na jej wykorzystanie w trudno dostępnych – najczęściej górskich – rejonach (Aronson, McNulty 2009).

W niniejszych badaniach wykorzystano klosze OTC o wymiarach przedstawionych na rycinie 2 do symulacji ocieplenia gleby. W grudniu 2019 r. na powierzchni badawczej założono 15 poletek. Cała powierzchnia badawcza objęła obszar lasu o powierzchni 1 ha. Wszystkie poletka zostały rozmieszone losowo, zachowując minimalną odległość między nimi wynoszącą 15 m. Na dziesięciu poletkach umieszczono klosze OTC, a pięć poletek stanowiło grupę kontrolną. Lokalizacja poletek kontrolnych została ustabilizowana w terenie. Klosze OTC pokryły łącznie 26 m^2 gleby, natomiast łącznie z poletkami kontrolnymi objęto badaniami 39 m^2 gleby.



Rycina 2. Schemat kloszy OTC wykorzystanych w badaniach. Opracowanie własne.

W celu monitoringu skuteczności zastosowanej metody ocieplenia na głębokości ok. 5 cm (odpowiadającej poziomowi przejściowemu między poziomem organicznym a mineralnym) umieszczono czujniki temperatury gleby oraz zawartości objętościowej wody (ang. *water volume content*, WVC) (5TM Sensor, Decagon), jeden pod kloszem OTC oraz jeden poza nim. Przed wykorzystaniem w terenie czujniki zostały umieszczone w glebie w warunkach laboratoryjnych (temperatura powietrza 20°C, wilgotność 60%) na pięć dni, aby upewnić się, że dostarczają identycznych wyników. Dokładność pomiaru temperatury wynosiła ±0,1 °C, a WVC ±2%. Pomiary były rejestrowane od momentu rozpoczęcia eksperymentu w grudniu 2019 r. do końca sierpnia 2021 r. w odstępach 15 min i zapisywane na automatycznym rejestratorze (Decagon Em50 Data Logger). Dane zebrane od początku eksperymentu do grudnia 2020 r. nie mogły być wykorzystane z powodu awarii rejestratora.

Materiał badawczy pobierany był cyklicznie co około 3 miesiące, począwszy od dnia założenia powierzchni, natomiast materiał roślinny zebrano dwukrotnie, pod koniec sezonów wegetacyjnych po pierwszym oraz drugim roku trwania symulacji. Sposób pobierania materiału badawczego został dokładnie opisany w rozdziale 6.1.3.

6.1.2. Czas trwania eksperymentu

Publikacje nr 1, 2 i 3 odnoszą się do tego samego eksperymentu symulacyjnego, przy czym uwzględniają one różne zakresy czasowe oraz ilość serii pobranych próbek. Eksperyment symulujący ocieplenie gleby rozpoczęto z początkiem grudnia 2019 r., a ostatnie dane użyte do publikacji wchodzących w skład niniejszej dysertacji zebrano w drugiej połowie września 2021 r. Podczas trwającego 656 dni eksperymentu pobrano łącznie dziewięć serii pomiarowych. Szczegółowy harmonogram poboru próbek przedstawiono w tabeli 1.

Tabela 1. Harmonogram poboru materiału badawczego w ramach przeprowadzanego eksperymentu symulacyjnego

Seria pomiarowa	Czas trwania symulacji [dni]	Data	Rodzaj pobieranego materiału	Materiał wykorzystany w publikacjach nr
0	0	5 grudnia 2019	mat. glebowy	1, 2
1	84	28 lutego 2020	mat. glebowy	1, 2
2	167	21 maja 2020	mat. glebowy	1, 2
3	258	20 sierpnia 2020	mat. glebowy	1, 2
4	292	22 września 2020	mat. glebowy, roślinny	3
5	363	3 grudnia 2020	mat. glebowy	1, 2

6	476	26 marca 2021	mat. glebowy	1, 2
7	578	6 lipca 2021	mat. glebowy	2
8	656	21 września 2021	mat. glebowy, roślinny	3

6.1.3. Pobór materiału badawczego

6.1.3.1. Gleba

Materiał glebowy był pobierany za pomocą cylindrycznego próbnika glebowego o średnicy 5 cm. Podczas każdej serii pomiarowej, na każdym z piętnastu poletek badawczych pobierano glebę z pięciu losowo wybranych punktów. Pobrana gleba była rozdzielana na poziom organiczny (O) o zróżnicowanej miąższości (wynikającej z lokalnych warunków, wynoszącej zwykle około 5 cm) oraz na mineralną warstwę gleby o miąższości 10 cm, odpowiadającą w przybliżeniu miąższości genetycznego poziomu A. Zastosowanie określenia „warstwa gleby” – a nie „poziom gleby” – wyklucza niepewność związaną z subiektywnym charakterem wyznaczenia granicy poziomu próchnicznego, umożliwiając tym samym porównywanie uzyskanych wyników (Gruba 2012). Dla jasności prezentowanych wyników przyjęto jednak określenia „poziom O” oraz „poziom A”. Na każdym poletku badawczym próbki z pięciu punktów łączono w próbkę zbiorczą, z zachowaniem podziału na poziomy O oraz A. Materiał zbierany był do plastikowych worków i szczelnie zamykany.

W przypadku badań zaprezentowanych w publikacji nr 3 analizowano wyłącznie próbki z poziomu A. Takie podejście podyktowane było faktem, że systemy korzeniowe roślinności runa leśnego nie występują w poziomie O.

6.1.3.2. Materiał roślinny

Materiał roślinny został zebrany podczas dwóch serii pomiarowych, po 292 (wrzesień 2020) oraz 656 dniach (wrzesień 2021) i objął zbiór nadziemnych części borówki czarnej *Vaccinium myrtillus* (L.), tj. łodyg oraz liści. Z każdego poletka badawczego podczas każdej z serii pomiarowych pobrano przynajmniej po pięć krzewinek borówki. Krzewinki zostały ucięte sekatorem na równo z gruntem. Zbioru roślin dokonano pod koniec sezonu wegetacyjnego, gdy były jeszcze zielone, przed zaobserwowaniem jakichkolwiek oznak starzenia się liści (tj. żółknięcie, nekrozy, zrzut aparatu asymilacyjnego). Ze względu na rozbudowany system korzeniowy borówki czarnej i jednoczesną konieczność utrzymania nienaruszonej struktury gleby podczas trwającego doświadczenia symulacyjnego nie pobierano próbek korzeni.

6.1.4. Prace laboratoryjne

6.1.4.1. Gleba

Przed przystąpieniem do suszenia usunięto z próbek większe kamienie oraz korzenie. Próbki gleby suszono w suszarce laboratoryjnej przez 3 dni w temperaturze 60 °C, a następnie przesiewano przez sito o średnicy oczek 2 mm.

Skład granulometryczny określono metodą dyfrakcji laserowej (Analysette 22, Fritsch, Idar-Oberstein, Niemcy). Próg detekcji wynosił 0,1%.

pH gleby określono metodą potencjometryczną w dejonizowanej wodzie (pH_{H2O}) oraz w 1 mol l^{-1} roztworze chlorku potasu (pH_{KCl}) w proporcji wagowo-objętościowej 1:5 (Buurman i in. 1996). Próg detekcji wynosił 0,01.

Zawartość azotu amonowego ($N\text{-}NH_4$) oraz azotanowego ($N\text{-}NO_3$) oznaczono po dwudziestominutowej ekstrakcji 10 g gleby z 50 ml roztworu KCl o stężeniu 2,0 mol l^{-1}, wykorzystując wstrzykowy analizator przepływowy FIAstar 5000 (FOSS, Hillerød, Dania). Próg detekcji wynosił 1 µg l^{-1}.

W celu oznaczenia zawartości rozpuszczonego węgla organicznego (DOC) oraz rozpuszczonego azotu organicznego (DON) próbki gleby (2,5 g dla poziomu O i 5 g dla poziomu A) wymieszano z 40 ml dejonizowanej wody i mieszano przez 1 godzinę. Roztwory były następnie sączone na sączkach laboratoryjnych i przepłukiwane do osiągnięcia 100 ml ekstraktu. Pomiar został przeprowadzony z wykorzystaniem analizatora Shimadzu TOC-VCPN ze sprzężoną jednostką TNM-1 (Shimadzu, Kyoto, Japonia). Progi detekcji dla DOC i DON wynosiły odpowiednio 4 µg l^{-1} i 20 µg l^{-1}.

Kwasowość hydrolityczną (całkowitą) gleby (TA) oznaczono w próbce 10 g gleby po godzinnym wytrząsaniu na mieszadle rotacyjnym z 30 ml roztworu octanu wapnia $(CH_3COO)_2Ca$ o pH = 8,2 i stężeniu 1 mol l^{-1} oraz dalszej ekstrakcji na sączkach laboratoryjnych do uzyskania objętości 100 ml. Z tak uzyskanego ekstraktu pobierano 20 ml i miareczkowano do pH = 8,2 wykorzystując 0,1 mol l^{-1} M roztwór wodorotlenku sodu (NaOH). Miareczkowanie przeprowadzono metodą potencjometryczną z wykorzystaniem automatycznego titratora Mettler Toledo (Mettler Toledo Inc., Columbus, OH, USA). Próg detekcji wynosił 0,01 $cmol_{(+)}$ kg^{-1} suchej masy gleby.

Oznaczenia wymiennych form sodu (Na), magnezu (Mg), wapnia (Ca) i potasu (K) dokonano po uprzedniej ekstrakcji 10 g próbki gleby 1 mol l^{-1} roztworem octanu amonu (CH_3COONH_4)

o pH = 7. Po godzinnym wytrząsaniu z 30 ml roztworu próbkę umieszczono na sączku laboratoryjnym i sączono do uzyskania 100 ml ekstraktu. Analiza została przeprowadzona z wykorzystaniem spektrometru spektrometrii atomowej emisyjnej z indukcyjnie sprzężoną plazmą ICP-OES Thermo iCAP 6500 Duo (Thermo Fisher Scientific, Cambridge, UK). Próg detekcji wynosił 0,01 $\mathrm{cmol_{(+)}\,kg^{-1}}$ suchej masy gleby.

Zawartość wymiennego glinu (Al) oznaczono po ekstrakcji 3 g gleby 30 ml 0,5 $\mathrm{mol\,l^{-1}}$ roztworu chlorku miedzi(II) ($CuCl_2$) po 2 h mieszania na mieszadle rotacyjnym. Analiza została przeprowadzona z wykorzystaniem spektrometru ICP-OES.

Niewielką część każdej z próbek zmielono na młynie kulowym (Fritsch) w celu otrzymania homogenicznego materiału. W próbkach tych oznaczono całkowitą zawartość węgla (C_t) oraz całkowitą zawartość azotu (N_t) za pomocą analizatora LECO CNS TrueMac (LECO, St. Joseph, MI, USA). Ponieważ badana gleba nie zawierała węglanów, wartości C_t uznano za równe całkowitej zawartości węgla organicznego. Kontrola jakości pomiarów była przeprowadzana z wykorzystaniem certyfikowanych materiałów odniesienia *Organic Analytical Standards* (OAS, Element Microanalysis Ltd., Okehampton, Wielka Brytania) nr B1280 (C = 0,83 ±0,05%, N = 0,07 ±0,01%), B2178 (C = 2,46 ±0,22%, N = 0,16 ±0,01%), AR4021 (C = 3,04 ±0,16%). Zarówno próbki jak i materiał odniesienia analizowano w dwóch powtórzeniach przy dopuszczalnej różnicy między pomiarami <10%. Materiał odniesienia badano na początku i na końcu każdej z serii pomiarowych. Limity detekcji wynosiły 0,008% dla C_t oraz 0,266% dla N_t.

Całkowite zawartości wapnia, miedzi (Cu), żelaza (Cu), potasu, magnezu, manganu (Mn), sodu, fosforu (P) i cynku (Zn) oznaczono po mineralizacji 1 g zmielonej próbki gleby w mieszaninie kwasów azotowego(V) (HNO_3) i chlorowego(VII) ($HClO_4$) w proporcji objętościowej 4:1 (Ostrowska i in., 1991). Próbki analizowano w dwóch powtórzeniach z wykorzystaniem spektrometru ICP-OES.

Zawartość kationów zasadowych (BC) obliczono według wzoru (1) (Ostrowska i in. 1991):

$$\mathrm{BC} = \mathrm{Ca} + \mathrm{K} + \mathrm{Mg} + \mathrm{Na}\ [\mathrm{cmol_{(+)}\,kg^{-1}}]. \tag{1}$$

Pojemność wymiany kationów (CEC) obliczono według wzoru (2) (Bednarek i in. 2005):

$$\mathrm{CEC} = \mathrm{BC} + \mathrm{TA}\ [\mathrm{cmol_{(+)}\,kg^{-1}}]. \tag{2}$$

Zawartość wymiennego wodoru (H) obliczono według wzoru (3) (Bednarek i in. 2005):

$$\mathrm{H} = \mathrm{TA} - \mathrm{Al}\ [\mathrm{cmol_{(+)}\ kg^{-1}}]. \qquad (3)$$

Stopień wysycenia kompleksu sorpcyjnego kationami zasadowymi obliczono według wzoru (4) (Bednarek i in. 2005):

$$\mathrm{BS} = \left(\frac{\mathrm{BC}}{\mathrm{CEC}}\right) \cdot 100\ [\%]. \qquad (4)$$

6.1.4.2. Materiał roślinny

Próbki nadziemnych części borówki czarnej po zbiorze zostały przetransportowane do laboratorium i przechowywane w workach foliowych w temperaturze 4°C do czasu przeprowadzenia analiz. Na początku obróbki krzewinki były przepłukiwane wodą dejonizowaną przez około 1 min w celu usunięcia ewentualnej depozycji atmosferycznej. Po opłukaniu rośliny suszono przez 48 h w temperaturze 60 °C, po czym dokonano frakcjonowania na liście i łodygi, które następnie zmielono.

W materiale roślinnym oznaczono zawartość form całkowitych następujących pierwiastków: C, N, P oraz Ca, Cu, Fe, K, Mg, Mn, Na i Zn. Wszystkie analizy wymienionych pierwiastków przeprowadzono w taki sam sposób jak dla materiału glebowego, opisany w rozdziale 6.1.4.1, przy czym użyto naważki 0,5 g.

6.1.5. Metody analizy statystycznej

Normalność rozkładów badanych zmiennych badano testem Shapiro-Wilka. W celu sprawdzenia skuteczności zastosowanej metody symulacji ocieplenia dokonano porównań wartości temperatury i WVC pomiędzy grupami (ocieplenie *vs*. kontrola), stosując test kolejności par Wilcoxona bądź test t. Zarówno dla całego okresu symulacji, jak i poszczególnych serii pomiarowych przeprowadzono test U Manna-Whitneya (w publikacjach nr 1 i 2) bądź test t (w publikacji nr 3) w celu oceny różnic pomiędzy grupami badawczymi. W publikacji nr 1 do badania efektu ocieplenia, czasu i ich łącznego wpływu na właściwości gleby i roślin zastosowano dwuczynnikową analizę wariancji (ANOVA), po uprzednim sprawdzeniu zasadności jej zastosowania (określenie poziomu homoskedastyczności reszt i jednorodności wariancji zbadanej testem Levene'a). W przypadku niespełnienia wspomnianych warunków zmienne poddawano transformacji logarytmicznej. W publikacji nr 3 w podobnym celu wykorzystano analizę kowariancji (ANCOVA), gdzie czas został potraktowany jako zmienna współwystępująca (kowariant). Korelacje pomiędzy zmiennymi były określane współczynnikami korelacji Pearsona. W celu określenia zależności pomiędzy dwiema zmiennymi w publikacji nr 2 wykorzystano regresję liniową. Do analizy czynnikowej wykorzystano analizę głównych składowych (PCA). Istotność statystyczną określono na poziomie $p < 0,05$, chyba że wskazano inaczej.

Analizy statystyczne wykonano za pomocą oprogramowania Statistica 13 (Dell Inc., 2016) oraz R (R Core Team 2023), wykorzystując biblioteki „ggplot2” (Wickham 2016), „factoextra” (Kassambara, Mundt 2020), „dplyr” (Wickham i in. 2022), „stringr” (Wickham 2022) i „ggpubr” (Kassambra 2022).

6.2. Doświadczenie laboratoryjne (publikacja nr 4)

6.2.1. Ogólne założenia eksperymentu

Celem eksperymentu przedstawionego w publikacji nr 4 było zbadanie zdolności gleby leśnej o zróżnicowanym pH i składzie kompleksu sorpcyjnego do sorpcji DOC, pochodzącego ze ściółek różnych gatunków drzew leśnych, w tym świerka pospolitego. Poznanie mechanizmów regulujących sorpcję DOC uwolnionego z poziomów organicznych gleb leśnych jest kluczowym elementem efektywnego prognozowania zasobów C w glebach leśnych w obliczu zmian środowiskowych, wywołanych np. zmianą klimatu.

Eksperyment składał się z dwóch niezależnych doświadczeń laboratoryjnych, badających sorpcję DOC pochodzącego ze ściółek sześciu głównych gatunków lasotwórczych w lasach strefy umiarkowanej przez glebę leśną. Istotą pierwszego doświadczenia (zwanego w dalszej części doświadczeniem I) było zbadanie pojemności sorpcji DOC w funkcji pH w zakresie kwaśnym gleby pochodzącej z poziomu skały macierzystej (poziom C). W drugim doświadczeniu (zwanym dalej doświadczeniem II) badano sorpcję DOC przez glebę leśną o pH w zakresie od kwaśnego do zasadowego, pochodzącą z poziomu mineralnego A. W obu doświadczeniach wykonano przemywanie gleby wodnymi ekstraktami ściółek o znanej początkowej zawartości DOC i określono stopień sorpcji DOC przez glebę. Podsumowanie obu doświadczeń przedstawiono w tabeli 2.

Tabela 2. Główne założenia metodyczne eksperymentu w publikacji nr 4

Charakterystyka	Doświadczenie	
	I	II
Poziom diagnostyczny	C (80–100 cm)	A (0–10 cm)
Gatunki drzew, z których pochodziły ściółki	świerk pospolity (*Picea abies* (L.) H.Karst), sosna zwyczajna (*Pinus sylvestris* L.),	

	jodła pospolita (*Abies alba* Mill.), buk zwyczajny (*Fagus sylvatica* L.), grab zwyczajny (*Carpinus betulus* L.), dąb szypułkowy (*Quercus robur* L.)	
Początkowa zawartość C w glebie [g kg^{-1}]	~0	~30
Liczba wariantów pH gleby	3	5
Zakres pH gleby	4,38–5,52	3,52–7,89
Liczba kolejnych płukań jednej próbki gleby	3	1
Analiza właściwości chemicznych przemytych próbek gleby	Tak	Tak
Analiza właściwości chemicznych ściółek	Tak	Tak

6.2.2. Pobór materiału badawczego

6.2.2.1. Gleba

Gleba użyta do doświadczenia I została pobrana z poziomu skały macierzystej (C, 80–100 cm) po uprzednim odsłonięciu poziomów diagnostycznych położonych powyżej. Gleba wykorzystana w doświadczeniu II została pobrana z poziomu mineralnego (A, 0–10 cm) po odsłonięciu nadkładu organicznego. Próbki pobierane były w maju za pomocą próbnika glebowego o średnicy 5 cm.

6.2.2.2. Ściółka

Ściółka do obu doświadczeń została zebrana z poziomów O gleb w sześciu dojrzałych, jednogatunkowych drzewostanach: świerka pospolitego, sosny zwyczajnej, jodły zwyczajnej, buka zwyczajnego, grabu pospolitego i dębu szypułkowego. Wszystkie drzewostany były w podobnym wieku ok. 70 lat. Zbiór materiału miał miejsce w maju, kiedy opad organiczny z poprzedniej jesieni uległ wstępnej dekompozycji. Próbki zbierano do szczelnych worków plastikowych, które po przetransportowaniu do laboratorium niezwłocznie umieszczono w chłodni w temperaturze 4 °C.

6.2.3. Prace laboratoryjne

6.2.3.1. Doświadczenie I

Gleba była suszona w temperaturze pokojowej przez pięć dni, po czym została przesiana przez sito o średnicy oczek 2 mm. Tak przygotowany materiał podzielony został na trzy części, po 300 g każda. W celu modyfikacji pH i zwiększenia udziału wodoru w CEC, jedna część została zakwaszona przez przepłukanie na sączku 400 ml 0,5 mol l^{-1} roztworem kwasu solnego (HCl). Druga część nie została poddana modyfikacjom. Trzecią część gleby przepłukano na sączku 400 ml 0,5 mol l^{-1} roztworem wodorotlenku wapnia ($Ca(OH)_2$) w celu zwiększenia pH oraz udziału wymiennego Ca w CEC. W każdym wariancie oznaczono pH, zawartość kationów wymiennych (Ca, K, Mg, Na) oraz całkowitą zawartość węgla (C_t) i azotu (N_t).

W celu uzyskania ekstraktów ściółkowych świeże próbki ściółek (o objętości 1 l każda) umieszczono w zlewkach, do których dodano 3 l wody dejonizowanej i mieszano przez 5 min szklaną bagietką. Po 24 h zawiesiny przefiltrowano przez sito o średnicy oczek 2 mm (z wykorzystaniem siły grawitacji), a następnie przez sączek membranowy z włókna szklanego 0,45 µm i filtrowano z wykorzystaniem pompy próżniowej. W uzyskanych ekstraktach oznaczono zawartość DOC, po czym rozcieńczono tak, aby uzyskać jednakową zawartość DOC (150 mg l^{-1}).

Próbki ściółki były suszone w suszarce laboratoryjnej przez 3 dni w temperaturze 60°C, po czym zostały zmielone w młynie kulowym.

Po 50 g z przygotowanych wcześniej trzech wariantów gleby o zróżnicowanym pH zmieszano ze 100 ml jednego z sześciu ekstraktów ściółkowych i wytrząsano przez 1 h. Otrzymane zawiesiny wytrząsano ręcznie przez 1 min, po czym wirowano w wirówce laboratoryjnej przez 30 min (3000 g). Po odwirowaniu zbierano supernatant, a do osadu pozostałego po dekantacji dodawano kolejne 100 ml roztworu ściółkowego. Tak przygotowane próbki wytrząsano przez 1 h oraz wirowano przez kolejną 1 h. Po drugim zebraniu supernatantów całą procedurę powtórzono trzeci raz. Operacja została przeprowadzona dla każdego wariantu gleby i każdego rodzaju ściółki. Po każdej z trzech rund mieszania i wirowania zawiesin supernatanty zbierano i filtrowano przez sączek membranowy z włókna szklanego za pomocą pompy próżniowej w celu oznaczenia zawartości DOC.

Metody oznaczania wymienionych parametrów chemicznych były takie same, jak te opisane w rozdziale 6.1.4.1.

6.2.3.2. Doświadczenie II

Gleba była suszona w temperaturze pokojowej przez pięć dni, po czym została przesiana przez sito o średnicy oczek 2 mm. Tak przygotowany materiał podzielono na pięć części, po 300 g każda. Dwie części zostały zakwaszone, używając roztworu 1 mol l^{-1} HCl (jedna część jako silnie zakwaszona wykorzystując 400 ml HCl, natomiast druga część jako słabo zakwaszona z wykorzystaniem 200 ml HCl). Kolejne dwie części zostały zalkalizowane (jedna jako lekko zalkalizowana z wykorzystaniem 200 ml 1 mol l^{-1} roztworu wodorotlenku wapnia $Ca(OH)_2$ oraz druga jako silnie zalkalizowana z wykorzystaniem 400 ml roztworu $Ca(OH)_2$. Próbki gleby umieszczano na bibule filtracyjnej i przemywano odpowiednio roztworem kwasu/zasady. Ciecz ekstrahowano przy użyciu siły ciężkości. Następnie zmierzono pH, zawartość wymiennych kationów zasadowych, wymiennego glinu i wodoru, kwasowość hydrolityczną (całkowitą) oraz obliczono CEC dla każdego wariantu gleby.

Ekstrakty ściółkowe przygotowano tak samo, jak w przypadku doświadczenia I (por. rozdział 6.2.3.1).

W ściółkach oznaczono: pH, zawartość kationów zasadowych, wymiennego glinu, materii organicznej, całkowitą zawartość C i N, kwasowość hydrolityczną (TA).

Metody oznaczania wymienionych parametrów chemicznych były takie same, jak te opisane w rozdziale 6.1.4.1. Zawartość materii organicznej w ściółkach oznaczono metodą strat prażenia, po wyprażaniu próbek w piecu muflowym w temperaturze 400°C przez 4 h.

6.2.4. Metody analizy matematycznej

Aby określić wyjściową zawartość DOC w glebie, każdy wariant gleby mieszano z wodą destylowaną (DOC_{gleba}) i dokonano oznaczenia. Następnie obliczono DOC sorbowany przez glebę, korzystając ze wzoru (5):

$$\mathrm{DOC_{sorbowany}} = \mathrm{DOC_{ściółka}} - \left(\mathrm{DOC_{mix}} - \mathrm{DOC_{gleba}}\right), \tag{5}$$

gdzie: $DOC_{ściółka}$ to zawartość DOC w wodnych roztworach ściółek, DOC_{mix} to zawartość DOC w roztworze po przepłukaniu gleby ekstraktem ze ściółki, DOC_{gleba} to wyjściowa zawartość DOC w glebie. Jako że próbki gleby w eksperymencie II zawierały śladowe ilości DOC, założono, że $DOC_{gleba} = 0$. Stąd obliczenie $DOC_{sorbowany}$ przyjęło postać wzoru (6):

$$\mathrm{DOC_{sorbowany} = DOC_{ściółka} - DOC_{mix}}. \tag{6}$$

Normalność rozkładów badanych parametrów w poszczególnych grupach została zbadana testem Shapiro-Wilka. W doświadczeniu II przeprowadzono analizę wariancji (ANOVA) po uprzednim sprawdzeniu zasadności jej zastosowania, a także test HSD Tukeya dla DOC sorbowanego przez poszczególne rodzaje gleb i dla poszczególnych gatunków ściółki. Porównania parametryczne właściwości chemicznych dwóch grup ściółek (iglaste *vs.* liściaste) wykonano za pomocą testu t, natomiast do porównań nieparametrycznych między tymi grupami zastosowano test Kołmogorowa-Smirnowa (K-S). W celu określenia zależności między sorpcją DOC a pH gleby wykonano analizę regresji nieliniowej dla wszystkich obserwacji ($n = 70$) z doświadczenia II. Zastosowano metodę regresji najmniejszych kwadratów z estymacją Gaussa-Newtona, a najlepszym dopasowaniem okazała się funkcja kwadratowa dana wzorem (7):

$$y = b_1(x - b_2)^2 + b_3, \tag{7}$$

gdzie: y to zasorbowany DOC, x to pH gleby, natomiast b_1, b_2, b_3 to parametry regresji. Wszystkie analizy statystyczne zostały wykonane w oprogramowaniu R (R Core Team, 2023), wykorzystując biblioteki „ggplot2” (Wickham, 2016) i „factoextra” (Kassambra i Mundt, 2020).

7. Wyniki

7.1. Wpływ symulowanego ocieplenia na zawartość całkowitych oraz labilnych form C i N oraz pH w glebach drzewostanów świerkowych w Zachodnich Karpatach

Wpływ symulowanego ocieplenia na całkowite oraz labilne (DOC, DON) formy C i N, a także na pH gleby w drzewostanach świerkowych Zachodnich Karpat przedstawiono w pracy *Initial responses of soil chemical properties to simulated warming in Norway spruce (Picea abies (L.) H.Karst.) stands in Western Carpathians* (Kupka D., Pan K., Gruba P. 2023). Dane zebrane w okresie grudzień 2020 – marzec 2021 (476 dni) dowodzą, że średnia temperatura gleby na głębokości ok. 5 cm (w warstwie przejściowej między poziomami O i A) była 0,4°C wyższa w glebie poddanej ociepleniu w porównaniu do grupy kontrolnej. Również zawartość objętościowa wody (WVC) w ocieplanej glebie była istotnie niższa w porównaniu do grupy kontrolnej (3,2% różnicy). Wielkość różnic w przypadku obu badanych właściwości gleby kształtowała się różnie na przestrzeni miesięcy; także w ciągu dnia obserwowano zróżnicowanie amplitud. Analizując dane z całego eksperymentu (nie uwzględniając poszczególnych serii pomiarowych), można stwierdzić, że ocieplenie wyraźnie wpłynęło na właściwości chemiczne gleby, bardziej w poziomie O niż w A. Stwierdzono statystycznie istotny wzrost wartości pH gleby (pH_{H2O} i pH_{KCl}) w obu poziomach ocieplanej gleby. W poziomie O ocieplanej gleby zawartość NO_3-N była statystycznie mniejsza, natomiast zawartość NH_4-N wzrosła o 54% w porównaniu ze średnią z gleb kontrolnych. Zawartości DOC i DON uległy istotnemu zwiększeniu w ocieplanym poziomie O, natomiast w ocieplanym poziomie A zawartość DOC uległa niewielkiemu zmniejszeniu. Dwuczynnikowa analiza wariancji przeprowadzona dla wszystkich serii pomiarowych wykazała statystyczną istotność dla wszystkich czynników modelu (czas, ocieplanie, czas × ocieplanie) w poziomie O. Dla poziomu A stwierdzono statystyczną istotność wpływu czasu i ocieplenia, której nie zaobserwowano dla połączonego efektu czasu i ocieplenia. Wyniki jednowymiarowe analizy wariancji wykazały statystycznie istotny wpływ czasu i ocieplenia na zawartość NH_4-N i NO_3-N w poziomie O. Wykazany został także istotny wpływ ocieplenia na wartość pH w obu badanych poziomach oraz na DOC i DON w poziomie A. Analizując przebieg zmian parametrów chemicznych pod wpływem ocieplenia w funkcji czasu stwierdzono wzrostowy trend w przypadku zawartości DOC i DON w poziomie O ocieplanych gleb. Zawartości te różniły się statystycznie tylko w jednej serii pomiarowej (marzec, 476 dni trwania eksperymentu). Identyczna zależność wystąpiła w przypadku zawartości NH_4-N, która przez cały okres symulowanego ocieplenia cechowała się trendem wzrostowym w ocieplanej glebie, ale w tylko jednej serii pomiarowej (marzec, 476 dni trwania eksperymentu), była statystycznie wyższa w porównaniu z grupą kontrolną. Istotną zmianą był wzrost pH_{H2O} ocieplonego poziomu O w czterech seriach pomiarowych (pH_{KCl} w dwóch seriach) oraz wzrost pH_{H2O} ocieplonego poziomu A w trzech seriach pomiarowych (pH_{KCl} w jednej serii).

7.2.Wpływ symulowanego ocieplenia na relacje jonowymienne wierzchnich warstw gleby drzewostanów świerkowych w Karpatach Zachodnich

W pracy *Simulated warming decreases acidity of organic horizons of forest soils under Norway spruce (Picea abies (L.) H.Karst.) stands in the Western Carpathians* (Kupka D., Pan K., Gruba P. 2023) przeanalizowano mechanizmy odpowiedzialne za wzrost pH wierzchniej warstwy gleby drzewostanów świerkowych pod wpływem ocieplenia. Punktem wyjścia dla niniejszej pracy było wykazanie w publikacji 1, że gleba poddana ociepleniu cechuje się wyższym pH w stosunku do grupy kontrolnej. Analizą objęto okres 578 dni ocieplenia, podczas którego średnia temperatura ocieplanej gleby była 0,5°C wyższa, a objętościowa zawartość wody była o 1,9% niższa w stosunku do grupy kontrolnej. W momencie zakładania doświadczenia średnie pH gleb ogrzewanych i kontrolnych było podobne (ocieplanie *vs.* kontrola: poziom O 3,55 *vs.* 3,53; poziom A 3,66 *vs.* 3,62). Po około trzech miesiącach zaobserwowano znaczny wzrost wartości pH ocieplanej gleby w obu poziomach. W poziomie O wzrost pH był istotny w czterech kolejnych seriach pomiarowych: w lutym 2020 r. (ΔpH = 0,14), maju 2020 r. (ΔpH = 0,13), sierpniu 2020 r. (ΔpH = 0,21) i grudniu 2020 r. (ΔpH = 0,14). W dwóch kolejnych seriach pomiarowych w 2021 r. średnie pH ocieplanej gleby oraz grupy kontrolnej w tym poziomie były podobne. W poziomie A w warunkach ocieplenia w lutym 2020 r. stwierdzono wyższe pH niż w grupie kontrolnej (ΔpH = 0,29). W kolejnych seriach pomiarowych pH ocieplanego poziomu A było znacznie wyższe niż w grupie kontrolnej: maj 2020 r. (ΔpH = 0,23), sierpień 2020 r. (ΔpH = 0,19, brak istotności statystycznej), grudzień 2020 r. (ΔpH = 0,19), marzec 2021 r. (ΔpH = 0,17, brak istotności statystycznej) i lipiec 2021 r. (ΔpH = 0,21). Co zaskakujące, podczas ocieplania nie stwierdzono istotnych różnic w zawartości kationów zasadowych pomiędzy grupami badawczymi; niemniej zawartość ta wzrastała we wszystkich poletkach wraz z upływem czasu. Również zawartość wymiennego Al była zwykle wyższa w ocieplanej glebie, szczególnie w poziomie O, jednak nie stwierdzono istotnych różnic pomiędzy grupami badawczymi w żadnej z serii pomiarowych. Wyższą zawartość Al stwierdzono w próbkach gleby o pH > 3,8, które pochodziły z poletek poddanych ociepleniu. Zawartość wymiennego H w poziomie O nie różniła się istotnie pomiędzy grupami podczas trwania ocieplenia. Jednakże, znaczy spadek zawartości H w ocieplanej glebie zaobserwowano w poziomie A wkrótce po rozpoczęciu eksperymentu, który to utrzymywał się przez prawie cały czas trwania doświadczenia.

Wpływ ocieplenia gleby na skład jonowy CEC oraz pH był bardziej wyraźny, gdy wyrażono udział wymiennych kationów zasadowych (BC), H i Al w CEC jako funkcję pH. W poziomie O stosunek BC CEC^{-1} znacząco wzrósł wraz ze wzrostem pH ($r = 0,71$ dla ocieplenia, $r = 0,86$ dla kontroli, $p < 0,001$). Udział wymiennego H w CEC zmniejszał się znacząco ($r = -0,47$ dla ocieplenia, $r = -0,58$, $p < 0,001$ dla kontroli) wraz ze wzrostem pH. Frakcja wymiennego Al w CEC nie była istotnie skorelowana z pH w poziomie O. Nachylenia tych istotnych zależności były stosunkowo niewielkie (BC CEC^{-1}: ocieplenie 2,17, kontrola 3,01, $p < 0,001$; H CEC^{-1}: ocieplenie -1,89, kontrola -1,52, $p < 0,001$). W poziomie A zawartość BC była niewielka, a związek między frakcją BC CEC^{-1} a pH nie był istotny. Natomiast wzrost pH w poziomie A był silnie dodatnio skorelowany ze wzrostem frakcji Al w CEC ($r = 0,81$ dla ocieplenia, $r = 0,51$ dla kontroli, $p < 0,001$) i jednoczesnym spadkiem frakcji H w CEC ($r = -0,81$ dla ocieplenia, $r = 0,47$ dla kontroli, $p < 0,001$). W przypadku poziomu O dane z obu grup badawczych cechowały się podobnymi zakresami, podczas gdy w poziomie A wszystkie próbki o pH > 3,8 zostały pobrane z ocieplanej gleby.

W pracy zastosowano modelowanie pH za pomocą równania Hendersona-Hasselbalcha (8) (H-H), które pierwotnie opisywało dysocjację słabych kwasów (McBride 1994):

$$\mathrm{pH} = \mathrm{pK_{app}} + n\mathrm{Log}\frac{(A^{-})}{(\mathrm{HA})} \tag{8}$$

gdzie: $\mathrm{pK_{app}}$ oznacza ujemny logarytm stałej dysocjacyjnej, *n* jest empiryczną stałą reakcji stechiometrycznej, która oznacza ilość moli H reagujących z jednym molem naładowanych grup funkcyjnych glebowej materii organicznej, A^{-} to ilość zdysocjowanych grup funkcyjnych, a HA oznacza ilość grup niezdysocjowanych. Na potrzeby gleboznawstwa równanie (8) zostało zmodyfikowane (Bloom, Grigal 1985), przyjmując następującą postać (9):

$$\mathrm{pH} = \mathrm{pK_{app}} + n\mathrm{Log}\left(\frac{\mathrm{BS_E}}{1 - \mathrm{BS_E}}\right) \tag{9}$$

gdzie: $\mathrm{BS_E}$ to stopień wysycenia kompleksu sorpcyjnego kationami zasadowymi, a $1\text{-}\mathrm{BS_E}$ stanowi frakcję kwasowości wymiennej, obejmującej jony H i Al.

Równanie (9) wykorzystano dla wyjaśnienia zmian pH w poziomie O, natomiast dla poziomu A zastosowano zmodyfikowaną wersję równania (8), uwzględniając Al jako pierwiastek o charakterze zasadowym w kwaśnych (pH < 4,5) glebach leśnych (Gruba, Mulder 2015) (10):

$$\mathrm{pH} = \mathrm{pK_{app}} + n\mathrm{Log}\left(\frac{\mathrm{Al} + \mathrm{BC}}{\mathrm{H}}\right) \tag{10}$$

W obu poziomach uzyskano istotne, negatywne liniowe zależności. Ocieplana gleba cechowała się konsekwentnie wyższymi wartościami $\mathrm{pK_{app}}$ (przedstawionymi jako punkty przecięcia funkcji liniowej): ogrzewanie *vs.* kontrola: poziom O 3,85 *vs.* 3,77; poziom A 3,68 *vs.* 3,61. Wartości *n* (odzwierciedlającego nachylenie funkcji) były natomiast do siebie zbliżone: ogrzewanie *vs.* kontrola: poziom O 0,364 *vs.* 0,478; poziom A 0,528 *vs.* 0,522.

7.3. Wpływ symulowanego ocieplenia na zawartość wybranych pierwiastków w nadziemnych częściach roślinności runa leśnego drzewostanów świerkowych w Karpatach Zachodnich na przykładzie borówki czarnej

W publikacji *Effect of warming on ground vegetation in Carpathian Norway spruce stands, exemplified by European blueberry (Vaccinium myrtillus L.) nutrient stoichiometry* (Kupka D., Pan K., Pietrzykowski M., Kraj W., Gruba P. 2023) przedstawiona została relacja pomiędzy symulowanym ociepleniem a chemizmem części nadziemnych (liście, łodygi) borówki czarnej oraz mineralną glebą (poziom A) ze szczególnym uwzględnieniem stechiometrii C:N:P. Oprócz wspomnianych pierwiastków analizą objęto także zawartości Ca, Cu, Fe, K, Mg, Mn, Na i Zn. Materiał przedstawiony w publikacji pobrano pod koniec dwóch następujących po sobie sezonów wegetacyjnych, po 292 i 656 dniach trwania eksperymentu klimatycznego. Zawartość C w liściach ocieplanych borówek po pierwszym sezonie była istotnie wyższa (o 4%) w porównaniu z roślinami z powierzchni kontrolnych, podczas gdy zawartość P w ocieplanych liściach było istotnie niższa (o 14%). Stechiometria C:N:P w liściach nie różniła się istotnie pomiędzy grupami, jednak zaobserwowano trend do wyższych wartości współczynników C:N, C:P i N:P w ocieplanych borówkach. Analiza kowariancji wykazała istotny wpływ ocieplenia na zawartość C w liściach ($F = 12{,}79$, $p < 0{,}01$). Łodygi ocieplanych borówek charakteryzowały się istotnie wyższym stosunkiem C:P w stosunku do kontroli, odpowiednio o 14% i 11% po pierwszym i drugim sezonie. Nie stwierdzono innych istotnych różnic między badanymi grupami pod względem zawartości C, N, P oraz wartości współczynników C:N i N:P. Ocieplony poziom A zawierał istotnie niższe stężenia C i N po obu sezonach (C: o 15% i 27% mniej w stosunku do kontroli, odpowiednio po pierwszym i drugim sezonie), a także P (o 15%) po drugim sezonie. Istotnie niższe wartości stosunku C:P zaobserwowano w ocieplanej glebie po drugim sezonie (o 13%), a istotne niższe wartości stosunku N:P po obu sezonach (o odpowiednio 15% i 11%) względem kontroli. Analiza kowariancji wykazała znaczący wpływ ocieplenia na zawartość N w glebie ($F = 60{,}83$, $p < 0{,}0001$), a także C ($F = 25{,}12$, $p < 0{,}0001$), stosunek C:N ($F = 114{,}77$, $p < 0{,}0001$), stosunek N:P ($F = 84{,}22$, $p < 0{,}0001$) i stosunek C:P ($F = 16{,}21$, $p < 0{,}001$).

Analiza zawartości metali w liściach borówki wykazała istotnie mniejszą zawartość Cu (o 11% po dwóch sezonach), Mg (o 17% po dwóch sezonach), Mn (o 45% po pierwszym sezonie) i Na (o 15% po pierwszym sezonie) w porównaniu do grupy kontrolnej. Podobny wzorzec zaobserwowano w łodygach ocieplanych borówek, które cechowały się istotnie niższą zawartością Fe (o 17% po pierwszym sezonie), Mn (o 37% po pierwszym sezonie) i Na (o 24%

po drugim sezonie). Analiza głównych składowych dla roślin (wyjaśniająca 59,7% zmienności) wykazała, że ocieplenie pozytywnie wpłynęło na zawartość C i N oraz stosunki C:P i N:P, ale negatywnie wpłynęło na zawartość Na i P oraz stosunek C:N. Liście były głównie skorelowane z zawartością Ca, Fe, K, Mg, Mn i Ni, podczas gdy łodygi były skorelowane z zawartością Cu, Na i Zn. Analiza głównych składowych dla gleby (wyjaśniająca 75,5% zmienności) wykazała, że ocieplenie pozytywnie wpłynęło na stosunek C:N, ale było negatywnie skorelowane z zawartością C i N oraz stosunkami C:P i N:P.

7.4. Zależność pomiędzy pH gleby a jej zdolnością do sorpcji rozpuszczonej materii organicznej

Publikacja *Effect of pH on the sorption of dissolved organic carbon derived from six tree species in forest soils* (Kupka D., Gruba P. 2022) porusza istotny temat zdolności gleby do sorpcji rozpuszczonej materii organicznej na tle zmian pH. Stanowi zatem swoiste rozwinięcie tematu w ujęciu eksperymentu laboratoryjnego, uwzględniając ściółkę nie tylko świerka zwyczajnego, ale i pięciu innych gatunków lasotwórczych, choć nie dotyczy bezpośrednio tematyki ocieplenia klimatu i jego wpływu na glebę.

Doświadczenie I. Wykorzystana w doświadczeniu gleba składała się w 76% z piasku, 21% z pyłu oraz 3% z iłu. Wyjściowe pH wynosiło 5,02, natomiast pH części zakwaszonej 4,38, a zalkalizowanej 5,52. Wszystkie trzy warianty gleby charakteryzowały się znikomą całkowitą zawartością C i N. Analiza zawartości DOC również wykazała śladowe ilości; założono zatem, że wartości te są równe zeru. Trzykrotne przelewanie różnych wariantów gleby ekstraktami ściółek badanych gatunków wskazało na ogólną tendencję, że sorpcja DOC spada wraz ze wzrostem pH gleby. We wszystkich przypadkach sorpcja DOC była największa podczas pierwszego przelewania, co było najbardziej widoczne podczas przelewania zakwaszonego wariantu gleby (przy którym sorpcja była największa). Wraz z kolejnymi przelewaniami zdolność gleby do sorpcji DOC malała. W przypadku przelewania ekstraktami ze ściółki dębowej, jodłowej i świerkowej zalkalizowany wariant gleby sorbował prawie taką samą ilość DOC.

Doświadczenie II. Gleba użyta do doświadczenia cechowała się podobnym uziarnieniem do gleby wykorzystanej w doświadczeniu I: 72% piasek, 23% pył, 5% ił. Zgodnie z założeniami warianty pH gleby (silnie zakwaszony o pH = 3,52, lekko zakwaszony o pH = 4,01, naturalny o pH = 4,65, lekko zalkalizowany o pH = 6,41 i silnie zalkalizowany o pH = 7,89) różniły się właściwościami chemicznymi. Zawartości wymiennych Ca, K i Mg wzrastały wraz z pH, a stężenie wymiennego Ca znacząco wzrosło przy pH > 6. Podobną zależność zaobserwowano w przypadku zawartości wymiennego Na, z wyjątkiem wariantu naturalnego. Wymienny Al osiągnął największą zawartość w wariancie silnie zakwaszonym i lekko zalkalizowanym. Wymienny H oraz kwasowość hydrolityczna zmniejszały się wraz ze wzrostem pH gleby. Najwyższą pojemność wymiany kationów stwierdzono w wariancie silnie zalkalizowanym. Ilość DOC uwalnianego podczas kontaktu gleby z wodą dejonizowaną różniła się pomiędzy wariantami pH gleby: naturalna (37 mg L^{-1}) < lekko zakwaszona (46 mg L^{-1}) < silnie zakwaszona (52 mg L^{-1}) < lekko zalkalizowana (62 mg L^{-1}) < silnie zalkalizowana (114 mg L^{-1}). Silnie zakwaszony wariant gleby cechował się największą zdolnością do sorpcji DOC uwolnionego przez ściółki, która była znacznie wyższa niż sorpcja przez wariant lekko zakwaszony, naturalny i lekko zalkalizowany. Sorpcja DOC przez wariant silnie zalkalizowany była na powrót wyższa, w porównaniu do wariantów lekko zakwaszonego, naturalnego i lekko zalkalizowanego. Można przyjąć ogólne założenie, że krzywa sorpcji DOC przez glebę w funkcji pH przyjmuje kształt paraboli z ramionami skierowanymi ku górze. Według modelu regresji nieliniowej, opracowanej na podstawie uzyskanych danych (11):

$$\mathrm{DOC_{sorbowany}} = 2{,}741 \cdot (\mathrm{pH} - 5.993)^2 + 17{,}916 \qquad (11)$$

sorpcja DOC zmniejsza się wraz ze wzrostem pH gleby do wartości 5,99, po czym zaczyna rosnąć.

W publikacji wykazano także, że ilość DOC sorbowanego przez glebę może być związana z właściwościami chemicznymi ściółki. Analiza głównych składowych, wyjaśniająca 85,8% zmienności wskazała trzy grupy ściółek: liściaste (buk, grab i dąb), iglaste (sosna, świerk), natomiast osobną grupę stanowiła ściółka jodłowa. Stwierdzono silną zależność między DOC sorbowanym przez glebę a zawartością wymiennego Al i Na w ściółkach. Ponadto zaobserwowano ujemną korelację między zasorbowanym przez glebę DOC, a zawartością C w ściółce.

8. Dyskusja

Modyfikacja właściwości gleb pod wpływem ocieplenia klimatu stanowi jeden z najważniejszych aspektów wpływu globalnych zmian na ekosystemy lądowe. Wyniki przedstawione w publikacjach wchodzących w skład niniejszej dysertacji sugerują, że zarówno gleba, jak i roślinność runa leśnego drzewostanów świerkowych są podatne na prognozowane zmiany klimatyczne. Szereg przedstawionych zmian w chemizmie badanych komponentów ekosystemu leśnego może wpłynąć na warunki siedliskowe i dostępność kluczowych substancji odżywczych dla drzew.

Dzięki zastosowaniu symulacji ocieplenia metodą otwartych kloszy OTC uzyskano w okresie monitoringu (grudzień 2020 – sierpień 2021) wzrost temperatury gleby o 0,5°C na głębokości ok. 5 cm. Uzyskany wzrost jest zbliżony do przewidywanego wzrostu temperatury wierzchniej warstwy gleb leśnych strefy umiarkowanej pod koniec obecnego stulecia, wynoszącego 0,3°C (przy założeniu scenariusza klimatycznego RCP8,5, prognozującego utrzymanie aktualnego tempa emisji gazów cieplarnianych) (Soong i in. 2020). Należy jednak zauważyć, że dokładna wartość wzrostu temperatury gleby jest trudna do przewidzenia, zwłaszcza na obszarach górskich, gdzie gleby charakteryzują się różnymi głębokościami, wysokościami nad poziomem morza i ekspozycją stoków. Wpływ naturalnego ocieplenia na glebę może różnić się od symulowanego. Klosze OTC mogą symulować w inny sposób zmiany wilgotności gleby i wpływać na ilość opadu ściółki niż ma to miejsce w naturze. Wynika to z konstrukcji klosza, którego górna część ma mniejszą powierzchnię niż podstawa. Nachylenie ścian klosza wynika z potrzeby ograniczenia wymiany ciepła między ogrzaną glebą a otoczeniem. Zmianie ulegają również parametry fali świetlnej docierającej do gleby po przejściu przez ścianę komory (Marion i in. 1997).

Przedstawione w dysertacji wyniki dowodzą istotnego spadku zawartości całkowitych C, N i P pod wpływem ocieplenia gleby (publikacja nr 3). Istotnie niższe wartości C, N i P w ocieplanej glebie były także przedmiotem licznych publikacji (Melillo i in. 2011; Hopkins i in. 2012; Feng i in. 2015; Marek i in. 2020) i wynikają głównie ze wzrostu intensywności respiracji gleby, silnie powiązanej z aktywnością mikroorganizmów glebowych. Przyspieszona mineralizacja glebowej materii organicznej pod wpływem ocieplenia została także potwierdzona istotnie większymi zawartościami labilnych form C i N. Wzrost zawartości labilnych form C i N pod wpływem ocieplenia wskazuje na gorszą stabilizację glebowej materii organicznej (Fröberg i in. 2006; Xu i in. 2010; Dawes i in. 2017). Większa zawartość labilnej materii organicznej, powstałej pod wpływem ocieplenia, może stanowić obfite źródło dostępnych dla roślin składników odżywczych, które mają kluczowe znaczenie dla ich produkcyjności (Altman i in. 2017). Zwiększona mineralizacja i odpływ P, który jest pierwiastkiem limitującym produktywność roślin, może stanowić poważny problem dla przyszłej stabilności cykli biogeochemicznych i odżywiania roślin w badanym drzewostanie (zarówno dla drzew, jak i runa leśnego). Biorąc pod uwagę to, że P w glebie ma kluczowe znaczenie dla metabolizmu roślin i syntezy cukrów (Wrage i in. 2010), zmniejszona zawartość tego pierwiastka w glebie pod wpływem ocieplenia jest zjawiskiem niepokojącym.

Przyspieszone tempo mineralizacji glebowej materii organicznej pod wpływem ocieplenia zostało także potwierdzone zmniejszeniem się wartości współczynnika stechiometrycznego C:P w ocieplanej glebie. Według Zechmeister-Boltenstern i in. (2015) znaczne zmniejszenie stosunku C:P w ogrzanej glebie wskazuje na wyższe tempo rozkładu glebowej materii organicznej, co jest również zgodne z zaobserwowanym zmniejszeniem zawartości glebowych C, N i P. Na szczególną uwagę zasługuje znaczny spadek współczynnika N:P w glebie pod wpływem ocieplenia. Wskaźnik ten został zaproponowany jako sygnał niedoboru N lub P (Liu i in. 2019). Spadek wartości tego współczynnika wynika głównie ze znacznego spadku zawartości N w ocieplanej glebie. Prawdopodobnym wyjaśnieniem tego zjawiska może być przyspieszenie mineralizacji glebowej materii organicznej pod wpływem ocieplenia. Proces mineralizacji uwalnia N w postaci azotu amonowego (NH_4-N), co zwiększa ilość dostępnego N w glebie. Ponieważ wartość współczynnika N:P powyżej 16 wskazuje na niedobór P, a poniżej 11 – na niedobór N (Koerselman, Meuleman 1996; Garrish i in. 2010), można stwierdzić, że w glebach lasów świerkowych strefy umiarkowanej (pierwotnie borykającymi się z dostępnością N) występuje jeszcze większe ograniczenie dostępności tego pierwiastka z powodu ocieplenia.

Ocieplenie gleby spowodowało wyraźny wzrost zawartości N-NH_4 w poziomie O, co było najbardziej widoczne na początku okresu wegetacyjnego (publikacja nr 1). W naturalnych warunkach zawartość N-NH_4 w glebie wiosną gwałtowanie spada z powodu wzrostu roślinności i pobierania przez nie N (Jaeger i in. 1999; Schmidt i in. 2007; Heinzle i in. 2021), co było widoczne w grupie kontrolnej. Zaobserwowany w ocieplanej glebie wzrost zawartości N-NH_4 wynika najprawdopodobniej ze zwiększonej aktywności bakterii amonifikacyjnych stymulowanych ociepleniem. Biorąc pod uwagę, że N jest uważany za jeden z głównych limitujących składników odżywczych w ekosystemach lądowych (Rustad i in. 2001), aktywność bakterii amonifikacyjnych ma istotne znaczenie w ekosystemowym cyklu N (Dannenmann i in. 2009; Wang i in. 2017). W rezultacie przyswajalny N w postaci NH_4-N jest dostarczany roślinom i może pozytywnie stymulować ich wzrost, zwłaszcza na kwaśnych glebach leśnych, gdzie NH_4-N jest głównym źródłem N dla roślin (Nihlgård 1985; Chen i in. 2020). Założenia te są zgodne z wcześniejszymi wnioskami dotyczącymi pozytywnego wpływu ocieplenia na produkcyjność siedlisk drzewostanów świerkowych w Europie (Thiele i in. 2017).

Amonifikacja jest procesem, podczas którego pobierane są jony H^+. Podczas następującego po niej procesu nitryfikacji uwalniany wodór kompensuje powstałą nierównowagę wodorową. W przeprowadzonym eksperymencie klimatycznym ocieplona gleba cechowała się znacznym wzrostem N-NH_4, ale nie zaobserwowano wzrostu zawartości azotu azotanowego (N-NO_3). Spadek zawartości jonów H^+ wskutek intensywniejszej amonifikacji w ocieplonej glebie mógł być praprzyczyną wzrostu pH, prowadzącą do zmian w kompleksie sorpcyjnym. Dokładniejszych informacji na temat wpływu ocieplenia na wzrost pH gleby uzyskano przez zbadanie zależności między pH a frakcjami H, Al i kationów zasadowych w kompleksie sorpcyjnym oraz z zastosowania modelowania pH równaniem Hendersona-Hasselbalcha. Wykorzystanie frakcji kompleksu sorpcyjnego jako danych wejściowych do modelowania pH za pomocą wspomnianego równania jest powszechną koncepcją (Bloom, Grigal 1985; Ciarkowska, Miechówka 2019; Ma, Xiao 2023). Przedstawione w niniejszej pracy wyniki sugerują, że w poziomie O wzrost pH był związany z równowagą między BC i H, podczas gdy udział Al był nieistotny. Dla tego poziomu zastosowano zatem równanie Hendersona-Hasselbalcha w jego klasycznej postaci, w której tylko BC uwzględniono jako kationy zasadowe. Podobne podejście dla poziomu O zasugerował Johnson (2002). Natomiast dla poziomu A zastosowanie znalazło zmodyfikowane równanie, w którym Al został uznany za kation zasadowy. Większa wartość parametru pK_{app} ocieplanego poziomu O w stosunku do kontroli pozwala stwierdzić, że ocieplana glebowa materia organiczna zawierała słabsze kwasy organiczne, mniej zdolne do dysocjacji. Innymi słowy, glebowa materia organiczna pod wpływem ocieplenia może cechować się wyższym pH w stosunku do materii organicznej z powierzchni kontrolnych o podobnym nasyceniu wodorem. Zjawisko to może wynikać z intensywniejszej humifikacji pod wpływem ocieplenia oraz przekształceniem kwasów fulwowych słabsze kwasy humusowe (Machado i in. 2020). Z kolei w poziomie A poddanym ociepleniu zwiększone nasycenie Al może wynikać ze zwiększonego transportu tego kationu z mineralnej fazy gleby, spowodowanego intensywniejszym wietrzeniem skały macierzystej i ruchem wody w górę. Podobne zależności zostały opisane przez Lawrence i in. (1995). Nie można również wykluczyć, że na aktywność Al wpływa aktywność mikroorganizmów glebowych, zwiększona przez ocieplenie. Opisane różnice między poziomami O i A w mechanizmach regulacji pH pod wpływem ocieplenia mogą także wynikać z różnic fizykochemicznych i biologicznych wspomnianych poziomów (poziom O składa się głównie z materii organicznej, podczas gdy poziom A składa się głównie z frakcji mineralnej).

Na uwagę zasługuje również zaobserwowany trend wzrostowy zawartości kationów zasadowych w glebie, zarówno ocieplanej, jak i należącej do grupy kontrolnej. Zjawisko to może być skorelowane z postępującymi zmianami klimatycznymi: wzrost temperatury atmosferycznej i spadek ilości opadów mogą kontrolować dynamikę zawartości kationów zasadowych w glebie (Xue i in. 2019). Zgodnie z wcześniejszymi badaniami (Erickson i in. 2014) zawartość kationów zasadowych w glebie wzrasta przy jednoczesnym spadku jej wilgotności. Zależność ta koresponduje z danymi klimatycznymi dla powierzchni badawczej z niniejszej dysertacji, gdzie z roku na rok obserwowano spadek ilości opadu atmosferycznego. W lutym 2020 r. miesięczna suma opadów wynosiła 164,9 mm, podczas gdy w lutym 2021 r. wynosiła 78,9 mm (Instytut Meteorologii i Gospodarki Wodnej 2023). Można zatem założyć, że zmieniający się klimat wpływa na wzrost zawartości kationów zasadowych poprzez przyspieszenie rozkładu glebowej materii organicznej, co pośrednio prowadzi do spadku siły kwasów organicznych i wzrostu pH.

Najważniejszymi z punktu widzenia niniejszej dysertacji wynikami przedstawionymi w publikacji nr 4 są dane, które rzucają światło na relacje między pH gleby a jej zdolnością do stabilizacji rozpuszczonej materii organicznej, pochodzącej ze ściółki świerkowej. Dwa niezależne od siebie eksperymenty laboratoryjne potwierdziły znaczący wpływ pH gleby i relacji jonowymiennych na zdolność gleby do sorpcji rozpuszczonej materii organicznej, pochodzącej ze ściółek głównych gatunków lasotwórczych w strefie umiarkowanej. Doświadczenie I dostarczyło informacji o sorpcji rozpuszczonej materii organicznej przez glebę o zróżnicowanym pH w zakresie kwaśnym (4,4–5,2). Gleba o najniższym pH sorbowała największą ilość rozpuszczonej materii organicznej, pochodzącej ze ściółek. W analizowanym przedziale wartości pH sorpcja zmniejszała się wraz ze wzrostem pH gleby, niezależnie od gatunku, z którego pochodziła ściółka. Ma to związek z malejącą protonacją grup funkcyjnych (Krettek, Rennert 2021). Ponadto wielkość sorpcji zmniejszała się z kolejnymi porcjami glebowej materii organicznej, dostarczanymi do gleby. Pojemność sorpcyjna zmniejszała się z powodu nasycenia powierzchni sorpcyjnych fazy mineralnej (Avneri-Katz i in. 2017; Krettek, Rennert 2021). Uzyskane w wyniku eksperymentu II dane sugerują, że wzrost zdolności sorpcji rozpuszczonej materii organicznej był związany głównie z pH gleby i nasyceniem pojemności wymiany kationów kompleksu sorpcyjnego przez jony H^+ i Ca^{2+}. Przy niskim pH głównym mechanizmem odpowiedzialnym za zwiększoną sorpcję rozpuszczonej materii organicznej była protonacja (wysycenie grup funkcyjnych wodorem). Protonacja grup funkcyjnych była wcześniej sugerowana jako prawdopodobny mechanizm kontrolujący stabilizację rozpuszczonej materii organicznej (Berggren i in. 1998). Sorpcja rozpuszczonej materii organicznej była zwiększona także w silnie alkalizowanym wariancie gleby (pH = 7,9). Miało to związek ze zwiększonym stężeniem jonów Ca^{2+} w kompleksie sorpcyjnym. Przy pH > 5 udział Ca^{2+} w kompleksie sorpcyjnym gleby znacznie wzrasta (Reuss i in. 1990; Prenzel, Schulte-Bisping 1995). Wyniki te są zgodne z wynikami Singh i in. (2016), którzy stwierdzili, że sorpcja rozpuszczonej materii organicznej była dodatnio skorelowana z zawartością Ca^{2+}. Mikutta i in. (2007) również wykazali, że Ca^{2+} miał znaczący wpływ na wiązanie rozpuszczonej materii organicznej z fazą mineralną gleby przy wyższym pH. Kationy poliwalentne, takie jak Ca^{2+}, są w stanie łączyć ze sobą ujemnie naładowane grupy funkcyjne rozpuszczonej materii organicznej, zmniejszając w ten sposób jej rozpuszczalność poprzez wiązania mostkowe lub flokulację (Kalbitz i in. 2000). Ponadto w obecności Ca^{2+} rozpuszczona materia organiczna może być stabilizowana poprzez siły van der Waalsa (Mikutta i in. 2007). Podobne zależności pomiędzy pH gleby a zawartością węgla zaobserwowano dla dużej bazy danych uzyskanej z gleb Polski o naturalnie szerokim zakresie pH. Gruba i Socha (2019) wykazali zwiększoną akumulację C w warstwie 0–10 cm mineralnej warstwy gleby przy pH poniżej 4,0 i powyżej ok. pH 6,0.

Symulowane ocieplenie miało także widoczny wpływ na chemizm nadziemnych części borówki czarnej. Borówki poddane ociepleniu cechowały się zwiększoną zawartością C w liściach w porównaniu do kontroli. Analiza głównych składowych wykazała pozytywną korelację ocieplenia z zawartością C i N w tkankach roślinnych. Wyniki te uzupełniają poprzednie odkrycia, wskazując, że w warunkach ocieplenia rośliny intensyfikują fotosyntezę, głównie poprzez większą wydajność karboksylacji (Contran i in. 2013; Hao i in. 2019). Alternatywnym wyjaśnieniem podwyższonego stężenia C w tkankach roślinnych pod wpływem ocieplenia może być zwiększony pobór tego pierwiastka (Newton i in. 1995; Reich i in. 2006; Sardans, Peñuelas 2012). Melillo i in. (2011) sugerowali, że ocieplenie gleb lasów strefy umiarkowanej może powodować wzrost zawartości C w tkankach roślinnych (wynikający ze zwiększonej dostępności N), przy jednoczesnym spadku zawartości C w glebie, co jest zgodne z wynikami uzyskanymi w doświadczeniu stanowiącym podstawę niniejszej dysertacji. Doświadczenie wykazało także znaczący wpływ ocieplenia na stechiometrię C:P w łodygach ocieplanych roślin. Wcześniejsze badania nad stechiometrią C:N:P na tle ocieplenia klimatu sugerują, że wpływ ocieplenia jest różny w zależności od organu rośliny (Yang i in. 2011; Sardans i in. 2017; Du i in. 2019). Wzrost stosunku C:P w ogrzanych łodygach *V. myrtillus*, zgodnie z licznymi badaniami podsumowanymi przez Zechmeister-Boltenstern i in. (2015), może być związany ze zwiększonym wykorzystaniem wody i składników odżywczych. Ocieplenie spowodowało istotny spadek zawartości Cu, Mg, Mn i Na w liściach oraz Fe, Mn i Na w łodygach borówek. Zaobserwowane zjawisko może wynikać z tzw. „efektu rozcieńczenia” (ang. *dilution effect*). Wzrost pobierania C przez rośliny pod wpływem ocieplenia może prowadzić do spadku stężenia składników odżywczych w tkance roślinnej, ponieważ pobieranie to jest wolniejsze niż tempo wzrostu (Landuyt i in. 2019). W konsekwencji obniżone stężenie składników mineralnych może być związane z rozcieńczeniem wynikającym ze zwiększonej produkcji biomasy (Mcgrath, Lobell 2013; Sardans i in. 2017). Biorąc pod uwagę fakt, że wiele metali jest niezbędnych do prawidłowego funkcjonowania roślin, dostarczając substancji potrzebnych do procesów metabolicznych, ich zmniejszona zawartość spowodowana ociepleniem może zakłócić przyszły wzrost (Elbasiouny i in. 2022).

Zaobserwowane zmiany są początkowymi symptomami zmieniających się warunków siedliskowych drzewostanów świerka pospolitego w górskich obszarach klimatu umiarkowanego. Należy jednak zauważyć, że do zwiększonej śmiertelności i rozpadu drzewostanów świerkowych mogą przyczyniać się także inne czynniki poza ociepleniem klimatu. Czynniki takie jak intensywne susze, ekstremalne wiatry, zwiększona depozycja N w atmosferze, gradacje szkodników i emisje przemysłowe mają znaczący wpływ na pogorszenie żywotności drzewostanów (Jandl 2020; Socha i in. 2023).

9. Wnioski

1. W badaniach wykazano wpływ ocieplenia klimatu na chemizm roślinności runa leśnego (na przykładzie borówki czarnej) oraz wierzchniej warstwy gleby drzewostanów świerkowych. Uzyskane wyniki mogą przyczynić się do stworzenia scenariusza zmian w chemizmie gleb lasów świerkowych na tle zmian klimatycznych. Praca może stanowić materiał pomocniczy w planowaniu hodowlanym na terenach górskich, na których głównym gatunkiem lasotwórczym jest na chwilę obecną świerk pospolity.
2. Uzyskany w doświadczeniu symulacyjnym średni wzrost temperatury gleby o 0,5°C na głębokości ok. 5 cm koresponduje z prognozowanymi warunkami według scenariusza klimatycznego RCP8,5, zakładającego niezmienione do końca 21. stulecia tempo wzrostu emisji gazów cieplarnianych.
3. Wykazano, że podniesienie się temperatury wierzchniej warstwy gleb drzewostanów świerkowych wpływa negatywnie na stabilność glebowej materii organicznej, prowadząc do szybszej jej mineralizacji.

4. Przeprowadzone doświadczenie wykazało, że wzrost temperatury gleby prowadzi do spadku zawartości węgla, azotu i fosforu w wierzchniej warstwie gleby mineralnej. Niedobory w zawartości tych kluczowych dla procesów fizjologicznych roślin pierwiastków mogą stanowić poważne zagrożenie dla produkcyjności siedlisk leśnych i utrzymania drzewostanów w dobrej kondycji zdrowotnej.
5. Wzrost zawartości azotu amonowego w glebowej materii organicznej pod wpływem ocieplenia może wskazywać na zwiększoną aktywność mikroorganizmów glebowych. Amonifikacja jako proces pobierający jony wodorowe z kompleksu sorpcyjnego jest pierwotną przyczyną wzrostu pH wierzchnich warstw gleby. Wzrost ten skutkuje zmniejszeniem siły kwasów organicznych w poziomie O, cechujących się mniejszą zdolnością do dysocjacji. Osłabienie siły kwasów organicznych pod wpływem ocieplenia ma związek z intensywniejszą mineralizacją glebowej materii organicznej. Wzrost pH gleby w poziomie A ma związek z amfoterycznym charakterem glinu, który w obecnych warunkach zachowuje się jak kation zasadowy. Wzrost wysycenia kompleksu sorpcyjnego kationami glinu w ocieplonej glebie wynika najprawdopodobniej z migracji tego pierwiastka z głębszych warstw gleby.
6. Ocieplenie wpływa na chemizm nadziemnych części borówki czarnej, będącej jednym z najpospolitszych gatunków runa leśnego w lasach świerkowych Karpat Zachodnich. Stwierdzony wzrost zawartości węgla w tkankach roślinnych przy jednoczesnym spadku zawartości mikro- i makroelementów wskazuje na zaburzenia procesów fizjologicznych pod wpływem ocieplenia. Zaburzenia w stechiometrii C:N:P ocieplonych roślin sugerują zwiększone pobieranie wody i składników odżywczych.
7. Wzrost pH wierzchniej warstwy gleby pod wpływem ocieplenia może przyczynić się do spadku zdolności gleby do stabilizacji rozpuszczonej materii organicznej pochodzącej ze ściółki.

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11. Wykaz załączników

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Initial responses of soil chemical properties to simulated warming in Norway spruce (*Picea abies* (L.) H.Karst.) stands in the Western Carpathians

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ABSTRACT

The present-day decline observed in Norway spruce stands in the Western Carpathians is particularly worrying given the large forest area of this species. Relatively little attention has been paid to the contribution of soil chemistry to the Norway spruce decline in the context of global warming. We conducted a field experiment to simulate warming for almost 500 days in the Western Carpathians, in a mature Norway spruce stand. Soil samples were collected approximately every 3 months from the organic and mineral topsoil horizons. Total carbon, total nitrogen, pH, dissolved organic carbon (DOC), total dissolved nitrogen (TDN), nitrate (NO_3-N), and ammonium (NH_4-N) nitrogen concentrations were estimated. Warming caused increase in DOC, TDN, NH_4-N, and pH in organic horizon, as well as increased pH in mineral topsoil. A trend for increasing total carbon and total nitrogen concentrations in the warmed organic horizon was observed. Increased concentrations of DOC and TDN may be associated with decrease in SOM stability or increase in production of biomass of the understory vegetation or soil microbial activity. Increased pH in warmed soil may be associated with the increased NH_4-N and may contribute to the decrease in soil ability to adsorb DOC. We conclude that soil warming negatively stimulates soil organic matter stability, resulting in higher concentrations of easy migrating labile forms, mostly organic acid anions. Observed changes may contribute to the deterioration of Norway spruce stands.

1. Introduction

Global climate change is a threat to the stability of forest ecosystems. The global surface temperature was 0.99 °C higher during the decades 2001–2020 than in the period 1850–1990 (Gulev et al., 2021). Moreover, the increase in surface temperature since 1970 is faster than in any other 50-year period over the last 2000 years (Gulev et al., 2021). Surface warming will continue in the future and cause many consequences, such as a concomitant increase in the frequency and duration of drought events in ecosystems (Christensen et al., 2007; Sheik et al., 2011). The average global temperature is predicted to rise by 2.1–3.5 °C at the end of 21st century. A large number of records indicate that global warming will lead to frequent extreme weather events, such as rainfall and drought (Ballester et al., 2010; Gulev et al., 2021).

Global climate change is negatively affecting the stability of forest ecosystems, owing to changes in the local climate and, therefore, site conditions. The most seriously affected are mountainous temperate-zone forest ecosystems (Hagedorn et al., 2010b). In recent years, the disruption of the stability of mountain forests has been noted in Poland and has mainly concerned Norway spruce (*Picea abies* (L.) H.Karst.) stands in the Carpathians (Čermák et al., 2019; Krejza et al., 2020; Małek et al., 2012). The intensification of Norway spruce decline observed in Central Europe is particularly worrying given the high proportion of this species in montane forest areas. Spruce forest is among the main coniferous forest types in Europe (Hanewinkel et al., 2013; Thiele et al., 2017; Yousefpour et al., 2010). The extensive forest area of Norway spruce in Central Europe is connected with its high growth potential, valued wood quality, and moderate site requirements (Hanewinkel et al., 2013; Spiecker, 2000; Thiele et al., 2017). Based on the predictions of numeric models, an increase in average annual air temperature of 1.1–1.2 °C by 2050 may lead to (1) changes in the vertical range of the climatic floors by 100–150 m upwards, (2) an increase in the frequency of drought periods, especially in autumn, and (3) contraction in the distribution of Norway spruce (Durło, 2012; Dyderski et al., 2018). The projected climatic change in Central Europe may also cause deterioration in the growth conditions for Norway spruce in

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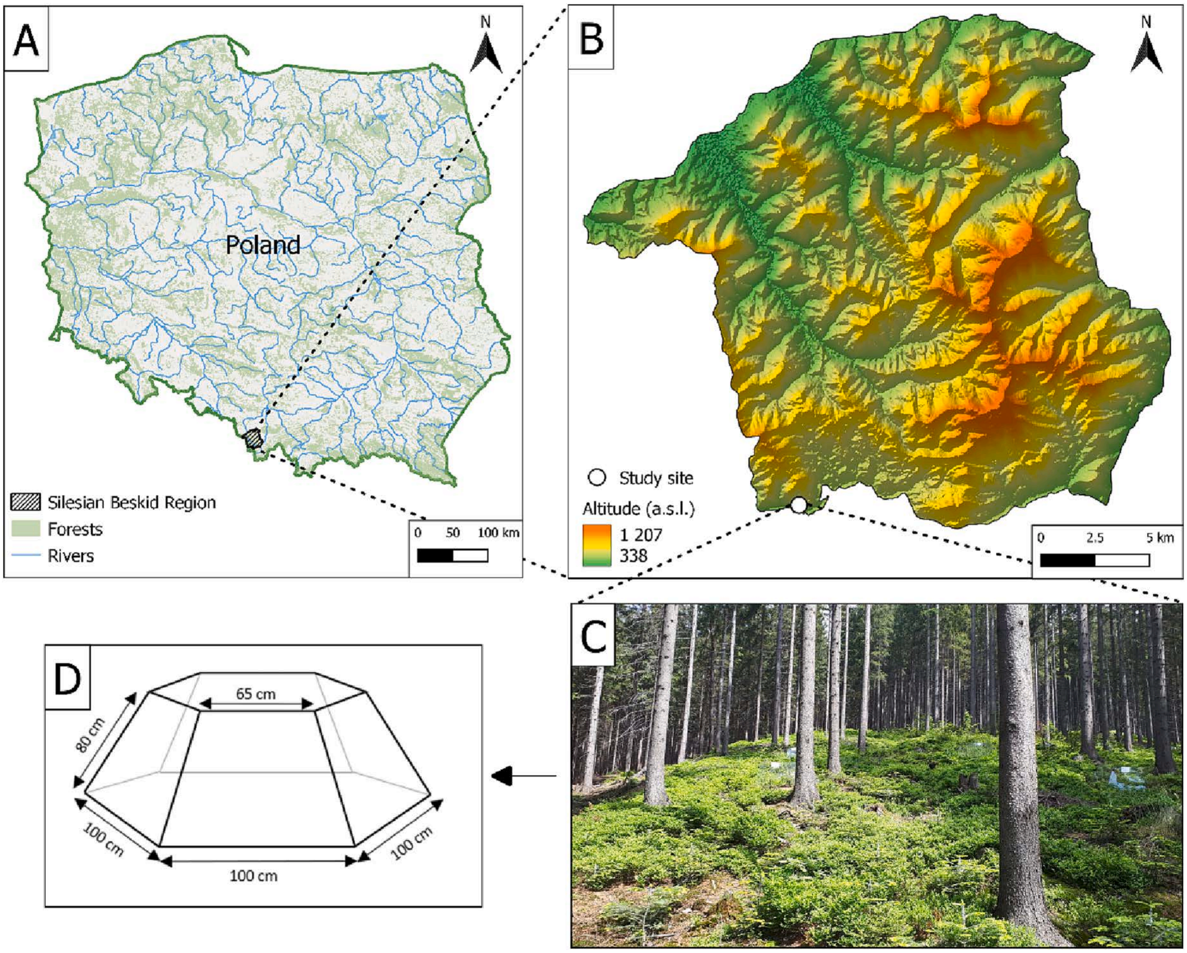

Fig. 1. Study site. a) Map of Poland indicating the Silesian Beskid Region; b) study site in the Silesian Beskid Region; c) study site with installed open-top chambers (OTCs); d) schematic diagram of an OTC used in the warming experiment. Maps were generated using QGIS version 3.22.7 software (QGIS Association, 2022).

mountainous regions, which will have a dramatic effect on the productivity of stands of this species (Dyderski et al., 2018; Hanewinkel et al., 2013; Socha, 2015).

Global warming also leads to disruptions of soil homeostasis caused by increasing soil temperature (Davidson and Janssens, 2006; Koven et al., 2015). Particular attention has focused on forest soils in the context of global change, because the soil is a massive C reservoir (Crowther et al., 2016; Koven et al., 2017; Lal, 2004). The response of the C cycle in terrestrial ecosystems to climate change is characterized by considerable uncertainty (Arora et al., 2013; Koven et al., 2017). Soil characteristics and their response to climate change are dependent on climatic and geographic factors, as well as on soil particle size distribution and parent material (Bockheim and Munroe, 2014; Bojko and Kabala, 2017; Egli et al., 2007; Johnson et al., 2011; Labaz et al., 2014; Spiecker, 2000). Other chemical properties of forest soils may also be influenced by climate change - although numerous studies have demonstrated the effect of warming on soil nitrogen (N) and its fractions (ammonium and nitrate), dissolved organic matter (DOM), soil respiration, or microbial activities (Chen et al., 2020; Dawes et al., 2017; Heinzle et al., 2021; Li et al., 2018), the results are characterized by high variability, mostly because different ecosystems have been analyzed.

Numerous studies have focused on the response of mountain forest soils to climate change. However, constructing a coherent feedback pattern is not possible owing to variation in the properties of mountain soils, climatic factors, vertical zonality, geological impact, vegetation cover, and soil (micro)biological activity (Bojko and Kabala, 2017; Ghosh et al., 2014; Józefowska and Miechówka, 2015; Labaz et al., 2014). Inconsistency in the effect of warming on SOM motivates the need for additional studies, focused on particular types of ecosystems (Li et al., 2018; Prietzel et al., 2016; Xu et al., 2010). However, to the best of our knowledge, no in situ studies have focused on the initial changes in soil C, N, and pH of Western Carpathian spruce stands in response to warming. The response is particularly important because spruce litter is abundant in organic C (resulting from relatively high resistance to decomposition in undisturbed ecosystems) compared with broadleaf or grassland litter (Bojko and Kabala, 2017).

The aim of the present research was to investigate the initial responses of Norway spruce forest soil in mountainous areas of the Western Carpathians to simulated soil warming. Tracking changes in forest soils under the influence of projected climate change can be more effective through the simultaneous use of field-based active or passive simulation experiments (Sun et al., 2013). The most commonly used passive method is made from translucent Plexiglas open-top chamber (OTC), which uses relatively small greenhouses with an open top (Aronson and McNulty, 2009). The soil enclosed by the chamber is protected against wind, eliminating potential disruptions to warming efficiency (de Frenne et al., 2010). The relatively low cost, convenient transportation and installation, and no need for an external power source are all advantages of the OTC method, allowing it to be used in remote or mountainous areas (Aronson and McNulty, 2009).

In this study, we conducted an experiment to simulate soil warming for almost 500 days. We used the OTC method for simulation of climate warming. We hypothesize that the warming in spruce stands in Western Carpathians will lead to: (1) the decrease in the content of total C and N; (2) the increase in the content of dissolved organic C and total dissolved N; (3) significant changes in the content of N forms (ammonium and nitrate), as well as soil pH. The results will fill an important gap in knowledge of the effects of climate change on the soil of Norway spruce stands, which comprise an important proportion of montane temperate forests.

2. Methodology

2.1. Study site

The research site was established in the Silesian Beskid Region (SBR), in the Wisła Forest Inspectorate (49°33′47.7″N, 18°52′06.5″E), in southern Poland (Fig. 1a and b). The site was located on a southeast-facing slope at an elevation of 520–550 m above sea level. The SBR is the westernmost part of the Western Beskid Mountains, located in the Western Carpathians. The area belongs to the montane climate region, with significant variation in the frequency of weather types throughout the year. This is because the SBR is located in a zone where oceanic and continental influences clash. The average air temperature for this elevational zone in the SBR is 6.5 °C, total precipitation is 1089 mm, and number of days with precipitation is 185 days. The warmest month is July (mean 14.9 °C) and the coldest is January (mean −3.7 °C). The average number of days with snow cover per year is 105 days (Januszek et al., 2015).

The mountain ranges of the SBR are composed of Carpathian Flysch formations. The mountains are formed mainly of sandstones, shales, conglomerates, and Tertiary formations (a series of sandstone and shale layers) (Aleksandrowicz, 1991; Gruba and Mulder, 2008). The parent material contains admixtures of muscovite, feldspars, and glauconite. The colour of the mineral topsoil is reddish owing to iron weathering (Gruba and Mulder, 2008). In accordance with the World Reference Base for Soil Resources, soils in the study area were classified as Skeletic Dystric Cambisols (Loamic) (IUSS Working Group WRB, 2015). Cambisols are the dominant reference soil group in the SBR, which are favored by the abundance of aluminosilicates in the Carpathian Flysch layers and a moderate climate (Gruba, 2001).

The study site was established in a mature (80 years old), managed Norway spruce stand (Fig. 1c). The stand comprised a single storey without developed undergrowth on the forest floor. The groundcover consisted mainly of European blueberry (*Vaccinium myrtillus* L.) with an admixture of mosses, such as leucobryum moss (*Leucobryum glaucum* (Hedw.) Ångstr.) and red-stemmed feathermoss (*Pleurozium schreberi* (Brid.) Mitt.).

2.2. Warming simulation

In December 2019 we established 15 research plots at the study site. The entire research site covered a forest area of approximately 1 ha. To simulate warming, in ten plots we placed hexagonal OTCs constructed with 3-mm-thick translucent Plexiglas (Fig. 1d). Five plots remained without an OTC and constituted the control group. All plots were randomly selected with a minimum distance between plots of 15 m. The top area of the OTC was 1.1 m^2 and the floor area was 2.6 m^2. To test their effectiveness for warming simulation, we placed soil temperature and water volume content (WVC) sensors (5TM Sensor, Decagon) at a depth of approximately 5 cm (between the organic and mineral soil horizons), with one sensor in an OTC plot and one in a control plot. Prior to the measurement, sensors were placed in the soil material in laboratory conditions (air temperature 20 °C, humidity 60%) for five days to ensure they provide similar results. The accuracy of temperature measurement was ±0.1 °C and the water volume content ±2%. The measurements were recorded from the beginning of December 2020 to the end of March 2021 at 15 min intervals and stored on an automatic logger (Decagon Em50 Data Logger). Data measured from the beginning of the experiment (December 2019) till December 2020 could not be used due to the data logger failure.

Table 1
Experimental timeline of the study.

Measurement series	Days from the beginning of the experiment	Date
0	0	December 5, 2019
1	84	February 28, 2020
2	167	May 21, 2020
3	258	August 20, 2020
4	363	December 3, 2020
5	476	March 26, 2021

2.3. Soil sampling

Soil samples were collected approximately every 3 months, from the start of the experiment in early December 2019 (“0” series); samples from this series formed a baseline to track subsequent changes. For clarity, measurement series were numbered sequentially (Table 1).

Soil samples were collected using a cylindrical auger with a diameter of 5 cm. During each measurement series, from each of the 15 plots, five replicates were collected using a diagonal sampling method and mixed to form one sample for each plot. The soil was collected from two horizons: the organic layer (O horizon) and mineral topsoil of 0–10 cm depth (A horizon). In total, 180 soil samples were collected in six series.

2.4. Laboratory analysis of soil samples

Before the laboratory analysis, living roots were removed from the soil samples. The samples were air-dried at 60 °C for 3 days and then sieved through a 2 mm mesh. The particle size distribution (the proportions of sand, silt, and clay) was estimated using laser diffraction (Fritsch Analysette 22, Idar-Oberstein, Germany). The limit of quantification for each fraction was 0.1%.

The soil pH was measured in distilled water (pH$_{H2O}$) and in 1 M potassium chloride (pH$_{KCl}$) (ratio 1:5, w/v), after 24 h of equilibration, with a potentiometric method using a combined electrode (Buurman et al., 1996). The limit of quantitation was 0.01.

Soil nitrate (NO_3-N) and ammonium nitrogen (NH_4-N) concentrations were measured, after extraction of 10 g soil with 50 mL of 2.0 M KCl for 20 min, using a flow injection auto analyzer (FIAstar 5000, FOSS, Hillerød, Denmark). The detection limits for both forms was 1 μg L^{-1}.

To determine the concentrations of dissolved organic carbon (DOC) and total dissolved nitrogen (TDN), dried soil samples (2.5 g for the O horizon and 5 g for the A horizon) were first mixed with deionized water (40 mL) and shaken for 1 h. The extraction mixtures were then filtered and rinsed with the water to obtain a volume of 100 mL. Measurement was performed using a Shimadzu TOC-V$_{CPN}$ analyzer with total nitrogen unit TNM-1 (Kyoto, Japan). The detection limits for DOC and TDN were 4 μg L^{-1} and 20 μg L^{-1}, respectively.

Subsamples of soil were ground with a ball mill (Fritsch) to obtain homogeneity. We used these milled samples to measure the concentration of total carbon (C_t) and total nitrogen (N_t) using a LECO CNS Tru-Mac analyzer (LECO, St. Joseph, MI, USA). Given that the soil was carbonate-free, we assumed that total carbon equaled organic carbon. The quality control of measurements was assured by analysis of Organic Analytical Standards (OAS, Elemental Microanalysis Ltd.) nos. B1280 (C = 0.83% ± 0.05%, N = 0.07% ± 0.01%), B2178 (C = 2.46% ± 0.22%, N = 0.16% ± 0.01%), AR4021 (C = 3.04% ± 0.16%). Both standards and samples were analyzed with two technical replicates, with an acceptable difference between the measurements of<10%. Standards were analyzed at the beginning and the end of each measurement run. The limit of quantitation was 0.008% for C and 0.266% for N.

Table 2
Mean and range of soil temperature and WVC by month under simulated warming with an open-top chamber and in the control plots.

Month	*n*	Temperature (°C)				WVC (%)			
		Warming	Control	Δ	Significance	Warming	Control	Δ	Significance
December 2020	2739	4.6 (2.0–8.1)	4.1 (1.5–6.7)	0.4	***	20.9 (19.0–21.9)	22.9 (20.0–32.1)	2.0	***
January 2021	2688	1.7 (0.4–7.3)	1.3 (0.5–3.7)	0.3	***	22.6 (17.9–28.4)	28.1 (22.2–38.3)	5.5	***
February 2021	2976	2.5 (0.5–5.9)	2.2 (1.1–5.0)	0.3	***	21.6 (20.2–23.9)	25.5 (22.4–38.1)	3.9	***
March 2021	2975	2.8 (0.2–12.3)	2.3 (0.2–9.5)	0.5	***	23.4 (22.0–25.0)	24.7 (22.4–32.2)	1.3	***

n, number of measurements; Δ, absolute value of the difference between treatments; Significance, assessed with the Wilcoxon test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

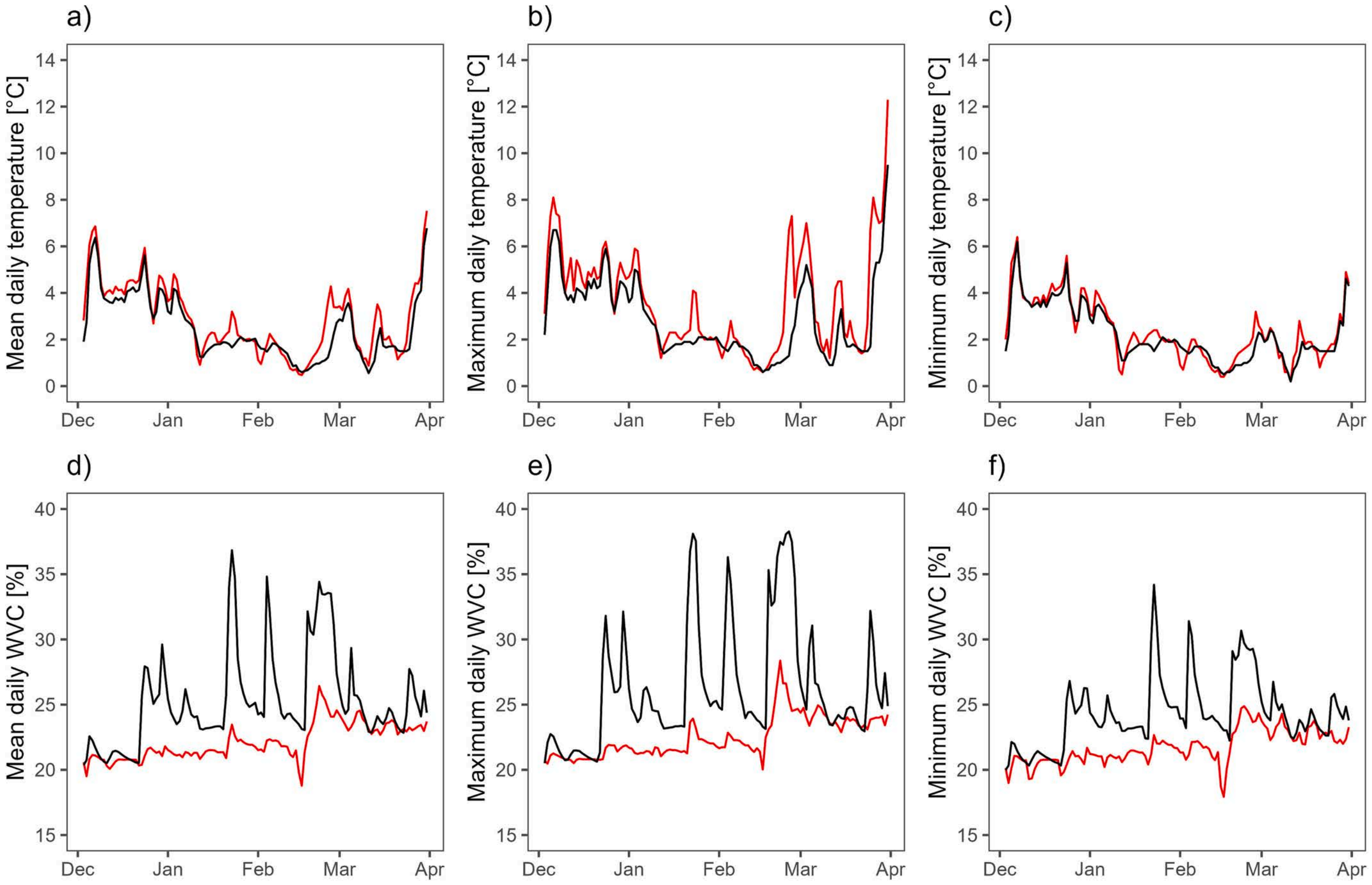

Fig. 2. Changes in soil temperature (a–c) and WVC (d–f) in the warmed soil (red line) and in the control (black line) monitored from early December 2020 to late March 2021.

2.5. Statistical analysis

To test the effectiveness of the warming simulation method used, comparisons of temperature and water volume content (WVC) between the groups (warming vs. control) were performed using the Wilcoxon test. Either for the entire simulation period or particular sampling series, a Mann–Whitney *U* test was performed to assess the difference between the warming treatment and control. To show results for the soil chemical properties, medians and interquartile ranges were used because the distributions were non-normal (as assessed with the Shapiro–Wilk test). Repeated-measures analysis of variance (ANOVA) was used to test the effects of warming, time, and their combined effect on the soil properties (C_t, N_t, pH in H_2O and KCl, NO_3-N, NH_4-N, DOC, and TDN). Before analysis, we checked that the assumptions of ANOVA were met for all data. Heterogeneous data were subjected to log-transformation. Correlations between soil properties were estimated using Pearson correlation analysis. Significance was defined as $p < 0.05$ unless otherwise specified. The statistical analyses and figure creating were conducted using R statistical software (R Core Team, 2020) with the package "ggplot2" (Wickham, 2016). We also used Statistica 13 software (Dell Inc., 2016).

3. Results

3.1. Soil microclimate

The simulation method significantly increased soil temperature and decreased soil WVC. A measurement of soil temperature and WVC conducted between December 2020 and March 2021 provided information on the effectiveness of the warming simulation. During winter season, the snow cover covering the plots with sensors was of similar thickness due to identical physiographic conditions and canopy closure, so it was assumed that microclimatic conditions were the same. Considering the entire measurement period, the average soil temperature under the OTC was significantly higher (Wilcoxon test, $p < 0.001$, $n = 11378$), attaining 2.9 °C (range 0.2–12.3 °C), in comparison with that of the control (2.5 °C, range 0.2–9.5 °C). The WVC was significantly

Table 3
Principal soil properties (median and interquartile range) for the study plots at the beginning of the experiment (“0” series).

	C_t (g kg^{-1})	N_t (g kg^{-1})	pH_{H2O}	pH_{KCl}	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	DOC (g kg^{-1})	TDN (g kg^{-1})
	Organic (O) horizon							
OTC	259.4 (176.6–328.4)	11.5 (7.3–13.0)	3.5 (3.5–3.6)	2.8 (2.8–3.0)	0.4 (0.3–0.6)	170.5 (78.7–219.4)	3.8 (2.5–4.4)	0.3 (0.2–0.4)
Control	342.2 (299.2–351.0)	14.5 (13.6–14.6)	3.6 (3.4–3.6)	2.7 (2.7–2.8)	0.6 (0.4–0.6)	161.5 (159.6–209.1)	3.9 (3.8–4.1)	0.33 (0.30–0.33)
	Mineral (A) horizon							
OTC	26.9 (18.5–32.6)	1.3 (1.0–1.5)	3.7 (3.6–3.8)	3.1 (3.0–3.3)	0.11 (0.06–0.11)	9.2 (8.7–12.8)	0.5 (0.3–0.8)	0.06^a (0.06–0.07)
Control	32.0 (31.8–33.6)	1.5 (1.5–1.5)	3.5 (3.5–3.8)	2.9 (2.9–3.)	0.06 (0.05–0.11)	10.1 (9.1–12.8)	0.7 (0.6–0.8)	0.07^b (0.07–0.08)

C_t, total carbon; N_t, total nitrogen; pH_{H2O}, pH measured in distilled water; pH_{KCl}, pH measured in 1 M KCl; NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; OTC, open-top chamber.
For each horizon, $n_{OTC} = 10$, $n_{control} = 5$. Different superscript letters within a column indicate a significant difference (Mann–Whitney *U* test, $p < 0.05$).

decreased in the OTC (Wilcoxon test, $p < 0.001$, $n = 11378$), attaining on average 22.1% (range 17.9%–28.4%). In contrast, the WVC in the control was 25.3% (range 20.0%–38.3%). When considering the individual months, a significant increase in soil temperature and decrease in WVC under the OTC was observed in each month (Table 2). The warmest month was December, whereas the coldest month was January, both in the OTC and the control. The greatest differences in mean soil temperature between the studied groups were observed in December and March. The lowest mean WVC for both groups was observed in January. The highest mean WVC in the plots with an OTC was recorded in March, whereas for the control it was observed in January. In addition, January was the month with the greatest difference in mean WVC between the two groups.

The pattern of soil temperature changes under the OTC and in the control was generally similar. This was particularly evident for mean daily soil temperature and minimum daily soil temperature (Fig. 2a and c). The maximum daily soil temperature differed notably between the OTC and the control, with significantly higher peaks in the former (Fig. 2b). The pattern for WVC (Fig. 2d – f) was characterized by considerably more abrupt fluctuations in the control plots, which were evident as numerous pronounced peaks. The WVC for the OTC (mean, minimum, and maximum values) was relatively constant and showed

Table 4
Median (and interquartile range) of soil chemical properties from all measurement series during the warming simulation period.

Parameter	Unit	O horizon			A horizon		
		OTC	Control	Sig.	OTC	Control	Sig.
C_t	g kg^{-1}	279.3 (244.3–332.6)	265.1 (224.0–330.0)	N.S.	39.3 (34.9–48.9)	47.7 (40.8–50.2)	N.S.
N_t	g kg^{-1}	10.3 (9.3–11.6)	10.6 (9.2–11.6)	N.S.	1.6 (1.3–2.1)	1.8 (1.5–2.3)	N.S.
pH_{H2O}		3.7 (3.6–3.8)	3.6 (3.5–3.6)	***	3.7 (3.5–3.9)	3.6 (3.5–3.6)	***
pH_{KCl}		2.9 (2.8–3.0)	2.8 (2.7–2.9)	***	3.1 (2.9–3.5)	2.9 (2.8–3.0)	***
NO_3-N	mg kg^{-1}	3.0 (1.2–5.1)	4.6 (2.2–11.5)	*	1.0 (0.7–1.4)	0.9 (0.6–1.4)	N.S.
NH_4-N	mg kg^{-1}	135.0 (101.9–181.9)	87.6 (66.4–104.8)	***	18.0 (15.6–23.0)	17.9 (13.9–21.1)	N.S.
DOC	g kg^{-1}	3.3 (2.9–3.9)	2.9 (2.5–3.4)	*	0.7 (0.5–0.8)	0.8 (0.7–0.9)	**
TDN	g kg^{-1}	0.3 (0.2–0.3)	0.2 (0.2–0.3)	*	0.06 (0.05–0.07)	0.06 (0.06–0.07)	N.S.

C_t, total carbon; N_t, total nitrogen; pH_{H2O}, pH measured in distilled water; pH_{KCl}, pH measured in 1 M KCl; NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen; OTC, open-top chamber.
For each horizon, $n_{OTC} = 50$, $n_{control} = 25$.
N.S., no significance; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Mann–Whitney *U* test).

Table 5

Results of two-way analysis of variance (ANOVA) showing *p*-values for responses of soil properties to experiment time, warming and combined effect of these factors; n = 75 for each horizon.

Factor	C_t	N_t	pH_{H2O}	pH_{KCl}	NO_3-N	NH_4-N	DOC	TDN
	O horizon							
Time	**<0.001**	0.381	**0.004**	**0.007**	**<0.001**	0.199	**<0.001**	0.555
Warming	0.771	0.968	**<0.001**	**<0.001**	**0.003**	**<0.001**	0.119	0.055
Time × Warming	0.981	0.378	0.577	0.944	**0.026**	**<0.001**	0.467	0.293
	A horizon							
Time	0.087	**<0.001**	**0.028**	0.781	**0.006**	**<0.001**	**<0.001**	**<0.001**
Warming	**0.018**	0.069	**<0.001**	**<0.001**	0.898	0.743	**<0.001**	**0.014**
Time × Warming	0.838	0.793	0.942	0.968	0.925	0.919	0.543	0.454

C_t, total carbon; N_t, total nitrogen; pH_{H2O}, pH measured in distilled water; pH_{KCl}, pH measured in 1 M KCl; NO_3-N, nitrate nitrogen; NH_4-N, ammonium nitrogen; DOC, dissolved organic carbon; TDN, total dissolved nitrogen. Significant *p*-values ($p < 0.05$) are highlighted in bold.

little fluctuation compared with the control.

Analysis of the differences in mean daily temperature and WVC between the warming treatment and control allowed us to determine the times of the day at which the OTCs were most effective for warming simulation. The greatest difference in soil temperature (more than 0.8 °C) was observed at approximately 14:00 (see Appendix A). The OTCs were least effective for soil warming in the early morning between 06:00 and 08:00. The smallest differences in WVC were observed in a similar period. The greatest differences in WVC between the two groups were observed in the period between 16:00 and 18:00.

3.2. Soil properties

3.2.1. The “0” series

Before the warming simulation started, the soil was characterized by high homogeneity of the chemical properties between the warmed plots and the control (Table 3). Both soil horizons were strongly acidic, with pH_{H2O} 3.5 in the O horizon and 3.6 in the A horizon. Regarding the particle size distribution, the analyzed samples were rich in the fine fraction (silt + clay). The average content of sand was 35%, silt 56%, and clay 9%. Soils of the warmed plots did not differ from the control in particle size distribution (OTC: 36% sand, 55% silt, 9% clay; control: 34% sand, 57% silt, 8% clay).

3.2.2. Entire warming simulation period

Analyzing the samples collectively from the entire warming experiment, it can be observed that warming clearly affected the chemical properties of the soil, mostly in the O horizon than in A. Table 4 summarizes the soil chemical properties for the entire simulated warming period from series 1 to series 5. Statistically significant increases in soil pH (either measured in H_2O or in KCl) in both horizons of the warmed soil were observed. In the O horizon of the warmed soil, the NO_3-N concentration decreased, whereas the NH_4-N concentration increased by 54%, compared with those of the control. The DOC and TDN concentrations were significantly increased in the warmed O horizon, but DOC was slightly decreased in the warmed A horizon. A Pearson correlation analysis between pH_{H2O} and the other chemical properties in the O horizon yielded interesting results. In the warmed soil, positive significant correlations were observed between pH_{H2O} and C_t ($r = 0.45$), N_t ($r = 0.33$), NH_4-N ($r = 0.52$), DOC ($r = 0.35$), and TDN ($r = 0.68$). In the control, the only significant correlation observed was a negative correlation between pH_{H2O} and NH_4-N ($r = -0.43$).

Two-way ANOVA of the soil chemical properties, for all measurement series including the “0” series, was performed (Table 5). For the O horizon, the *p*-values for all factors of the complete model were significant (time: $p < 0.0001$; warming: $p < 0.0001$, time × warming: $p < 0.0001$). For the A horizon, significance was observed for time ($p < 0.0001$) and warming ($p < 0.0001$), but not for the combined effect of time and warming ($p = 0.986$). The *p*-values of ANOVA for particular soil properties showed a significant combined effect of experiment duration and warming for NH_4-N and NO_3-N in the O horizon. Based on the ANOVA results, warming also affected soil pH in both horizons, and DOC and TDN in the A horizon.

3.2.3. Seasonal changes in soil properties during the warming experiment

The magnitude of changes in the studied chemical parameters under the influence of warming depended on the season, and the largest changes were observed for pH and NH_4-N. The values of the soil properties in each series were expressed as coefficients in relation to the values for the “0” series (Table 3). We observed a trend for increase in the concentrations of C_t and N_t in the O horizon of soils subjected to warming (Fig. 3a and b). Although in all series C_t and N_t concentrations were higher in the warmed soil, only in series 5 (March, 476 days of warming) were statistically significant differences observed. Similarly, higher medians for C_t and N_t characterized the A horizon of warmed soils, but the differences were not significant (Fig. 4a and b). In both horizons, soils under OTCs were characterized by greater variation in C_t and N_t. The concentrations of DOC and TDN in the O horizon showed a similar pattern to C_t and N_t, respectively. Median ratios of DOC and TDN in soils under OTCs were higher compared with those in soils in the control group, but significant differences were observed only in series 5 (Fig. 3c and d). In the A horizon, no significant differences in DOC and TDN concentrations were observed between the two groups (Fig. 4c and d). The NH_4-N concentration was higher in the second series (May, 167 days of warming), but significantly higher only in the fifth series, in comparison with the control (Fig. 3g). The NO_3-N concentration showed more dynamic changes in the O horizon of soils from the control group (Fig. 3f). In contrast, in the A horizon, the NH_4-N and NO_3-N concentrations showed no consistent relationship with simulated warming (Fig. 4e and f).

The pH of the soil subjected to warming was generally higher compared with that of the soil in the control group for both horizons (Fig. 5). More strongly significant differences were observed for pH_{H2O} than for pH_{KCl}. The smallest differences between groups were observed in the fifth series (March, 476 days).

4. Discussion

4.1. O horizon

4.1.1. Soil C and N

In the present experiment, warming caused an increase in C_t and N_t concentrations in the O horizon, as well as DOC and TDN concentrations at the beginning of the vegetative growth period, after 476 days of warming simulation compared with those in the soil of the control plots (Fig. 3; however, it should be noted that DOC and TDN analysis were performed on air-dried soil samples - such kind of sample preparation can enhance the release of DOC and TDN, mostly by the disruption of

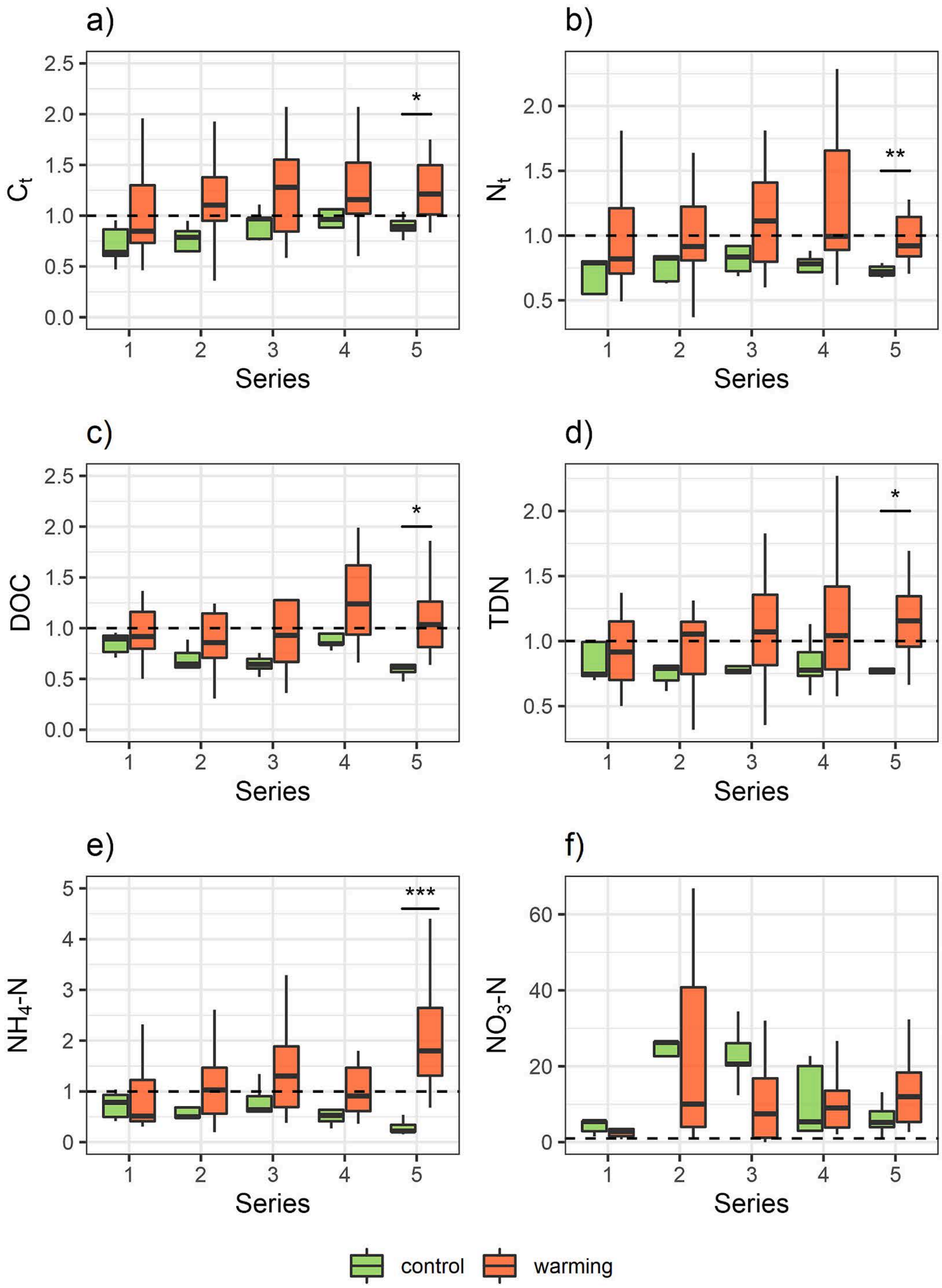

Fig. 3. Changes in chemical properties of the soil O horizon, relative to the measurements at the beginning of simulated warming ("0" series), in warmed (orange) and control (green) soils. The dashed line indicates the "0" series baseline. For the sampling series timeline see Table 1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Mann–Whitney *U* test). The black solid line within the box indicates the median, boxes indicate the interquartile range, and whiskers indicate minimum and maximum values.

soil microbial biomass (Kaiser et al., 2001)). In addition, a trend for these concentrations to increase in warmed soil over the experimental period was observed. Similar results were obtained by other authors, showing that warming can increase soil C and N concentrations (e.g., Liu et al., 2022; Prietzel et al., 2016). However, other studies have reported opposite results, indicating that C and N concentrations may decrease under soil warming (e.g., Hagedorn et al., 2010a; Heinzle et al., 2021). These seemingly contradictory results might arise from the influence of various factors, such as elevation, ecosystem type, duration of the experiment, or the methodology employed. In addition, to the best of our knowledge, direct comparison of the results of studies from different climatic zones or of different forest types is unreasonable owing to the high diversity of ecosystems. The small changes in total forms of C and N in the soil are difficult to detect, mostly because of natural soil variability and high background levels (Belay-Tedla et al., 2009). The statistically significant increase in C_t and N_t (which is contrary to the assumed hypothesis) with the simultaneous increase in DOC and TDN in the O horizon of warmed soil (compared to the control) is puzzling. The O horizon does not contain stable SOM fraction; hence we hypothesize that these observations are linked to the labile SOM fraction and its chemical composition. A likely reason of increased C_t and N_t concentrations in warmed soil could be the increased groundcover biomass production and, and thus, higher C influx, according to Giardina et al. (2014).

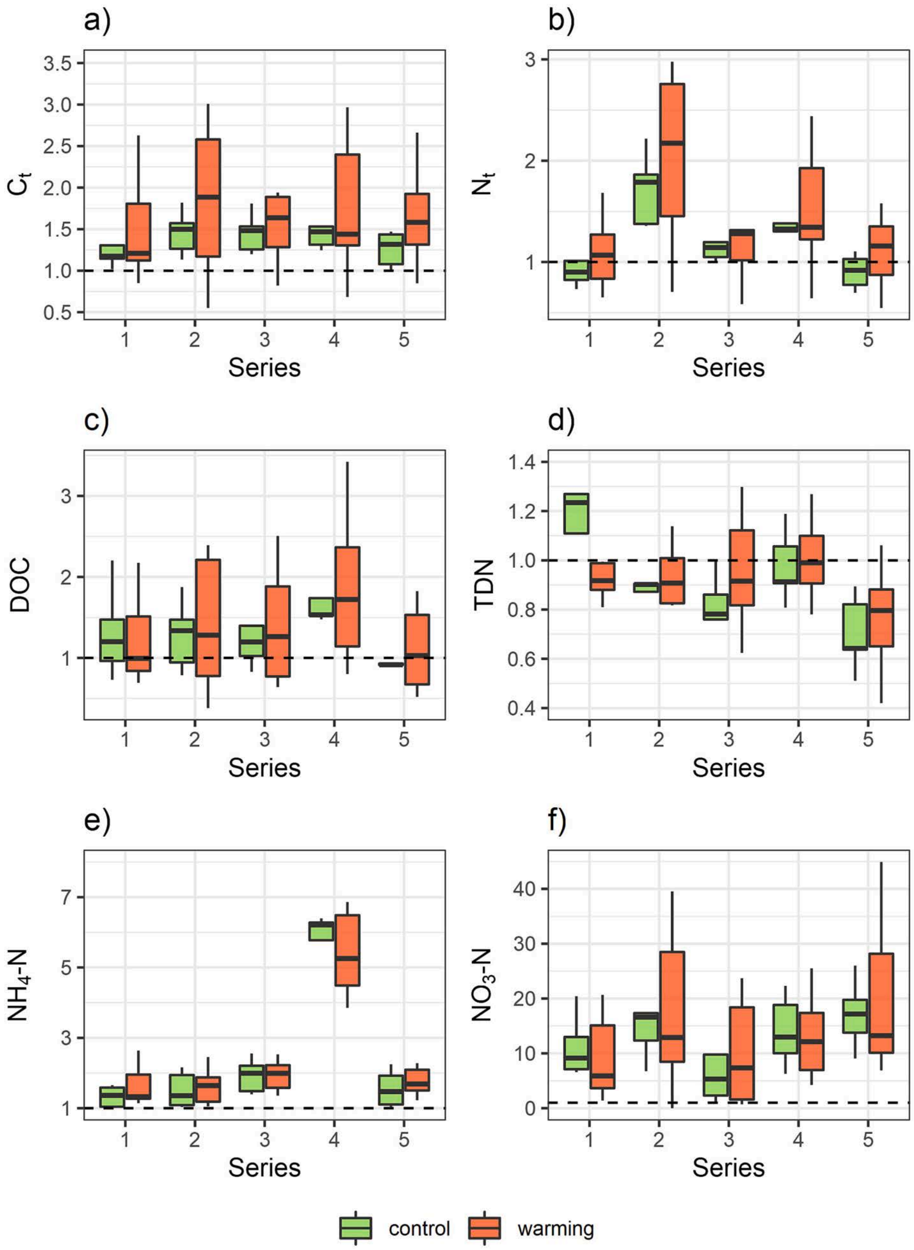

Fig. 4. Changes in chemical properties of the soil A horizon, relative to the measurements at the beginning of simulated warming, in warmed (orange) and control (green) soils. The dashed line indicates the "0" series baseline. For the sampling series timeline see Table 1. The black solid line within the box indicates the median, boxes indicate the interquartile range, and whiskers indicate the minimum and maximum values.

We hypothesize that the increased DOC and TDN concentrations in the O horizon under simulated warming may be associated with the increased SOM input, derived from increased biomass of groundcover vegetation (i.e., the litterfall and the fine root biomass). We observed that European blueberry shrubs growing on the warmed plots reached larger sizes, compared to the shrubs growing on the control plots (an analysis of the groundcover vegetation chemistry is the subject of separate publication). Our assumptions are in accordance with the results reported by other authors (e.g., Belay-Tedla et al., 2009; Chen et al., 2018; Rocci et al., 2021; Rustad et al., 2001; Six et al., 2001). The condition of high C and N availability in soil (manifested in the current study as increased DOC, TDN and NH_4-N concentration), associated with warming, may result in a positive response of groundcover vegetation productivity and, thus, increased SOM input (Shi et al., 2020). In addition, increase in DOC and TDN concentrations in response to an external disturbing factor significantly decreases the stability of SOM (Kupka et al., 2021). Such an assumption contributes to the findings of climate models, indicating that warming increases the plant biomass production (Thiele et al., 2017).

Another possible cause of increased C and N availability under warming conditions is that under soil warming, microbial communities responsible for decomposition of plant-derived organic compounds are promoted (Feng et al., 2008; Pisani et al., 2015; Rocci et al., 2021). Increased labile SOM fractions in warmed soils may also indicate poorer SOM stabilization, which may affect the microbial communities (Fröberg et al., 2006; Xu et al., 2010). Increased concentrations of DOC and TDN in the warmed O horizon indicate higher mobility of dissolved organic matter (Dawes et al., 2017; Fröberg et al., 2006; Xu et al., 2010). Thus, higher amounts of labile SOM, influenced by the warming, may act as an abundant source of available nutrients, which are crucial for plant productivity (Altman et al., 2017). To clarify the dynamics of C and N under warming, it would be appropriate to focus further research

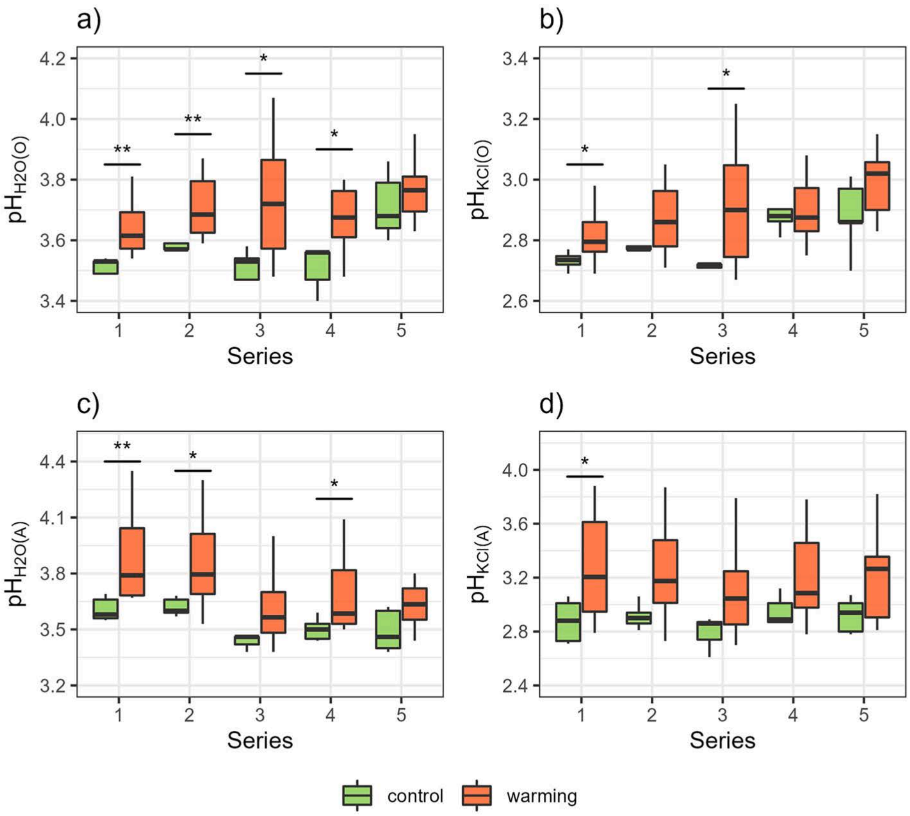

Fig. 5. Soil pH in the soil O horizon (a, b) and A horizon (c, d) measured in H_2O and KCl in warmed (orange) and control (green) soils. For the sampling series timeline see Table 1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (Mann–Whitney *U* test). The black solid line within the box indicates the median, boxes indicate the interquartile range, and whiskers indicate the minimum and maximum values.

on the uptake of these elements by aboveground vegetation. It should also be taken into account that the changes we observed in the soil C stability are an initial response and may change over time. According to Rustad and Fernandez (1998), SOM changes over a prolonged period will depend on altered litter quality and possibly modified forest stand composition.

4.1.2. Soil NH_4-N and NO_3-N

Simulated warming resulted in increased NH_4-N concentrations in the O horizon, which was most evident in the last sampling series at the beginning of the vegetative growth period (Fig. 3e). Considering the entire experimental period, NH_4-N concentrations were higher in the warmed O horizon (Table 4). Under natural conditions, the concentration of soil NH_4-N in spring rapidly decreases because of plant growth and N uptake (Heinzle et al., 2021; Jaeger et al., 1999; Schmidt et al., 2007), which was evident in the control group. In contrast, NH_4-N concentration increased significantly in the warmed soil. Given that N is considered to be among the main limiting nutrients in terrestrial ecosystems (Rustad et al., 2001), the activity of ammonification bacteria (responsible for hydrolysis and mineralization of N compounds), resulting in the formation of inorganic N, is crucial in the ecosystem N cycle (Dannenmann et al., 2009; Wang et al., 2017). As a result, assimilable N in the form of NH_4-N is provided to plants and can positively stimulate their growth, especially in acid forest soils, where NH_4-N is the primary source of N for plants (Chen et al., 2020; Nihlgård, 1985). These assumptions are in line with previous findings that warming will positively influence the site index of Norway spruce stands in Europe (Thiele et al., 2017). We cautiously assume that increased labile, easily available N in the form of NH_4-N in the soil O horizon of Norway spruce stands may contribute to the decreased stress resilience of spruce stands in Western Carpathians, consistent with the ammonium hypothesis (Nihlgård, 1985). N availability among other soil chemical properties has been shown to have a significant effect on the growth and development of Norway spruce in Central Europe (Cienciala et al., 2016). Expanding the ongoing research into exploring the biochemical responses of spruce trees to increased N is critical to understanding how the entire ecosystem functions in the face of climate change.

Despite a significant increase in NH_4-N under the influence of warming in the O horizon, no significant increase in NO_3-N concentration was detected (Fig. 3f). Moreover, a significant decrease in NO_3-N concentration during the entire warming period was observed (Table 4). Enhanced availability of NH_4-N should lead to increased nitrification (Barnard et al., 2005). The relationship between N forms and climate change is currently considered to be indirect, thus the soil NO_3-N concentration may potentially be influenced by many factors (Barnard et al., 2005). In contrast, there is a view that the effects of climate change do not significantly impact on NO_3-N. Some authors have suggested that the increase in concentration of one form of N (i.e., NO_3-N or NH_4-N) in the absence of an increase in the other may be due to individual plant assimilative preferences (Xu et al., 2010). Another possible explanation of such phenomenon may be the difference in the ability of soil sorption complex to bind different labile N forms. NO_3-N is sorbed mainly via outer-sphere adsorption, while NH_4-N via inner-sphere adsorption (basal planes) (Strawn, 2021). However, further research focusing on losses of NO_3-N or NH_4-N from sorption complex to water would be necessary.

4.1.3. Soil pH

Soil pH is considered to be the result of several factors, including vegetation, weathering of the bedrock, time, and climate (Smith et al., 2002). We observed a higher pH in soils subjected to warming in both studied horizons (Fig. 5, Table 4). The relationship between warming and soil pH varies among ecosystems. For example, Liu et al. (2022) observed a negative correlation between pH and warming for

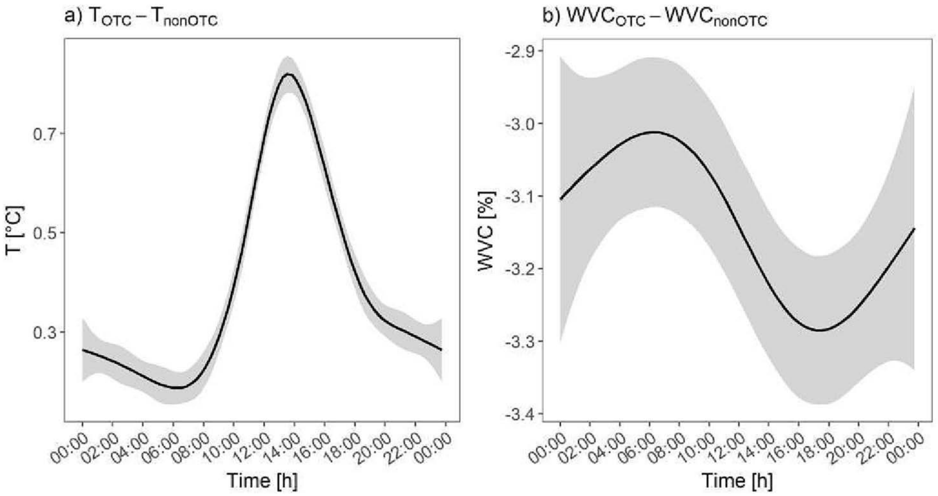

Fig. A1. Mean difference with 95% confidence interval (grey belt) in the soil temperature (a) and water volume content (b) between OTC and control (nonOTC).

agricultural soils. Some authors suggest that the increase in soil pH caused by soil warming and drying enhances the availability of NH_4-N (Singh et al., 2011; Smith et al., 2002). Recent findings by Kupka and Gruba (2022) demonstrate that an increase in pH of the acid soils of Norway spruce forest decreases the sorption of DOC, thereby reducing C sequestration. We hypothesize that the warming-induced increase of soil pH observed in the present study may contribute to decreased sorption capacity and, therefore, to leaching of DOC and TDN. A full understanding of the relationship between soil acidity and warming is crucial, as pH is proven to have a significant effect on spruce growth in central European forests (Cienciala et al., 2016).

The positive correlation between pH and NH_4-N as influenced by warming in the O horizon (compared with the negative correlation in control group) is also of interest. The change in this correlation may be due, in our opinion based on the results of other studies (Liu et al., 2022; Nie et al., 2018), to a change in soil microbial communities involved in N transformation, caused by soil warming and a decrease in soil WVC. We suggest, however, that it is not possible to thoroughly investigate the causes of the increase in soil pH using only the parameters analyzed in the present study because it may lead to erroneous conclusions. Soil pH, as an indicator of the amount of H^+ ions in the soil sorption complex, is a result of the ion-exchange relationships (Gruba and Mulder, 2015; Ross et al., 2008), hence these relationships should be thoroughly investigated.

4.2. *A horizon*

The A horizon of the studied soils showed fewer significant changes in response to warming than the O horizon, except for increased pH and decreased DOC (Table 4). The probable reason for the lower susceptibility of the A level to changes, apart from its greater depth, is its mineral character and thus lower content of organic matter. The stock of mineral-associated C is reported to be resistant to the warming (Giardina et al., 2014), which could explain obtained results. Considering the time aspect, changes in chemical properties of warmed soils were mostly similar to the changes in the control group (Fig. 4). Over the entire research site, the thickness of the organic layer covering the A horizon was approximately 5 cm. Thus, we conclude that in habitats with a well-developed O horizon, it will, as an initial response, insulate the deeper mineral soil horizons from the direct effects of climate warming. Therefore, we assume that the initial effect of soil warming on the A horizon was indirect and resulted mainly from changes in the O horizon. The results obtained by us, proving a lower impact on warming at deeper soil horizons, are consistent with the results obtained for the soils of other terrestrial ecosystems (Dove et al., 2021; Jiang et al., 2020; Wang et al., 2022). We surmise that greater input of readily decomposable SOM will lead to leaching into the soil profile, thus modifying its properties.

5. Conclusions

This study demonstrated an initial effect of warming on SOM in Norway spruce stands in the Western Carpathians. The warming method we used provided significant increase in soil temperature and decrease in soil WVC. The decrease in SOM stability and changes in soil chemical features may constitute a challenge for forest management in the near future. We conclude that climate change negatively stimulates SOM stability, resulting in higher concentrations of labile dissolved forms (DOC and TDN). Warming-induced increase in NH_4-N concentration can contribute to enhancement of the site index, but at the same time may lead to deterioration of forest stand resilience. The presence at the study site of the most common soil type under Norway spruce in the Western Carpathians allows us to relate our results to a larger area with high probability. Further research should be focused on the impact of warming on soil microbial communities, as well as soil ion-exchange relations. Particularly important is further investigation of warming-induced soil pH increase, which can seriously aggravate C sequestration. The present results fill a gap in research on the effects of climate change on Norway spruce forest soils.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A

Mean difference with 95% confidence interval (grey belt) in the soil temperature (a) and water volume content (b) between OTC and control (nonOTC). Fig. A1.

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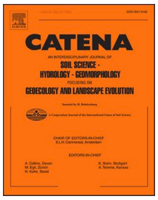

Simulated warming decreases acidity of forest topsoil under Norway spruce (*Picea abies* (L.) H.Karst.) stands in the Western Carpathians

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ABSTRACT

Global warming is affecting the montane forests of the temperate zone, causing a number of changes in all elements of the ecosystem, including the soil. Norway spruce forests, weakened by ongoing decay over several decades, are likely particularly sensitive to climate-induced changes. The response of soil cation exchange capacity components of spruce stands to warming is not well understood. We have assumed that warming will cause significant changes in pH, disrupting ion-exchange relationships in the soil. To the best of our knowledge, the present study is the first devoted to examining the mechanisms of soil pH change in temperate forest under the influence of warming. The forest in-situ experiment, simulating the soil warming for 578 days, was performed. Laboratory analyses included measurements of pH, and concentrations of carbon and exchangeable forms of calcium, potassium, magnesium, sodium, and aluminum. Shortly after the beginning of simulated warming (84 days), we observed a significant increase in the pH of the topsoil horizons. On average, in the warmed organic (O) horizon, the pH increased by 0.21, and in the mineral (A) horizon by 0.29 pH units. Based on previous studies, the pH increase was most likely triggered by an increase in ammonium-nitrogen content in the warmed soil. Using the Henderson–Hasselbalch equation to model the pH revealed that, in the O horizon, the pH increase is a result of the presence of weaker organic acids, less able to release hydrogen ions during dissociation. In the A horizon, the increase in pH was explained by an increase in abundance of aluminum ions, probably migrating upward from deeper soil layers. We consider that the ion-exchange mechanisms described here will contribute to an improved understanding of the complex processes of spruce forest dieback in the context of climate warming.

1. Introduction

The rate of increase in global mean surface temperature over the last 50 years is unprecedented for at least 2000 years (Gulev et al., 2021). Temperatures during the second decade of the twenty-first century likely exceeded the warmest 100-year scale range for the present interglacial since the last peak ca. 6500 years ago (Gulev et al., 2021). Observed changes are negatively affecting montane forest ecosystems, leading to disruption of their stability and decreasing homeostasis (Hagedorn et al., 2010). Particularly threatened are montane Norway spruce (*Picea abies* (L.) H.Karst) forests in the Carpathians (Altman et al., 2017; Durło, 2015; Socha and Durło, 2012), which are considered to be the most vulnerable to warming forest stands (Alberton et al., 2017; Lindner et al., 2008; Szewczyk et al., 2011). Based on the IPCC report, average summer air temperatures in the Western Carpathians by the end of the twenty-first century will increase by between 1.5–2 °C (RCP4.5 scenario, assuming a marked decline in atmospheric greenhouse gas content by mid-century) and 4.0–4.5 °C (RCP8.5 scenario, presuming that the present pace of greenhouse gas emission expansion remains unchanged) (Alberton et al., 2017). Despite the fact that many soil processes are temperature-sensitive, little is known about how global warming affects soil temperatures in general (Soong et al., 2020). In addition, due to the wide variation in exposures and altitudes in the Carpathians, there is a lack of accurate information on projected soil warming. However, the projected increase in temperature for temperate zone soils at a depth of 1 cm by the end of the century under the RCP8.5

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scenario is predicted to be about 0.3 °C (Alberton et al., 2017; Soong et al., 2020).

During recent decades, spruce stands in temperate forests have declined, due to to industrial emissions (Jandl, 2020), nitrogen (N) deposition (Oulehle et al., 2016), and, recently, global climatic changes (Altman et al., 2017; Cienciala et al., 2016; Dyderski et al., 2018). Given that Norway spruce is one of the most important coniferous trees in montane forests of Central Europe (Hanewinkel et al., 2013; Thiele et al., 2017), the observed decline is particularly worrying. The shrinkage of the spruce natural range will entail a change in forest species composition and management strategies (Yousefpour et al., 2010).

Although numerous attempts have been made to describe the influence of global changes on Norway spruce stands in Europe (e.g., Altman et al., 2017; Cienciala et al., 2016; Dyderski et al., 2018; Tumajer et al., 2017), relatively little attention has been paid to the forest soil under Norway spruce stands. The effect of warming is strongly connected with soil properties (Bojko and Kabala, 2017; Labaz et al., 2014; Spiecker, 2000). However, given that mountain soils differ in many aspects (such as climatic properties, vertical zonality, vegetation composition, or microbiota activity), it is difficult to create a coherent scenario of a soil's response to climate warming (Bojko and Kabala, 2017; Labaz et al., 2014). Among proposed methods for simulating the conditions of a changed climate, the open-top chamber (OTC) warming simulation method is widely used and employs translucent Plexiglas® greenhouses with an open top. The method has been used for>30 years and is characterized by its ease of application, especially in difficult terrain (e.g., mountainous, remote areas) (Aronson and McNulty, 2009). Such an experiment was initiated in a Norway spruce stand in the Western Carpathians in December 2019, focusing on the response of the soil in the stand to simulated warming. The initial results of this project, reported by Kupka et al. (2023), showed that warming caused increases in total forms of carbon (C), N, as well in labile: dissolved organic N, C, and ammonium nitrogen (NH_4-N) contents in the organic (O) horizon. An additional important finding is that simulated warming rises the pH of soil organic matter (SOM) and the mineral topsoil. In an era of climate warming and the need for its mitigation, it is also noteworthy that an increase in acidic forest soil pH causes a decrease in the soil's ability to stabilize dissolved organic matter, thereby reducing the potential for C sequestration (Kupka and Gruba, 2022). To fully understand the change in pH under the influence of warming, a thorough study of changes in ion-exchange relationships in the soil sorption complex is crucial.

In acidic forest soils, which are abundant in SOM, the pH may be outlined by the Henderson–Hasselbalch (H-H) equation (Eq. (1), which originally described the dissociation of weak acids (Gruba and Mulder, 2015; McBride, 1994):

$$pH = pK_{app} + n\mathrm{Log}\frac{(A^-)}{(HA)} \tag{1}$$

where pK_{app} indicates the negative logarithm of the dissociation constant, n is the empirical stoichiometry reaction constant, which indicates the amount of hydrogen (H) moles reacting with one mole of charged sites connected with SOM, A^- is the amount of dissociated groups, and HA is the amount of non-dissociated groups. To make this equation easier to apply in soil science, it was modified by Bloom and Grigal (1985):

$$pH = pK_{app} + n\mathrm{Log}\left(\frac{BS_E}{1 - BS_E}\right) \tag{2}$$

where BS_E is the effective base saturation, and $1 - BS_e$ is the fraction of exchangeable acidity, including H and aluminum (Al).

Equations (1) and (2) have been used to model the pH of a soil solution in numerous studies (Ciarkowska and Miechówka, 2019; Ma and Xiao, 2023). However, Skyllberg (1999) reported that in acidic forest soils with pH below 4.5, Al behaves as a base cation, competing with H for exchange sites of SOM. Such an approach assumes using the effective cation exchange capacity (CEC), being the sum of cations (Al, calcium [Ca], H, potassium [K], magnesium [Mg], and sodium [Na]) extractable by unbuffered salts. Subsequently, Gruba and Mulder (2015) confirmed the base character of Al in acidic soils and proposed that the pH can be described as an effect of changes in the relationships between exchangeable base cations (BC), organically bound Al (i.e., extracted from soil using $CuCl_2$ solution), and H. Thus, the parameter A^- equals the sum of exchangeable BC and Al ($CuCl_2$-extractable, i.e., exchangeable plus organically bound). According to these authors, the parameter $1 - BS_E$ equals the total H. Thus,

$$pH = pK_{app} + n\mathrm{Log}\left(\frac{Al + BC}{H}\right) \tag{3}$$

Most previous studies have assumed a decrease in soil pH as an effect of soil warming (e.g., Hubova et al., 2017; Keenan et al., 2014; Rengel, 2011). To the best of our knowledge, no previous research has focused on the changes in CEC composition with increase in soil pH in the context of soil warming. Studies of the impact of global changes on soil CEC have focused mainly on its decline (Fang et al., 2017). Warming has been suggested to accelerate the rate of SOM turnover (e.g., Chen et al., 2020), N transformation (e.g., Dawes et al., 2017; Liu et al., 2022), and the release of cations (e.g., Duan et al., 2013; Slessarev et al., 2016). In addition, increased evaporation resulting from warming may accelerate upward transport of cations from the mineral phase to SOM (e.g., Bechtold et al., 2012).

The aim of this study was to comprehensively analyze the components of soil CEC influenced by warming. In our simulation experiment, we assumed an increase in topsoil temperature of about 0.5 °C, which is consistent with the RCP8.5 scenario and the resulting soil warming forecast (Alberton et al., 2017; Gulev et al., 2021; Soong et al., 2020). Changes in CEC induced by warming may profoundly modify the soil properties, thereby creating unfavourable site conditions for Norway spruce. In an era of global climate change, a thorough investigation of the dynamics of CEC components is significant for simulating the response of the forest ecosystem and for policymakers to keep the steadiness of forest ecosystems. The present approach, which focuses on changes in the ion-exchange relationship of CECs, has not been widely used in recent times, hence there are few contemporary reports on ion-exchange relationships, let alone their relation to climate change. We hypothesized that soil warming causes an increase in soil pH, which is the effect of changes in soil CEC composition. In particular, we expected that soil warming leads to increase in the saturation of exchange sites of SOM with Al^{3+} followed by decrease in the fraction of H^+.

2. Materials and methods

2.1. Study site and warming experiment

The study site was located in the Polish part of the Western Carpathians, in the Silesian Beskid Region, on a south-west-facing slope at an altitude of ca. 535 m above sea level (Fig. 1). The main physiographic parameters at the study site are summarized in Table 1.

The single-storey forest stand on the study site was formed of 80-year-old Norway spruce (*Picea abies* (L.) H.Karst). The stand is managed by the State Forest Inspectorate. The forest floor vegetation was poorly developed and contained mainly mosses and European blueberry (*Vaccinium myrtillus* L.). The soil at the site is a Cambisol, which is the most common reference soil group in the Western Carpathians, owing to the moderate montane climate and abundance of aluminosilicates in the parent material (Gruba and Mulder, 2008).

In early December 2019, 15 research plots were established at the research site in an area of ca. 1 ha. All the plots were chosen at random while ensuring a minimum spacing of 15 m between them. Ten plots were each covered with an OTC to simulate soil warming, while five

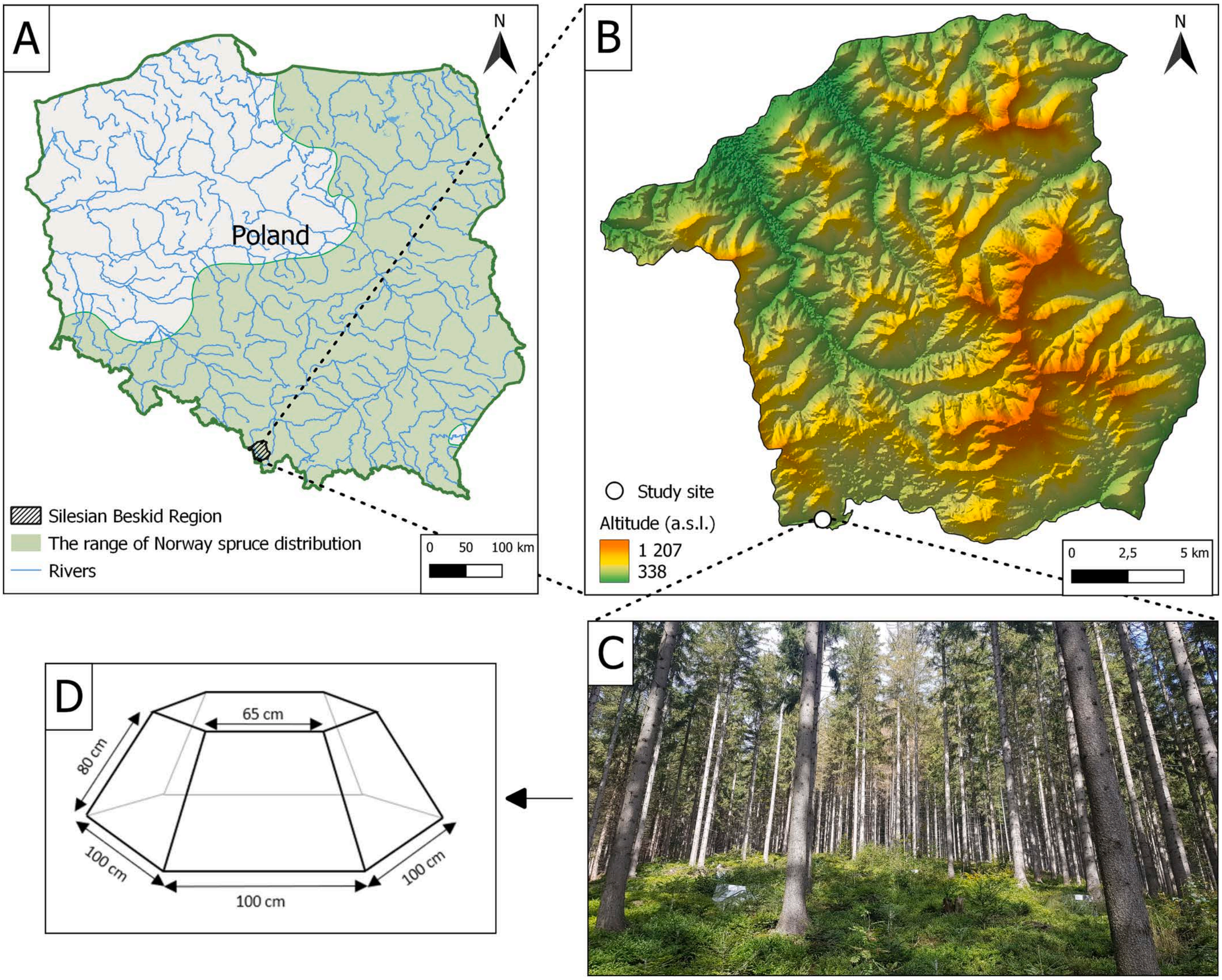

Fig. 1. Localization and view of the study site. A - Map of Poland indicating the natural range of Norway spruce and location of the Silesian Beskid Region. B - Hypsometric map of the Silesian Beskid Region with the research site indicated. C - View of the forest stand. D - Schematic illustration of the open-top chamber. Maps A and B were created with QGIS version 3.22.7 software (QGIS Association, 2023). The Norway spruce range shapefile, published by Caudullo et al. (2017) under the CC BY 4.0 license, was used in mapping.

Table 1
Description of the study site, based on Aleksandrowicz (1991), Gruba and Mulder (2008), IUSS Working Group WRB (2022).

Characteristic	Description/value
Coordinates	49°33′47.7″N 18°52′06.5″E
Altitude	520–550 m a.s.l.
Slope and aspect	20°, southeast, homogenous
Average air temperature	6.5 °C
Total precipitation	1089 mm
Temperature of warmest month	14.9 °C, July
Temperature of coldest month	−3.7 °C, January
Parent material	Carpathian Flysch (layers of sandstones, shales, conglomerates)
Soil WRB reference group	Skeletic Dystric Cambisol (Loamic)

plots (without OTC) formed the control group. The OTC was made from translucent 3-mm-thick Plexiglass®. The total warmed soil area was 26 m^2, while the area of control was 13 m^2. Periodically (every ca. 3 months), soil samples were collected with a metal soil auger of 5 cm diameter. The samples comprised material from the O horizon and the uppermost 10 cm of the mineral topsoil (A horizon). The organic subhorizons (Oi, Oe, Oa) were taken jointly, since the Oi subhorizon was extremely thin; it consisted mainly of needles and was not present at every sampling point. In each plot the samples were collected randomly from five points and mixed to form one composite sample. The entire experiment comprised seven sampling series covering a period of 578 days (December 2019–July 2021).

2.2. *Laboratory analysis*

Prior to the analysis, living roots and large stones were removed. The soil was dried for three days at 60 °C and then sieved, using a 2 mm mesh. Percentages distribution of sand, silt, and clay particles was analyzed using a laser diffractometer (Fritch Analysette 22, Idar-Oberstein, Germany).

The total C concentration was analyzed using a LECO CNS TrueMac analyzer (LECO, St. Joseph, MI, USA) on soil samples previously ground with a Fritsch ball mill. Because the soil was free from carbonates, total C equaled organic C. The detection limit was 0.008%.

The soil pH was analyzed using a potentiometric method with a combined electrode, after equilibration for 24 h with distilled water (1:5, w/v) (Buurman et al., 1996). The detection limit for pH measurement was 0.01.

Total acidity (TA) of the soil was measured after extraction of 10 g soil with 30 mL calcium acetate ($(CH_3COO)_2Ca$). Soil on paper filters was rinsed with the extractant to obtain an extract of 100 mL volume. An aliquot (20 mL) of the extract solution was used for potentiometric titration with an automatic titrator (Mettler Toledo Inc., Columbus, OH, US) to pH 8.2 with 0.1 M sodium hydroxide (NaOH). The detection limit of titration was 0.01 $cmol_{(+)}\ kg^{-1}$ dry soil mass.

The exchangeable forms of Na, Mg, Ca, and K were extracted with 1 M ammonium acetate (CH_3COONH_4), pH 7. After mixing 10 g of the soil sample with 30 mL extractant and shaking for 1 h, extraction was performed using paper filters. Samples on the filters were rinsed to obtain an extract of 100 mL volume. Quantification was performed using an inductively coupled plasma–optical emission spectrometer (ICP-OES; Thermo iCAP 6500 Duo, Thermo Fisher Scientific, Cambridge, UK).

The amount of exchangeable form of Al was assessed using the ICP-OES technique after extraction of 3 g of the soil samples with 30 mL of 0.5 M copper (II) chloride ($CuCl_2$) with shaking for 2 h. The detection limit of the ICP-OES was 0.01 $cmol_{(+)}\ kg^{-1}$ dry soil mass.

The base cations concentration (BC) was calculated according to the formula (4) (Ostrowska et al., 1991):

$$BC = Ca + K + Mg + Na\ \left[cmol_{(+)}\ kg^{-1}\right] \tag{4}$$

Cation exchange capacity (CEC) was calculated according to the formula (5) (Bednarek et al., 2005):

$$CEC = BC + TA\ \left[cmol_{(+)}\ kg^{-1}\right] \tag{5}$$

The hydrogen concentration (H) was calculated according to the formula (6) (Bednarek et al., 2005):

$$H = TA - Al\ \left[cmol_{(+)}\ kg^{-1}\right] \tag{6}$$

Base saturation (BS) was calculated according to the formula (7) (Bednarek et al., 2005):

$$BS = \left(\frac{BC}{CEC}\right) * 100\ [\%] \tag{7}$$

2.3. *Soil temperature and water volume content*

The soil temperature and water volume content (WVC) were measured between December 2020 and July 2021. One sensor (5TM, Decagon) was placed in an OTC plot and one in a control plot at 5 cm depth (at the transition between the O and A horizons). Every 15 min, temperature and WVC measurements were automatically recorded and stored on a data logger (Em50 Data Logger, Decagon). Prior to their installation, the sensors were put into the soil under laboratory conditions (20 °C, relative humidity about 60%) for 5 days to check the consistency of the measurements.

2.4. *Statistical analysis*

The normality of data distributions within each group was assessed using the Shapiro–Wilk test. Differences between treatment groups (warming vs. control) in soil temperature and WVC were calculated using the *t*-test. Obtained soil data were divided into groups according to the soil horizon, sampling series, and treatment (warming vs. control). The Mann–Whitney *U* test ($p < 0.05$) was used to test differences between the groups (warming vs. control). To analyze the relationships between pH and CEC components, linear regression was performed. All statistical analyses were performed in R software (R Core Team, 2023) using the packages ‘ggplot2′ (Wickham, 2016), ‘dplyr’ (Wickham et al., 2022), ‘stringr’ (Wickham, 2022), ‘ggpubr’ (Kassambra, 2022), and ‘facotextra’ (Kassambara and Mundt, 2020). The numbers following mean values as well as error bars in figures refer to standard errors.

3. Results

3.1. *Soil temperature and water volume content*

The soil temperature and WVC data are summarized in Table 2 and Fig. 2. During the monitoring period, the average soil temperature (at 5 cm depth) in the warmed plot was 0.5 °C higher compared with that in the control plot. The average WVC during the study period was 1.9% lower in the warmed soil compared with that of the control. A detailed analysis of the effectiveness of the applied warming method at the study site is presented in a separate publication (Kupka et al., 2023).

3.2. *Initial soil properties*

At the beginning of the experiment, none of the investigated properties differed between the OTC and control plots (as indicated by the Mann-Whitney *U* test). Thus, here we present the overall averages for all plots ($n = 15$; Table 3). The O horizon was present in all plots, ranging from 2 to 4 cm in depth, with average C content of 27.1%. This horizon consisted of weakly decomposed plant materials, including mostly spruce needles and blueberry plant remnants. The sampled O horizons were strongly acidic with an average pH of 3.54. The average CEC was 65.6 (±14.0) $cmol_{(+)}\ kg^{-1}$. The average CEC/C ratio was 2.35 (±0.23). The CEC was dominated by exchangeable H^+ (77%) and Al^{3+} (14%), whilst the sum of BC (Ca + K + Mg + Na) was only 7% of the CEC. The mineral horizon, sampled to 10 cm depth, in most cases was identical to the observed depth of the A horizon. The texture of the A horizon was sandy loam composed of sand 35%, silt 55%, and clay 9%. The average C content was 2.9% (±1.1%). The average CEC of the A horizon was 15.3 (±3.6) $cmol_{(+)}\ kg^{-1}$, which was remarkably lower than that of the O horizon, but the average pH was similarly low (3.66). The CEC/C ratio in the A horizon was 5.54 (±1.19). The CEC was dominated by Al (52%), exchangeable H comprised 46% of the CEC, and BC contributed to 2% of the CEC.

3.3. *Temporal changes during the experiment*

As mentioned above, at the beginning of the experiment, the average soil pH in the OTC and control plots was similar (Table 3). After 3 months, a significant increase in the pH of both the O and A horizons of warmed plots was observed (Fig. 3). In the O horizon, the increase was significant in four consequent sampling series: in February 2020 ($\Delta pH = 0.14$), May 2020 ($\Delta pH = 0.13$), August 2020 ($\Delta pH = 0.21$), and December 2020 ($\Delta pH = 0.14$). In next two sampling series in 2021, the average pH of the warmed and control samples was similar. Note that both warmed and control plots exhibited a trend for increase in pH over time. In the A horizon soil under simulated warming, a higher pH than that of the control plots was detected in February 2020 ($\Delta pH = 0.29$). In the subsequent sampling series, the pH of the A horizon in the warmed plots was significantly higher than that of the control plots: May 2020 ($\Delta pH = 0.23$), August 2020 ($\Delta pH = 0.19$, not significant), December 2020 ($\Delta pH = 0.19$), March 2021 ($\Delta pH = 0.17$), and July 2021 ($\Delta pH = 0.21$) (Fig. 3b).

Surprisingly, the soils of both groups (warming and control) of plots showed no significant differences in contents of major exchangeable cations (Fig. 4). The BC content did not differ between the groups; however, the BC content increased with time during the experimental period.

The content of exchangeable Al tended to be higher in the warmed plots, particularly in the O horizon. However, the average Al content did not differ between the groups in any sampling series (Fig. 5). A higher content of Al was observed in soil samples with pH > 3.8, which were noted only in the warmed plots. Significant differences in H content in O levels were not found between groups (Fig. 6). However, a significant

Table 2
Values of soil temperature and water volume content (WVC) during the measurement period in the warmed plot and the control plot (mean and range).

Date	*N* (amount of records)	Temperature [°C]				WVC [%]			
		Warming	Control	*p*	Δ	Warming	Control	*p*	Δ
Dec 2020	2739	4.6 (2.0–8.1)	4.1 (1.5–6.7)	<0.001	0.5	20.9 (19.0–21.9)	22.9 (20.0–32.1)	<0.001	2
Jan 2021	2688	1.7 (0.4–7.3)	1.3 (0.5 – 3.7)	<0.001	0.4	22.6 (17.9–28.4)	28.1 (22.2–38.3)	<0.001	5.5
Feb 2021	2976	2.5 (0.5–5.9)	2.2 (1.1–5.0)	<0.001	0.3	21.6 (20.2–23.9)	25.5 (22.4–38.1)	<0.001	3.9
Mar 2021	2975	2.8 (0.2–12.3)	2.3 (0.2–9.5)	<0.001	0.5	23.4 (22.0–25.0)	24.7 (22.4–32.2)	<0.001	1.3
Apr 2021	2880	5.1 (2.3–15.2)	4.4 (2.0–10.9)	<0.001	0.7	23.4 (21.6–24.8)	25.1 (22.0–33.8)	<0.001	1.7
May 2021	2976	9.5 (4.5–18.9)	8.9 (4.6–13.8)	<0.001	0.6	23.1 (21.4–26.4)	24.3 (21.7–35.4)	<0.001	1.2
Jun 2021	2875	14.0 (7.3–22.2)	13.5 (7.2–19.0)	<0.001	0.5	21.9 (20.0–23.7)	21.8 (19.8–29.8)	<0.001	−0.1
Jul 2021	2976	16.6 (13.2–22.0)	16.1 (12.9–19.6)	<0.001	0.5	17.7 (15.8–20.4)	17.4 (15.9–20.3)	<0.001	−0.3

p-value according to the *t*-test.

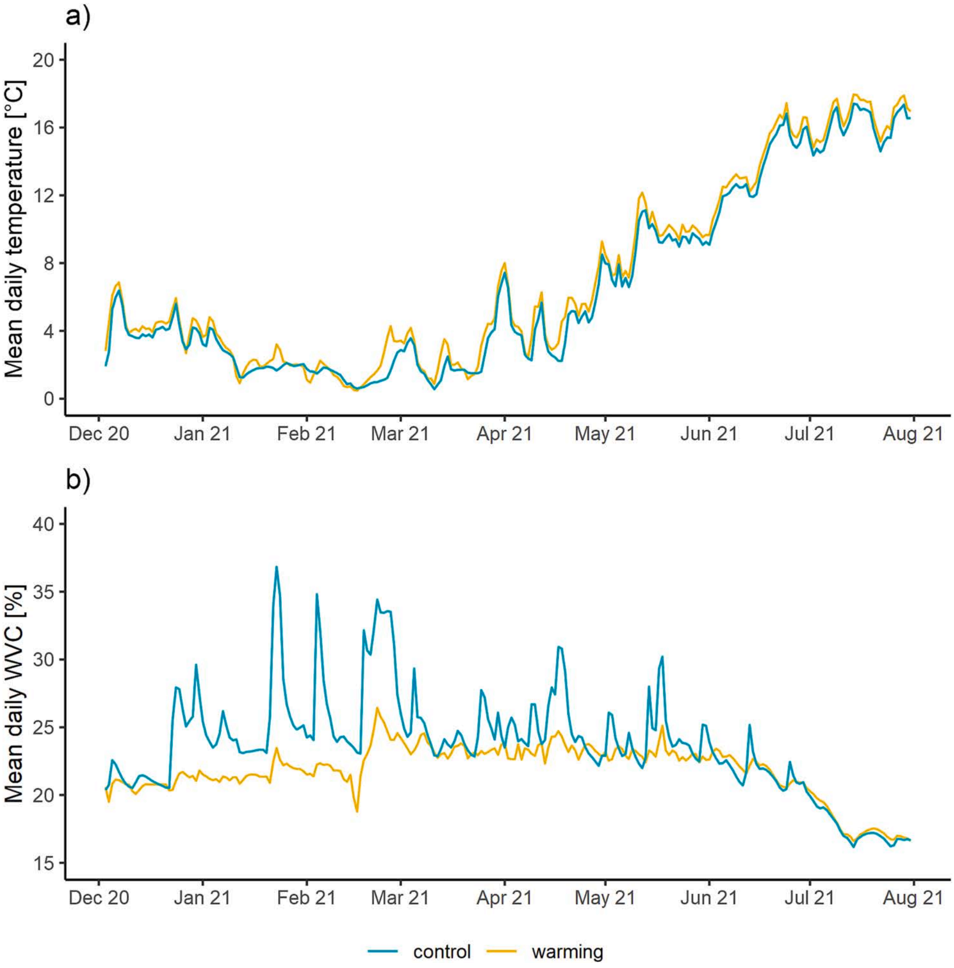

Fig. 2. Mean daily temperature (a) and mean daily WVC (b) in warmed plot and the control plot from December 2020 to July 2021.

decrease in H in the warmed plots was observed in the A level shortly after the onset of warming, which persisted for almost the entire duration of the experiment.

The influence of soil warming on soil CEC composition, as well as pH was more pronounced when expressed as the plot fraction of exchangeable BC, H, and Al as a function of pH. In the O horizon, the BC fraction of the CEC (BC CEC^{-1}, Fig. 7a) increased significantly ($r = 0.71$ for warming, $r = 0.86$ for control, $p < 0.001$) with increase in pH. The exchangeable H fraction of the CEC (H CEC^{-1}, Fig. 7e) decreased significantly ($r = -0.47$ for warming, $r = -0.58$ for control, $p < 0.001$) with increase in pH. The exchangeable Al fraction of the CEC (Al CEC^{-1}, Fig. 7c) was not significantly correlated with pH in the O horizon. Note that the slopes of these significant relationships (BC CEC^{-1}: warming 2.17, control 3.01, $p < 0.001$; and H CEC^{-1}: warming $-$ 1.89, control $-$

Table 3
Selected properties (mean and standard errors) of the initial soil series in the organic (O) and mineral topsoil (A) horizons of all plots.

Soil property	Unit	O		A	
		Warming	Control	Warming	Control
C	%	27.1 ± 8.3	31.2 ± 4.3	2.6 ± 1.2	3.4 ± 0.7
pH_{H2O}	–	3.55 ± 0.11	3.53 ± 0.07	3.66 ± 0.08	3.62 ± 0.15
Ca	$cmol_{(+)}$ kg^{-1}	3.7 ± 1.6	5.0 ± 1.6	0.1 ± 0.1	0.1 ± 0.0
K		0.7 ± 0.3	0.6 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
Mg		0.9 ± 0.4	0.9 ± 0.3	0.1 ± 0.0	0.1 ± 0.0
Na		0.1 ± 0.1	0.1 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
BC		5.4 ± 2.2	6.6 ± 1.9	0.2 ± 0.1	0.2 ± 0.1
Al		8.8 ± 1.6	8.9 ± 1.1	7.3 ± 2.5	8.4 ± 1.0
H		48.0 ± 13.5	58.1 ± 7.5	6.5 ± 2.7	8.5 ± 2.3
CEC		62.1 ± 15.0	73.5 ± 7.9	14.1 ± 3.8	17.1 ± 2.5

1.52, $p < 0.001$) were relatively small. In the A horizon, the BC content was very low, and the relationship between the BC fraction of the CEC and pH was not significant (Fig. 7b). However, the increase in pH in the A horizon was strongly positively correlated with increase in the Al fraction of the CEC (Fig. 7d; $r = 0.81$ for warming, $r = 0.51$ for control, $p < 0.001$) and decrease in the H fraction of the CEC (Fig. 7f; $r = -0.81$ for warming, $r = 0.47$ for control, $p < 0.001$). For the O horizon, data from the groups (warming and control) showed a similar range and pattern, whereas in the A horizon all samples with pH > 3.8 were collected from the warmed plots.

The data presented in Fig. 7 was used to apply the H-H equation in its canonical and modified versions. In the case of the O horizon, significant relationships were observed when the canonical version of the equation was applied (Fig. 8a). In the A horizon, the modified H-H equation, in which Al was included as a base cation, was capable of explaining the soil pH (Fig. 8b).

Data shown in Fig. 8 are for application of Equation (3), where the intercept and gradient are pK_{app} and n, respectively. Note that the gradients were relatively similar, whereas the intercept was consistently higher for data from plots subjected to simulated warming.

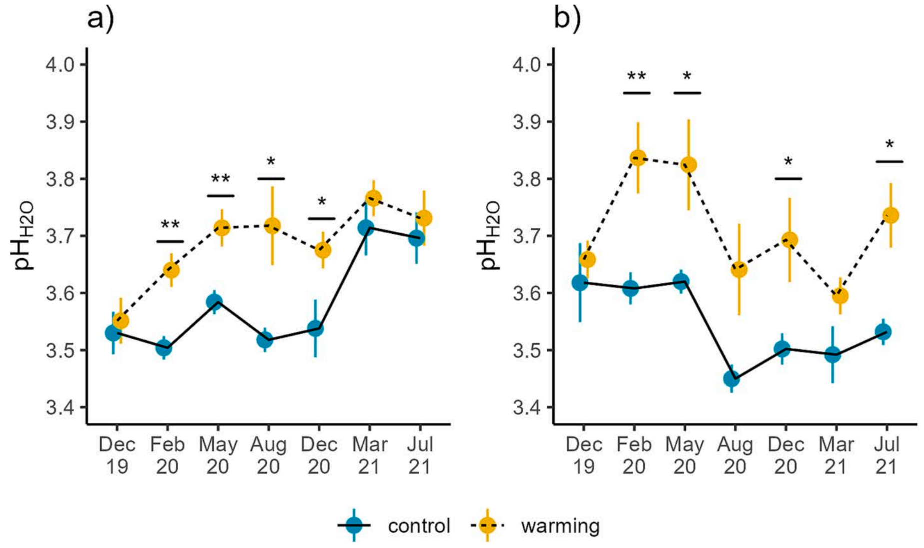

Fig. 3. Soil pH_{H2O} in the O (a) and A horizon (b) in warmed and control plots. * $p < 0.05$, ** $p < 0.01$ (Mann–Whitney U test).

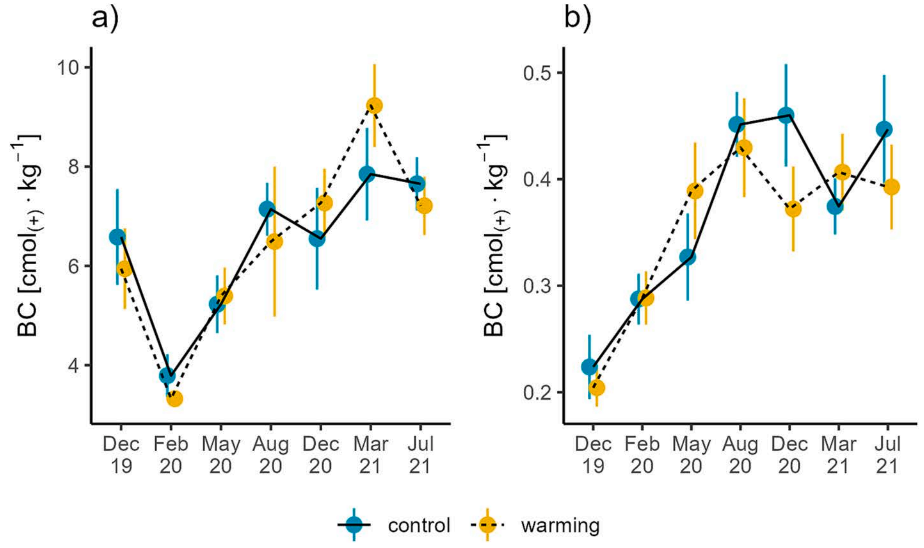

Fig. 4. Sum of base cations (BC) in soil from the O (a) and A horizon (b) in the warmed and control plots.

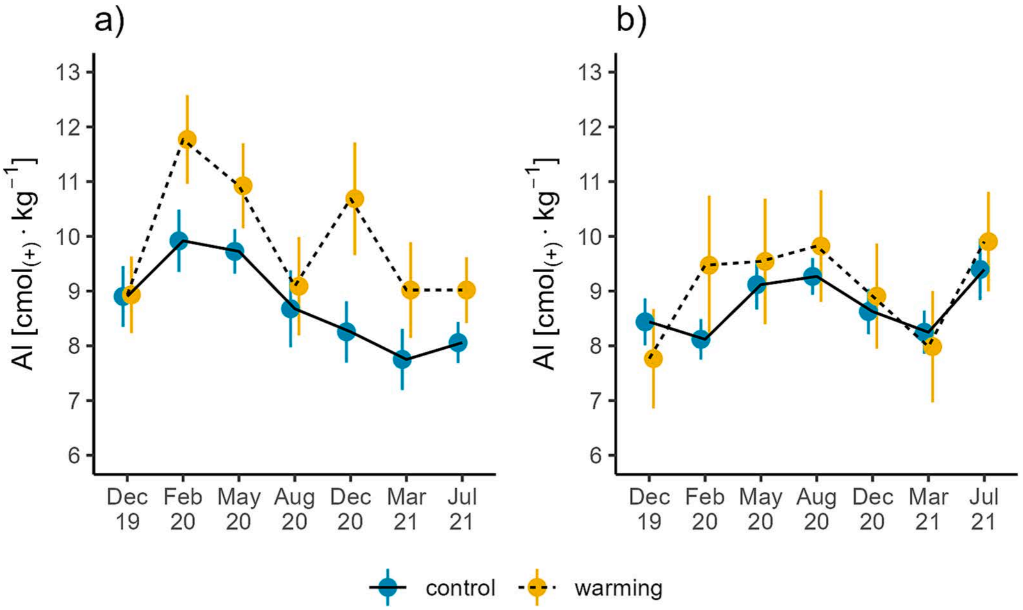

Fig. 5. Aluminum (Al) content in soil from the O horizon (a) and A horizon (b) in the warmed and control plots.

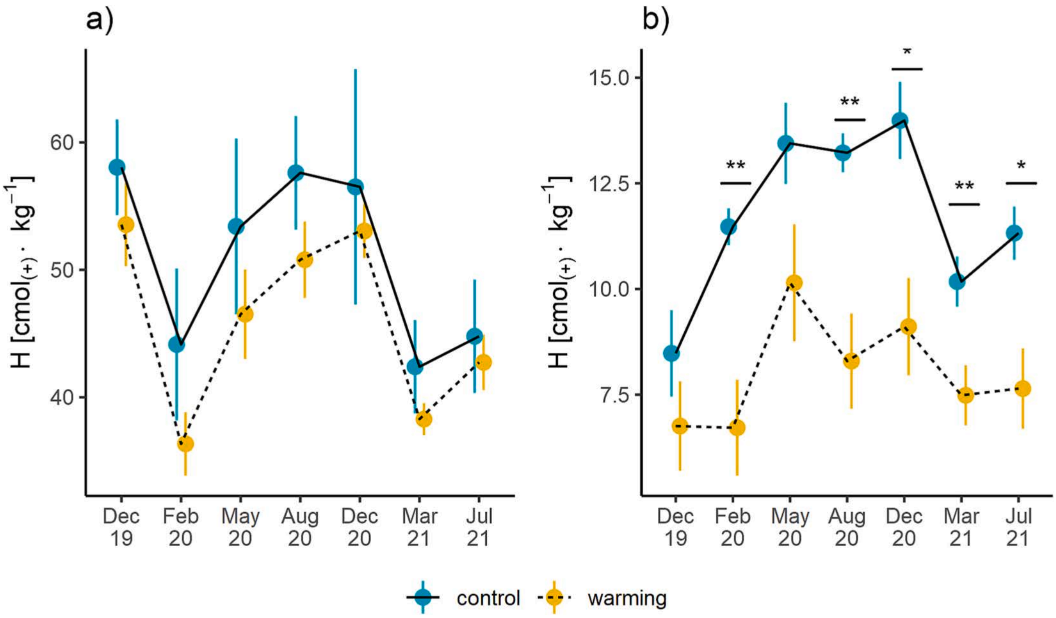

Fig. 6. Hydrogen (H) content in soil from the O horizon (a) and A horizon (b) in the warmed and control plots. * $p < 0.05$, ** $p < 0.01$ (Mann–Whitney *U* test).

4. Discussion

OTC soil warming method used in our research resulted in an average increase of 0.5 °C in average topsoil temperature during the measurement period (December 2020 - July 2021) (Table 2). The result is close to the predicted increase in topsoil temperature of temperate zone forest soils at the end of the current century, assuming an increase of about 0.3 °C (based on RCP8.5 scenario, (Soong et al., 2020)). However, it should be noted that the exact value of the increase in soil temperature is difficult to predict, especially in mountainous areas, where soils are characterized by different depths, elevations above sea level and slope exposures. The effect of natural warming on the soil can vary with respect to the warming technique used. OTC is commonly used in mountainous, inaccessible areas due to its low cost and easy setup. Although applicable to open spaces, it also gives significant increases in air and soil temperature in temperate zone forests (de Frenne et al., 2010). The used material (Plexiglass) has high solar transmittance at visible wavelengths and low transmittance in the infrared range (de Frenne et al., 2010; Li et al., 2018). In addition to the advantages, it should also be mentioned that OTC can simulate in a different way changes in soil moisture and affect the amount of litterfall than will occur in nature. This is due to the design of the chamber, the top of which has a smaller surface area than the base (Fig. 1D). The slope of the OTC walls is due to the need to limit heat transfer between the warmed soil and the environment. Also modified are the parameters of the light wave reaching the soil after passing through the wall of the chamber.

The present results confirmed our previous observation that warming of the soil in OTCs increases the topsoil pH in Norway spruce stands (Kupka et al., 2023). In general, the soil samples analyzed were strongly acidic, with a low content of BC and high contents of Al and H. Such low

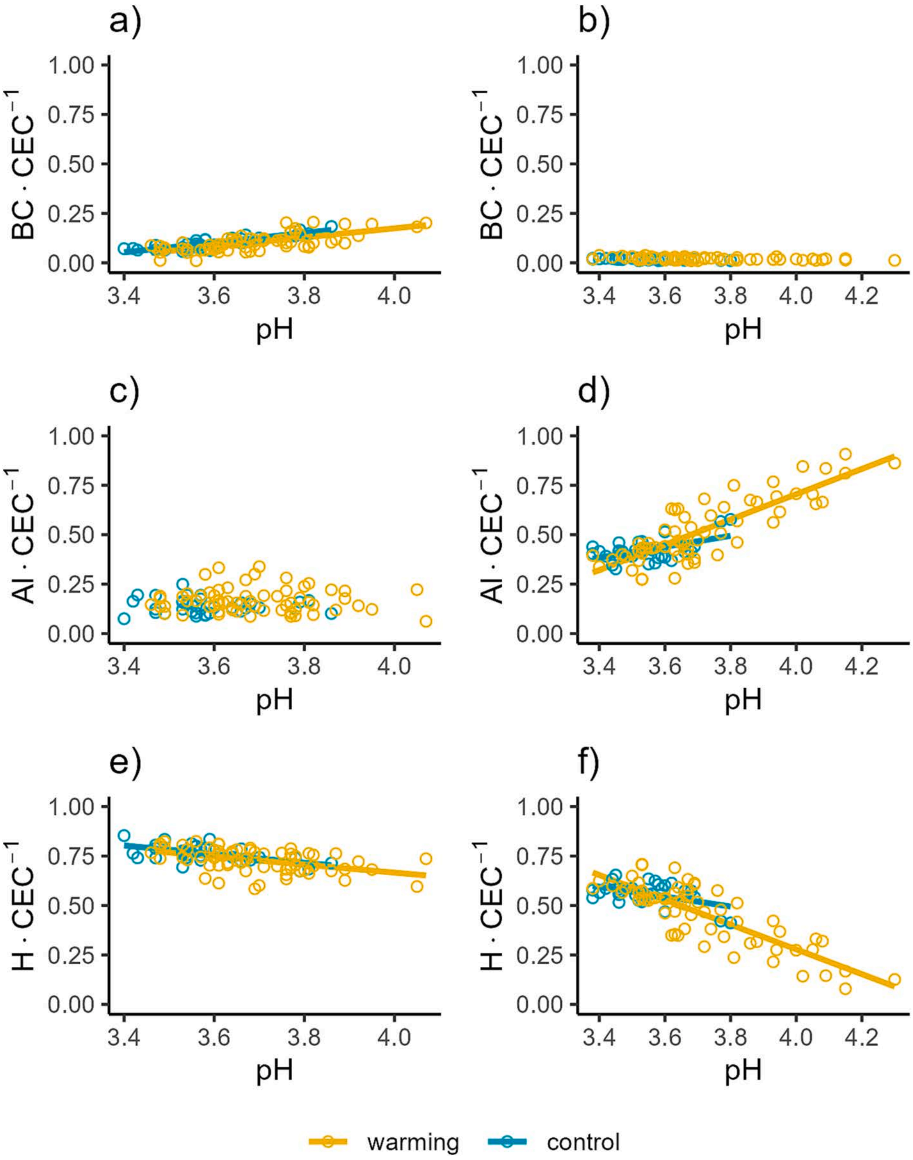

Fig. 7. Proportion of base cations (BC), aluminum (Al), and hydrogen (H) in the cation exchange capacity (CEC) in the O (a, c, e) and A horizon (b, d, f) in the warmed and control plots. Lines indicate a significant linear regression.

base saturation and pH are typical for soils under spruce stands, particularly those developed on carbonate-free parent materials (Johnson, 2002; Ross et al., 2008). High total CEC predominantly originates from deprotonated functional groups (mostly carboxylic) of SOM (McBride, 1994). Therefore, the CEC/C ratio expresses the ability of SOM to generate a negative charge and bind cations (Gruba and Mulder, 2015). According to McBride (1994), this potential can be roughly estimated as ~ 3 $\text{cmol}_{(+)}$ per 1% of soil C. The organic horizons usually have lower ability to generate exchange sites (Johnson, 2002), owing to the large contribution of chemically inactive C (e.g., fresh plant residues). In the A horizon we observed an average CEC/C ratio of 5.54, which suggests a significant contribution of the mineral clay fraction to CEC. Parameters of the linear relationship between CEC and C for the A horizon in the present investigation ($\text{CEC} = 2.5 * \text{C} + 8.3$) indicate the contribution of a permanent charge of ca. 8 $\text{cmol}_{(+)}\ \text{kg}^{-1}$ soil.

In acidic soils ($\text{pH} < \sim 4.5$) with low base ($\text{Ca} + \text{K} + \text{Mg} + \text{Na}$) saturation, Al has been recognized to be a base cation (Skyllberg et al., 2001), i.e. the cation competes with H for exchange sites and, therefore, regulates the soil pH in accordance with the modified H-H equation (Eq. (3)). Thus, we expect that the increase in pH is a result of increased saturation of CEC with Al followed by decrease in fraction of H.

In the present experiment we observed that, soon after the start of simulated warming (84 days), the warmed soil acidity had decreased compared with that of the control plots (Fig. 3). The difference attained a maximum 0.21 ΔpH in the O horizon and 0.29 ΔpH in the A horizon. Moreover, in the O horizon, in both the warmed soil and control plots, we observed a temporal trend for the pH to increase (Fig. 3a). This trend was not observed in the A horizon. Although slight (at least in the initial phase), but significant, such changes in pH can have a major impact on the balance of the entire ecosystem and the viability of organisms, being associated with nutrient supply (Kreuzwieser and Gessler, 2010). Increase in soil pH caused by warming is a poorly investigated phenomenon, particularly in a temperate coniferous forest ecosystem, where the mineral soil is protected from the temperature increase by the O horizon. The effect of global climate change on soil pH depends on direct climate components: CO_2 concentration (a higher CO_2 concentration increases acidity; Hubova et al., 2017), temperature (its increase stimulates C uptake and translocation of nutrients, thereby reducing pH; Keenan et al., 2014), and drought (which increases base cations owing to reduced uptake of plant nutrients; Duan et al., 2013; Slessarev et al.,

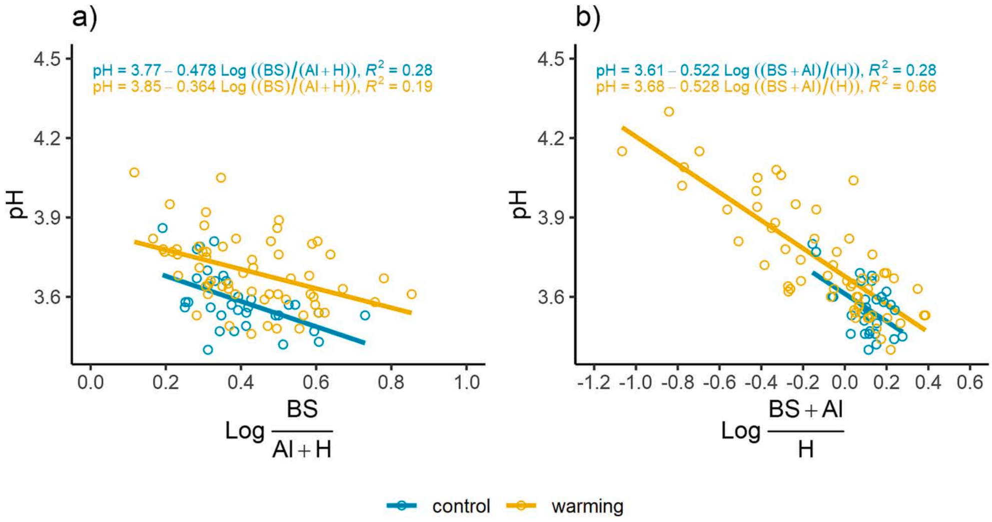

Fig. 8. Canonical version of the modified Henderson–Hasselbalch (H-H) equation for the O (a) and the A horizon (b). Lines indicate a significant regression.

2016). Hence, elucidation of the feedback response of soil to warming requires thorough research. We assume that the reason for the increase in pH under the influence of warming in the studied soil was an increase in N-NH_4 content, as described in a separate publication (Kupka et al., 2023) (Appendix A). Ammonification is a process during which H ions are consumed; during the subsequent nitrification process, H is produced, which compensates for the resulting H imbalance (Rengel, 2011). However, in the present study the warmed soil showed a significant increase in N-NH_4, but no increase in nitrate nitrogen (N-NO_3) was observed (Appendix A). Thus, we approach with care the assumption that the main reason for the increase in pH was a decrease in H^+ content caused by the imbalance between ammonification and nitrification (Kupka et al., 2023). However, it cannot be excluded that the described mechanisms represent only an initial response to warming. The intensity of nitrification may increase in the future, thus changing the availability of H and causing the pH to decrease. Moreover, the assumptions presented need to be thoroughly investigated, with a special focus on underlying processes connected with the activity of ammonifying and nitrifying bacteria.

Taking into account a direct comparison of the averages for given series, significant differences in average pH between the warmed and control soils were weakly linked to CEC composition, i.e., no significant decrease in the fraction of exchangeable H and increase in the fraction of Al was observed (Fig. 7). Based on many previous investigations (Gruba and Mulder, 2015; Johnson, 2002; Skyllberg et al., 2001), in the pH range below 4.5, the significant effect of base cations was not expected, mostly because of the very low BC content. However, direct comparison of averages shown in Fig. 6 confirmed the significant decrease of H content in the A horizon and, therefore, the effect of H bound to the soil solid phase on soil pH. Further insight into the effect of warming on the increase in soil pH was obtained by exploring the relationships between pH and the fractions of H, Al, and BC in the CEC (Fig. 7), and from application of the H-H equation (Fig. 8). The use of fraction of CEC as an input to the H-H equation to model the pH is a common concept (Bloom and Grigal, 1985; Ciarkowska and Miechówka, 2019; Ma and Xiao, 2023). Data presented in Fig. 7 imply that, in the O horizon, the increase in pH is associated with the balance between BC and H, whilst the Al fraction is insignificant. Thus, for the O horizon, the H-H equation was applied in its canonical form, in which only BC are included as base cations (Fig. 8). A similar approach for the O horizon was suggested by Johnson (2002). In contrast, for the A horizon, the modified H-H equation was found to be applicable, in which the Al fraction is included as base cations. Parameters of a linear relationship expressed by application of the H-H equation, as shown in Fig. 8, allow estimation of pK_{app} (the intercept) and the stoichiometry constant n (the gradient). Hence, the analysis suggested that, in the O horizon, warmed SOM had a larger pK_{app} (i.e., weaker organic acids; Gruba and Mulder, 2015) than that of SOM from the control plots, i.e., soil samples from warmed plots could have a higher pH than control soils with similar H saturation. This may be due to increased humification of SOM and transformation of fulvic acids to weaker humic acids (Machado et al., 2020). Moreover, in the A horizon, the samples with a higher pH contained more Al (Fig. 7d). Increased saturation with Al may be due to increased upward movement of this cation from the mineral soil phase driven by greater mineral weathering and upward movement of water. Such relationships were previously reported by Lawrence et al. (1995). It can also not be excluded that Al content is affected by the activity of soil microorganisms decomposing SOM, stimulated by warming. Aspects of SOM decomposition under soil warming are the subject of a separate publication (Kupka et al., 2023). The differences between soil horizons in the mechanisms of pH regulation under the influence of external modifying factors are also due to the physicochemical and biological differences between the O horizon (consisting mainly of organic matter) and the A horizon (consisting mainly of mineral soil).

Soil pH controls a majority of soil processes; thus, its increase may cause modification of soil properties and processes (McBride, 1994; Rengel, 2011; Zhou et al., 2019). Increase in pH decreases the activity of soluble Al and potentially decreases its toxicity to plant roots (Lofts et al., 2001; Van Schöll et al., 2004). At a higher pH, Al tends to be adsorbed to exchange sites of SOM rather than remain as active Al^{3+} in the soil solution (Ciarkowska and Miechówka, 2019; Jansen et al., 2004). Moreover, a higher pH may lead to increased availability of soil nutrients, particularly phosphorus (Azene et al., 2022; Rengel, 2011). In addition, the soil pH and metal ions are important for stabilization and destabilization of SOM (Kupka et al., 2021b, 2021a; Mulder et al., 2001). According to Kupka and Gruba (2022) and Gruba and Socha (2019), increase in the pH of acid soil leads to destabilization of SOM and increased release of C as dissolved organic C. Thus, we conclude that the changes we observed are the initial symptoms of changing habitat conditions of Norway spruce stands in montane temperate areas. However, it should be noted that other factors besides warming may also contribute to increased mortality and decay of spruce stands. Factors such as intensive droughts, extreme winds, increased atmospheric N deposition, pest species and industrial emissions have a significant impact on the deterioration of stands vitality (Jandl, 2020; Socha et al., 2023).

Noteworthy is the increasing trend of BC concentration in the soil of both O and A horizon, either in the warmed or control plots (Fig. 4). We assume that this phenomenon is correlated with climate change, as the precipitation decrease and temperature increase can control indirectly the variation and dynamics of BC concentration in the soil, particularly in SOM (Xue et al., 2019). According to previous observations, the concentration of BC in the soil is lower when the soil is wetter (Erickson et al., 2014). This corresponds with the climate data for the study plot, where a year-on-year decrease in precipitation was observed. In February 2020, the monthly precipitation was 164.9 mm, while in February 2021 it was 78.9 mm. Also, the number of days with snowfall was lower in February 2021 compared to February 2020 (11 days vs. 14, respectively; Institute of Meteorology and Water Management (2023)). We assume that changed climatic condition increase in BC concentration through acceleration the SOM decomposition, which is leading to a decrease in the strength of organic acids.

5. Conclusions

After 578 days, as a result of experimental warming of the soil by 0.5 °C, the pH of the O and A horizons increased, on average, by 0.21 and 0.29 pH units, respectively. The present data imply that subjecting the organic matter of the O horizon to experimental warming leads to change in the quality of SOM. Based on the Henderson–Hasselbalch equation, the warmed soil has an increased pK_{app}, i.e., it has weaker organic acids, less able to dissociate and therefore to release H^+ ions. The pH of the O horizon is attributed to the balance between BC and H. In the A horizon, the soil pH is explained by Al–H competition, i.e., increase in the pH is the effect of an increased fraction of Al in the CEC. This is probably the result of accelerated transport of Al from deeper soil layers induced by warming. The present results contribute to an improved understanding of changes in forest habitat conditions, which is crucial for future forest management planning.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. . Mean (with standard deviation) values of ammonium nitrogen (NH_4-N) and nitrate nitrogen (NO_3-N) from organic (O) horizon and mineral (A) topsoil from the study site (Kupka et al., 2023), after 476 days of warming

Property [mg kg^{-1}]	O horizon			A horizon		
	warming	control	p-value	warming	control	p-value
NH_4-N	246.8 ± 76.1	43.2 ± 9.2	**0.003**	18.1 ± 3.4	14.7 ± 3.2	0.076
NO_3-N	4.8 ± 2.4	3.1 ± 1.9	0.426	1.5 ± 0.5	1.3 ± 0.5	0.358

p-value according to Mann-Whitney test.

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Effect of warming on ground vegetation in Carpathian Norway spruce stands, exemplified by European blueberry (*Vaccinium myrtillus* L.) nutrient stoichiometry

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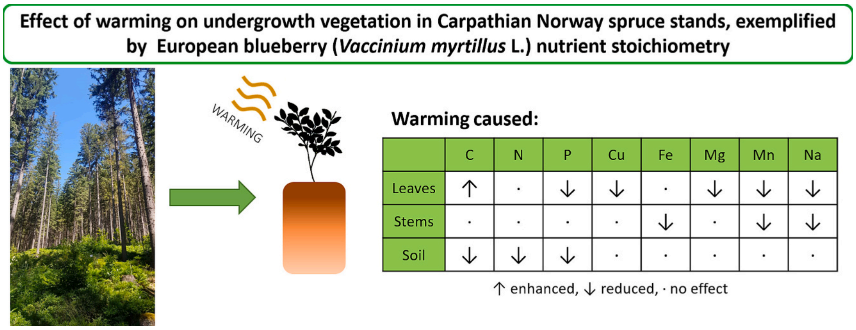
HIGHLIGHTS

- A two-season lasting warming experiment was performed in Norway spruce stand.
- Warming significantly decreased C, N and P content in mineral topsoil.
- Warmed European blueberry leaves had higher C and lower P content.
- Warming reduced Cu, Fe, Mg, Mn and Na content in aboveground parts of blueberries.
- N may be a limiting element for biomass production of forests threatened by warming.

GRAPHICAL ABSTRACT



Effect of warming on undergrowth vegetation in Carpathian Norway spruce stands, exemplified by European blueberry (*Vaccinium myrtillus* L.) nutrient stoichiometry

Warming caused:

	C	N	P	Cu	Fe	Mg	Mn	Na
Leaves	↑	·	↓	↓	·	↓	↓	↓
Stems	·	·	·	·	↓	·	↓	↓
Soil	↓	↓	↓	·	·	·	·	·

↑ enhanced, ↓ reduced, · no effect

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ABSTRACT

Despite its small share of total forest biomass, ground vegetation plays an important role in biogeochemical cycles, being able to modify carbon (C) and nutrients fluxes. Global climate warming may affect plant nutrient uptake and the carbon:nitrogen:phosphorus (C:N:P) stoichiometry, the release of nutrients from the soil and soil organic matter, as well as significantly influence the tree stand nutrient supply. In this context, the response of Norway spruce (*Picea abies* (L.) H.Karst) stands' ground vegetation to warming is uncertain. An open-top chamber soil-warming simulation, lasting two growing seasons, was conducted in a spruce forest. At the end of each of the two growing seasons, before leaf senescence, European blueberry (*Vaccinium myrtillus* L.) aboveground biomass (leaves and stems) and mineral topsoil samples were collected from the plots. The C, N, P, micronutrient, and macronutrient concentrations were estimated in the samples. Warming caused significant decreases in C, N, and P in the soil. Warming also decreased the C:P and N:P stoichiometric ratios in the soil and increased the C:P ratio in plant stems. Significant increase in foliar C and decrease in foliar P in warmed plots were observed. The most evident effect was reduction of N and P in the soil, which directly affected the plant C:P and soil N:P stoichiometry. Our results show that warming has caused a significant decrease in the content of some nutrients in the

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aboveground plant tissues of blueberries. Given that N is a limiting factor of ecosystems productivity, its reduction in the soil caused by warming may be a serious threat to proper nutrient uptake and cause disruption of biogeochemical cycles. The decrease in nutrient content in aboveground tissues due to warming can result in disruptions to physiological processes.

1. Introduction

The average global temperature is predicted to rise by 2.1–3.5 °C at the end of the current century (Gulev et al., 2021). In an era of global climate warming, relations between soil and plants may be modified (Koller and Phoenix, 2017). Adaptation of plants to modified growth conditions is associated with species-specific phenological, metabolic, and reproductive strategies (Dreesen et al., 2012; Moulton and Gough, 2011; Schuster and Monson, 1990). Predicted changes will affect the soil environment of Norway spruce (*Picea abies* (L.) H.Karst) forests in temperate zone, leading to the modification of its properties, such as soil nutrition. Soil nutrition is considered to be among the most important factors influencing plant productivity (Elbasiouny et al., 2022; Gong and Gao, 2019), especially in forests. Among forest stands, an important stratum is the ground vegetation, which plays important roles in forest ecosystems, participating in energy flow and the circulation of matter (Landuyt et al., 2019). The ground vegetation is mostly described as a forest stratum consisting of vascular plants, with a height of no >1 m (Gilliam, 2007). Despite the fact that the aboveground biomass of this stratum accounts for only about 1 % of the total forest, the ground vegetation has a significant impact in the cycling of crucial plant nutrients and energy flow (Landuyt et al., 2019; Muller, 2003). On average, the ground vegetation in temperate forests comprises over 80 % of the vascular plant diversity (Gilliam, 2007; Landuyt et al., 2019). It is also responsible for soil organic matter (SOM) input to the forest floor, both from litterfall and decomposition of well-developed root systems. An additional important function of this stratum is to increase retention properties, and counteract runoff and soil erosion (Djatmiko et al., 2021). The relationship between nutrient uptake from the forest floor and the chemical composition of litterfall is a significant factor altering soil surface properties. Among the most widespread ground plants in montane Norway spruce forests is European blueberry (*Vaccinium myrtillus* L.). Blueberry is a dwarf shrub used as a bioindicator in environmental studies (Coudun and Gégout, 2007; Kasiulienė et al., 2019; Mikkonen and Huttunen, 1981). It is a rhizomatous perennial, regenerating mostly by clonal growth and relatively rarely by seeds (Parlane et al., 2006; Ritchie, 1956). This shade-tolerant species grows on acidic soils, such as shallow, poorly developed montane soils (Ritchie, 1956). Given that the intensity of metabolism and reproductive performance of European blueberry are strongly associated with the temperature of the environment (Ritchie, 1954), climate warming may significantly alter the life strategies of these plants. Carbon (C), nitrogen (N), and phosphorus (P) are crucial macroelements for plant physiology and life functions (Wang et al., 2017). Moreover, maintenance of a stoichiometric balance of C:N:P by plants is reported to be the key element of proper functioning (Cleveland and Liptzin, 2007; Wang et al., 2017). An insight into the relationship between C, N, and P can provide information on biogeochemical cycles, net primary production, C sequestration potential, or SOM quality (Cleveland and Liptzin, 2007; Piaszczyk et al., 2019; Zechmeister-Boltenstern et al., 2015). It is also essential in understanding mechanisms of growing strategies under conditions of limited nutrients (Zechmeister-Boltenstern et al., 2015). The study of stoichiometric relationships has been applied in ecological research to a number of terrestrial ecosystems, soil, and soil microbial biomass (Cleveland and Liptzin, 2007; Du et al., 2019; Piaszczyk et al., 2019; Wang et al., 2017). In recent years, ecological stoichiometry in soil and plants has become a focus of interest in the face of climate change (Du et al., 2019; Zechmeister-Boltenstern et al., 2015). Global climate change can significantly modify the biogeochemical cycles of C, N and P, which can have an influence on the parameters of SOM, net primary production and the soil microbiota (Maaroufi and De Long, 2020). The feedback mechanisms between this ecological stoichiometry under changing environmental conditions deserves scientific attention. Especially, given that these stoichiometric relationships significantly affect geochemical nutrient cycling and influence the nutrient supply to forest trees (Zechmeister-Boltenstern et al., 2015).

Norway spruce (*Picea abies* (L.) H.Karst.) is among the most important forest-forming species in montane areas of Central Europe (Altman et al., 2017). Its large share is the result of intensive afforestation, aimed at combating wood shortage, soil erosion, and forest exploitation (Spiecker, 2003). Given the resulting inadequacy of site conditions to meet growth requirements, Norway spruce forests have become susceptible to the negative impact of external factors, such as atmospheric acidification, N deposition, and deterioration of air quality (Kopáček et al., 2016), resulting in the dieback of these forests since the 1970s (Sensuła et al., 2015). In addition, climate change is having a significant impact on site conditions of Norway spruce stands (Altman et al., 2017; Socha, 2015; Socha and Durło, 2012; Tumajer et al., 2017). Although several studies have assessed the impact of climate warming on the tree growth of Norway spruce stands in the temperate zone (Altman et al., 2017; Cienciala et al., 2016; Durło, 2012; Małek et al., 2012), to the best of our knowledge there has been no attempt to evaluate the impact on the ground vegetation of these stands.

The aim of the present study was to evaluate the effect of climate warming on the chemistry of ground vegetation in montane Norway spruce forests in the Western Carpathians. The choice of ground vegetation to study the response of the forest ecosystem to warming is motivated by several reasons. First, this stratum is an important component of the ecosystem, in addition to the tree layer, with a measurable impact on biogeochemical cycles despite its small size (Muller, 2003; Neufeld and Young, 2003; Welch et al., 2007). Second, warming-induced changes in the chemistry of the ground vegetation seem to be more rapidly responsive compared with adult trees. According to Muller (2003), litter from the ground vegetation decomposes more than twice as rapidly as tree litter. The authors focus on nutrient concentrations given that they are considered direct predictors of nutrient cycling (Landuyt et al., 2019). Findings presented in this paper are a part of research on the warming effects on SOM and soil properties, suggesting that warming significantly affects the chemical structure of SOM and modifies ion-exchange relationships, mostly by enhancing the pH and mobilizing N (Kupka et al., 2023). In the present research, three main questions were investigated: (1) how will warming affect the C, N, and P contents and the C:N:P stoichiometry of European blueberry in Norway spruce stands of the Western Carpathians? (2) How will warming affect their micro- and macronutrient contents? (3) Will warming significantly affect soil–plant relationships?

2. Materials and methods

2.1. Study site

The research site, covering an area of approximately 1 ha, was established in southern Poland, in the Silesian Beskid Region (SBR), on the altitude 520–550 m above sea level, on the slope facing southeast (20°), located in the Western Carpathians (49°33′47.7″N, 18°52′06.5″E) (Fig. 1). The parent material of the study site was characterized as Carpathian Flysch, formed from layers of shales, sandstones, Tertiary rocks and conglomerates. According to the World Reference Base for Soil

Resources, the soil in the study site was described as Skeletic Dystric Cambisol (Loamic) (IUSS Working Group WRB, 2022). The SBR area is characterized by a montane climate, with considerable variability in the weather throughout the year. The average annual air temperature is 6.5 °C, total annual precipitation is 1089 mm, and the number with precipitation during the year is 185. The coldest month is January (−3.7 °C), while the warmest one is July (14.9 °C) (according to Aleksandrowicz, 1991; Gruba and Mulder, 2008; Januszek et al., 2015).

The study site was established in a single-storey Norway spruce (*Picea abies* (L.) H. Karst) stand of age 80 years. The study site was a fragment of a vast forest complex covering the mountainous areas of the SBR. The average height of the trees was 32 m, and the average diameter at the breast height (DBH) was 34 cm. The tree stand closure was moderate (90 % of closure, with narrow open spaces between the crowns of trees) and uniform over the entire study site. The growing stock of the stand was 260 m^3 ha^{-1} (Polish Forest Data Bank, 2023). The stand is managed by the Wisła Forest Inspectorate. The groundcover of the forest consisted mainly of European blueberry with limited admixture of mosses, specifically red-stemmed feathermoss (*Pleurozium schreberi* (Brid.) Mitt.) and leucobryum moss (*Leucobryum glaucum* (Hedw.) Ångstr.).

2.2. Warming simulation

For the simulation of climate warming conditions, the open-top chamber (OTC) warming method was applied. This is a passive simulation method, which uses hexagonal greenhouses, made from translucent plexiglass. Such a method has been widely used (for >30 years), mostly in remote, mountainous areas (Aronson and McNulty, 2009; Kupka et al., 2023). Within an area of 1 ha, 15 plots were randomly established on the study site in early December 2019. The area of each plot was 2.6 m^2. The minimum distance between plots was 15 m. For the warming simulation, hexagonal OTCs were placed on 10 plots, whereas the remaining five non-covered plots constituted the control group. The OTCs were made from 3-mm-thick translucent Plexiglas®, covering a floor area of 2.6 m^2 with an open-top area of 1.1 m^2. The height of the OTCs was 80 cm. At least 15 European blueberry bushes grew in each of the fifteen plots. Blueberries growing in the plots subjected to warming were wrapped in OTCs. The effectiveness of the simulated warming was assessed by monitoring the soil temperature and water volume content (WVC). Two sensors (5TM Sensor, Decagon) were placed at 5 cm depth (at the transition between the O and A soil horizons), one under the OTC and one in the control plot. Before measurement in the field, the sensors were tested in the laboratory to ensure consistency in the provided results. The accuracy of temperature measurement was ±0.1 °C and that of WVC was ±2 %. The measurement series was performed from the beginning of December 2020 to the end of September 2021.

2.3. Sampling

Sampling was performed in two series, in each season close to the end of the vegetation period – at the beginning of September 2020 (after 10 months of simulated warming) and at the beginning of September 2021 (after 22 months of simulated warming). The sampling covered collecting the soil and aboveground parts of European blueberry. The detailed sampling procedures are described below.

2.3.1. Soil

In total, 30 samples were collected in the two series. Samples in both sampling series were collected from all 15 plots using a cylindrical auger with a diameter of 5 cm. The soil was collected from the mineral topsoil of 0–10 cm depth (comprising the A horizon). For each plot, one composite sample was formed after mixing five replicates.

2.3.2. European blueberry

The plants of European blueberry were cut directly above the ground. At least five specimens were collected from each plot. Sampling was done at the end of the growing season before leaf senescence. The samples were collected when the leaves were still green and showed no signs of discoloration or fall. Given the extensive root system of European blueberry and the need to keep the soil structure intact, the root systems were not sampled.

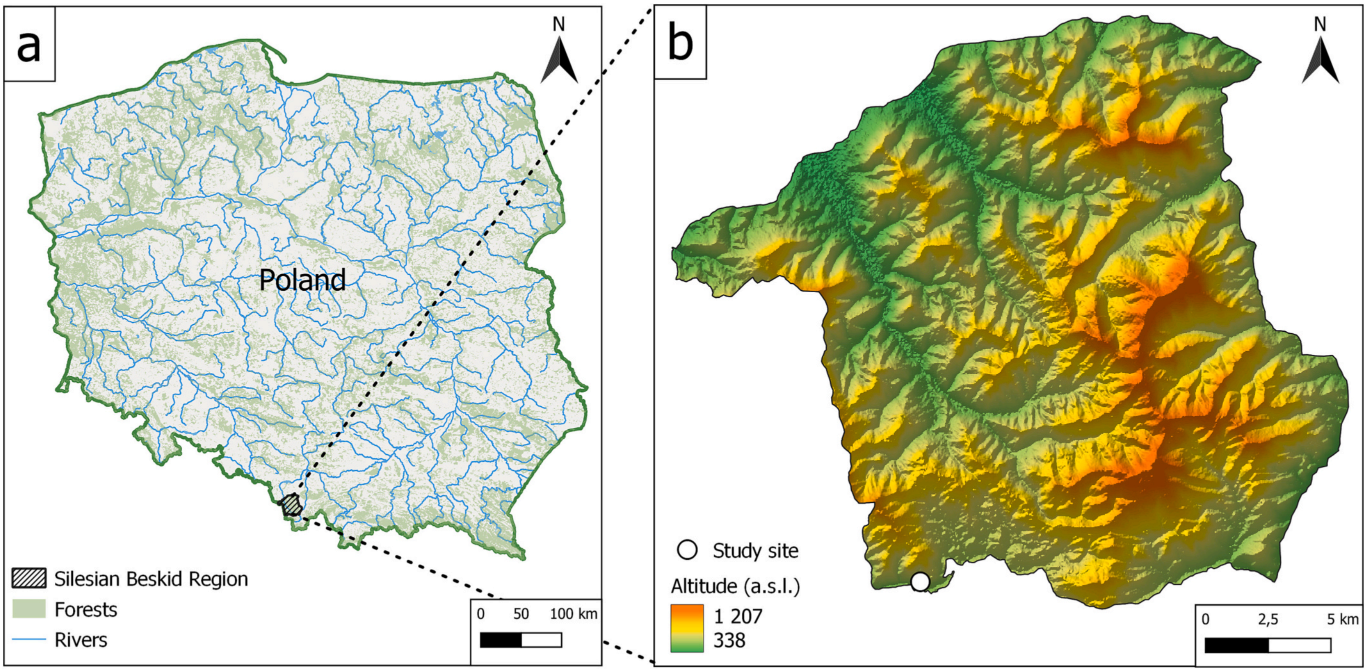

Fig. 1. a) Map of Poland indicating the Silesian Beskid Region (SBR). b) Location of the study site in the SBR. Maps were generated using QGIS version 3.26.3 (QGIS Association, 2023).

2.4. Laboratory analysis

2.4.1. Soil

Prior to the analysis, living root systems and large stones were removed from the samples. Soil samples were dried at 60 °C for 3 days and then sieved through a 2 mm plastic mesh. In such prepared samples the particle size distribution was examined using a laser diffractometer (Fritsch Analysette 22, Idar-Oberstein, Germany). The limit of quantification for each fraction was 0.1 %. Investigated soil samples were characterized by homogeneity of particle size distribution (warming plots: 36 % sand, 55 % silt, 9 % clay; control plots: 34 % sand, 57 % silt, 8 % clay).

For estimation of the chemical properties, soil samples were ball-milled for homogeneity. Carbon (C) and nitrogen (N) concentrations were measured using a LECO CNS TruMac analyzer (LECO, St. Joseph, MI, USA). Given that the soil was carbonate-free, it was assumed that total C equaled organic C. The limit of quantitation was 0.008 % for C and 0.266 % for N.

Total concentrations of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphorus (P), and zinc (Zn) were determined by digestion in a mixture (4:1, v/v) of nitric acid (HNO_3^-), d = 1.4, and 60 % perchloric acid ($HClO_4$) in accordance with procedure described by Ostrowska et al. (1991). The elements were quantified in two replicates using an inductively coupled plasma–optical emission spectrometer (Thermo iCAP 6500 Duo, Thermo Fisher Scientific, Cambridge, UK).

Quality control of the measurements was ensured by analysis of Organic Analytical Standards (Elemental Microanalysis Ltd.). Both standards and samples were analyzed with two technical replicates, with an acceptable difference between the measurements of <10 %. Standards were analyzed at the beginning and the end of each measurement run.

2.4.2. European blueberry

After collection from the research site, the plant materials were transported to the laboratory and stored in plastic bags in 4 °C until multi-elemental analysis. Subsequently, plants were washed in deionized water for about 1 min to remove any deposits on the surface. After washing, plants were dried for 48 h at 60 °C, separated into leaves and stems, and then milled to a fine powder using IKA MF10 basic microfine grinder with 2.0 mm mesh (IKA Werke, Staufen im Breisgau, Germany). Due to the small amount of test material from individual specimens, the samples from each plot were homogenized (divided into leaves and stems).

The following elements were quantified in the plant material: total forms of C, N, P, and the metals Ca, Cu, Fe, K, Mg, Mn, Na, and Zn. All analyses of chemical parameters of European blueberry were performed in the same manner as described for soil analysis in Section 2.4.1.

2.5. Statistical analysis

For the soil and plant chemical properties, means and standard deviations were calculated. The normality of distributions was examined using the Shapiro-Wilk test. To check the differences between the groups (warming vs. control) in each season, parametric comparisons between groups were performed using the t-test. The t-test was also used to determine the differences in temperature and WVC of the groups. An analysis of covariance (ANCOVA), in which time was considered as a covariate, was conducted to exclude the time aspect on changes in elemental content under the influence of warming.

The significance was defined at $p < 0.05$ unless otherwise specified. Principal component analysis (PCA) and calculation of Pearson correlation coefficients were performed to investigate relationships between warming and elements.

All statistical analyses were performed using R statistical software with the packages ‘ggplot2’ and ‘factoextra’ (Kassambara and Mundt, 2020; R Core Team, 2023; Wickham, 2016).

3. Results

3.1. Soil temperature and water volume content

The effectiveness of the method used to simulate warming and decrease in soil moisture at the current research site was described in a separate publication (Kupka et al., 2023). During the monitoring period (December 2020–August 2021), the average soil temperature under the OTC was 8.63 ± 5.84 °C and that in the control group was 8.12 ± 5.71 °C ($p < 0.0001$, t-test). Water volume content in the warmed plots was 21.36 % ± 2.09 % and that in the control group was 23.02 % ± 3.88 % ($p < 0.001$, t-test).

3.2. C:N:P stoichiometry

The C concentration in leaves of warmed plants after the first season of warming was significantly higher (4 %) compared with those of the control, while P concentration in warmed leaves was significantly lower (14 %) (Fig. 2a and c, respectively). Analysis of N concentrations in leaves showed no significant differences between groups (although the means after first warming season were lower in the warmed plants, see Fig. 2b). The C:N:P stoichiometry in leaves did not differ significantly between groups; however, a pattern of higher means was observed in the warmed group (Fig. 2d, e, f). ANCOVA showed a significant effect of warming on leaf C content (F = 12.79, $p < 0.01$).

Stems of warmed plants were characterized with a significantly higher C:P ratio in the warmed stems, 14 % and 11 % after both the first and second season of warming, respectively (Fig. 3e). No other significant differences were found between the study groups for the content of other parameters tested. ANCOVA revealed that the warming did not have a significant impact on the studied elements and their stoichiometry in the stems.

The soil (mineral level A) subjected to warming contained significantly lower concentrations of C and N in both measurement series (C – 15 % and 27 %; N – 21 % and 27 % after the first and second warming season, respectively), as well as of P (15 %) after the second season of warming (Fig. 4a, b, c, respectively). Significantly lower C:P and N:P ratios were observed in the warmed soil after the second (13 %) and both seasons of warming (15 % and 11 %), respectively (Fig. 4e, f). ANCOVA showed a significant effect of warming on soil N (F = 60.83, $p < 0.0001$), C (F = 25.12, $p < 0.0001$), C:N ratio (F = 114.77, $p < 0.0001$), N:P ratio (F = 84.22, $p < 0.0001$) and C:P ratio (F = 16.21, $p < 0.001$).

3.3. Macro- and micronutrients

Analysis of the metal concentration in European blueberry leaves proved that there was significantly less Cu (11 % after two seasons), Mg (17 % after two seasons), Mn (45 % after the first season), and Na (15 % after the first season) in the group subjected to warming in at least one measurement series (Fig. 5), compared to the control group. ANCOVA showed a significant impact of warming on the content of leaf Mn (F = 13.70, $p < 0.01$) and Na (F = 8.23, $p < 0.01$). A similar pattern was observed in the stems of warmed plants, which had significantly lower contents of Fe (17 % after the first season), Mn (37 % after the first season), and Na (24 % after the first season and 32 % after the second season) (Fig. 6). The results of ANCOVA indicate a significant impact of warming on the concentration of stem Mn (F = 4.81, $p < 0.05$). An analysis of these elements in the soil showed no significantly different concentrations between groups (data not shown).

3.4. Association between C, N, P, and metals

An analysis of correlations between C, N, and P concentrations, C:N:P stoichiometry, and investigated metals was performed for the analyzed

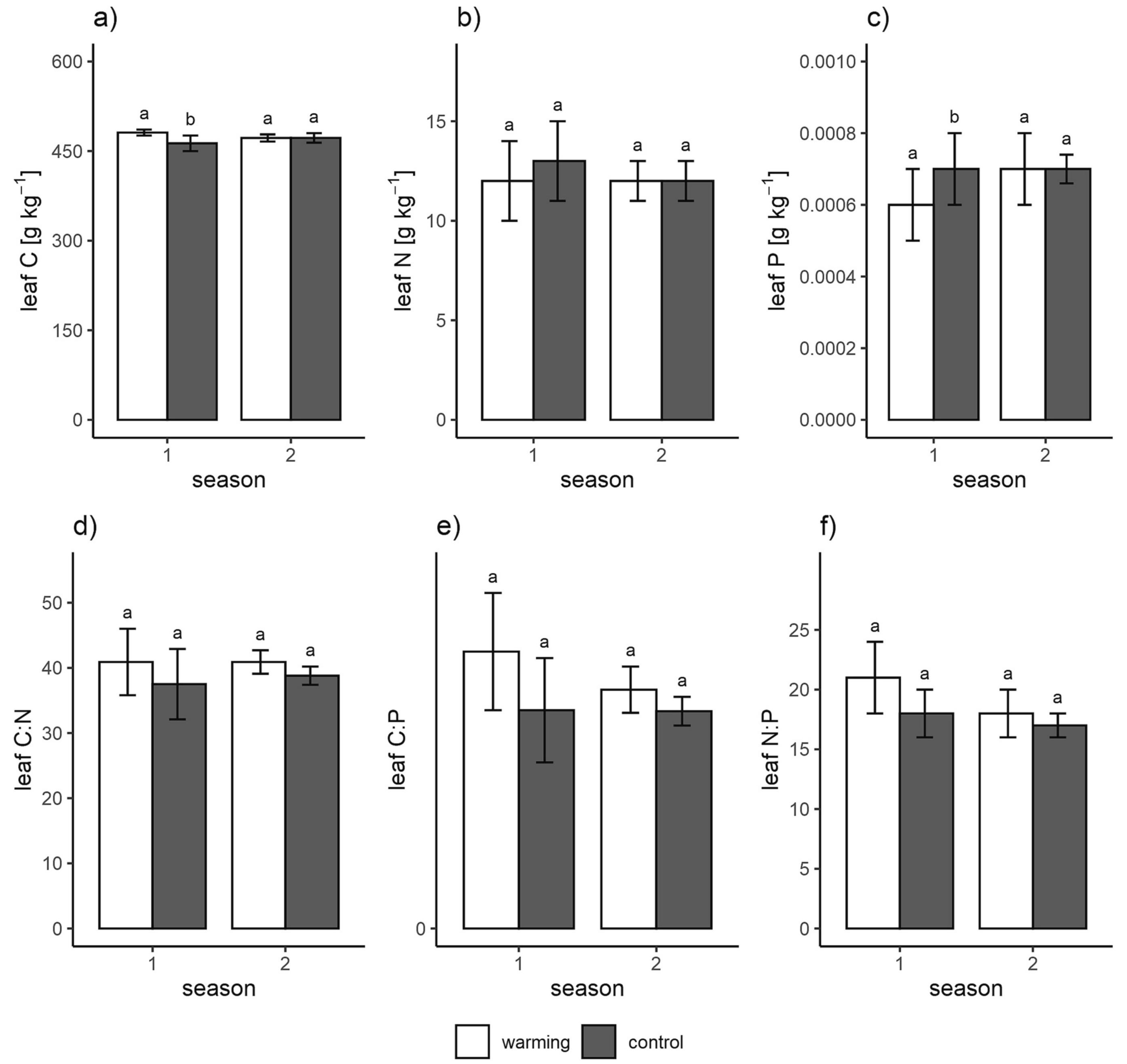

Fig. 2. Mean (with standard deviation) content of C, N, P, stoichiometric ratios C:N, C:P and N:P in leaves, after first and second warming season. Different letters indicate significant differences, according to t-test ($p < 0.05$). Season 1 – September 2020, after 10 months of simulated warming; season 2 – September 2021, after 22 months of simulated warming.

groups and altogether for both sampling series (Table 1). A general relationship can be drawn that C, N, and P (and the stoichiometric ratios) were significantly correlated with metals to a greater extent in the warming groups. This relationship was most apparent in the case of leaves. The most significant correlations were observed in warmed stems. The nature of the correlations (positive vs. negative) was characterized by high heterogeneity and depended on the metal and the research group.

A principal component analysis (PCA) was performed to examine relationships between the warming and investigated chemical properties, separately for plants and soil. Factors 1 and 2 used in the PCA for plants (Fig. 7a, b) collectively explained 59.7 % of the total variance (33.3 % and 26.4 %, respectively). The first principal component (Fig. 7a) clearly separated the leaf and stem samples. On the second principal component, samples from stems formed two separate groups distinguished by the year of sampling, with those collected after 1 year of warming in the lower left quadrant and those sampled after 2 years of warming in the upper left quadrant of the scatterplot. It is worth noting that in each of the three separate groups of samples (leaves, stems after 1 year, and stems after 2 years), the warmed individuals had higher loadings and the individuals from the control group had lower loadings on the second principal component. The projection of variables (Fig. 7b) showed that warming positively influenced the C and N contents, and the C:P, and N:P ratios in plants, but negatively influenced the Na and P contents and C:N ratio. Considering both projections (Fig. 7a and b), it can be concluded that leaves were mostly correlated with the contents of Ca, Fe, K, Mg, Mn, and Ni, whereas stems were correlated with the contents of Cu, Na, and Zn.

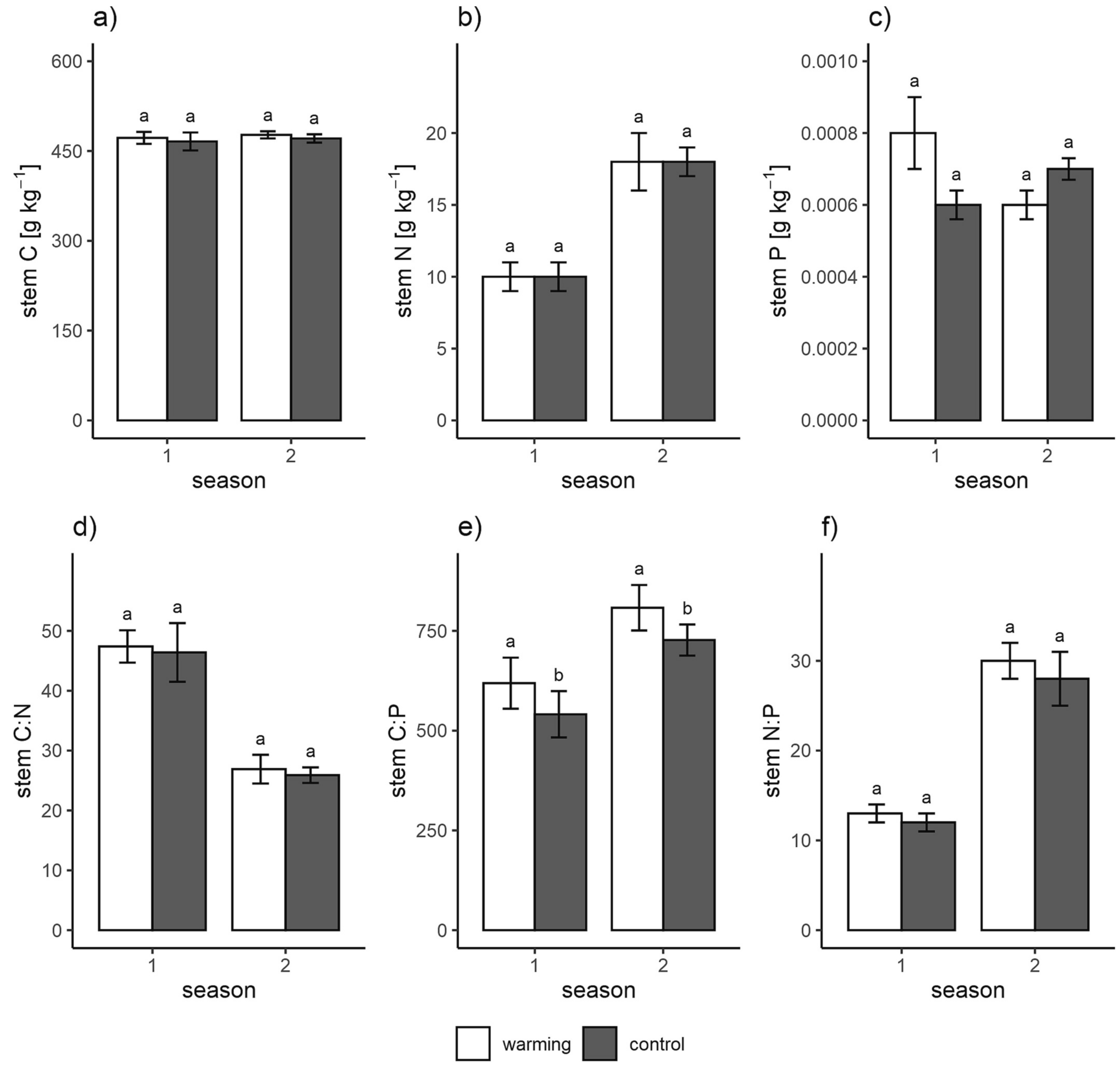

Fig. 3. Mean (with standard deviation) content of C, N, P, stoichiometric ratios C:N, C:P and N:P in stems, after first and second warming season. Different letters indicate significant differences, according to t-test ($p < 0.05$). Season 1 – September 2020, after 10 months of simulated warming; season 2 – September 2021, after 22 months of simulated warming.

Factors 1 and 2 for the PCA of soil samples (Fig. 7 c, d) collectively explained 75.5 % of the total variance (43.6 % and 31.9 %, respectively). Soil samples from both experimental groups (warming vs. control) were characterized by marked homogeneity on both factors 1 and 2 (Fig. 7c). However, it can be concluded that individuals from the warmed group were located mostly in the lower right quadrant of the scatterplot. The projection of variables indicated that warming positively influenced the C:N ratio in the soil, but was negatively correlated with C and N contents, and the C:P and N:P ratios. Thus, weak relationships were observed for warming and the contents of the investigated nutrients in the soil.

4. Discussion

As shown by Heath and Luckwill (1938), the occurrence and growth of European blueberry are strictly controlled by properties of the SOM; therefore, any change in SOM abundance and properties may impact on plants of this species. The present results demonstrate the significant influence of warming on the ground vegetation of montane Norway spruce forests of the Western Carpathians. In addition, the mineral topsoil at the study site responded significantly to the simulated warming, contributing to findings that the climate is among the most important direct controls of SOM decomposition (Powers et al., 2009; Zechmeister-Boltenstern et al., 2015).

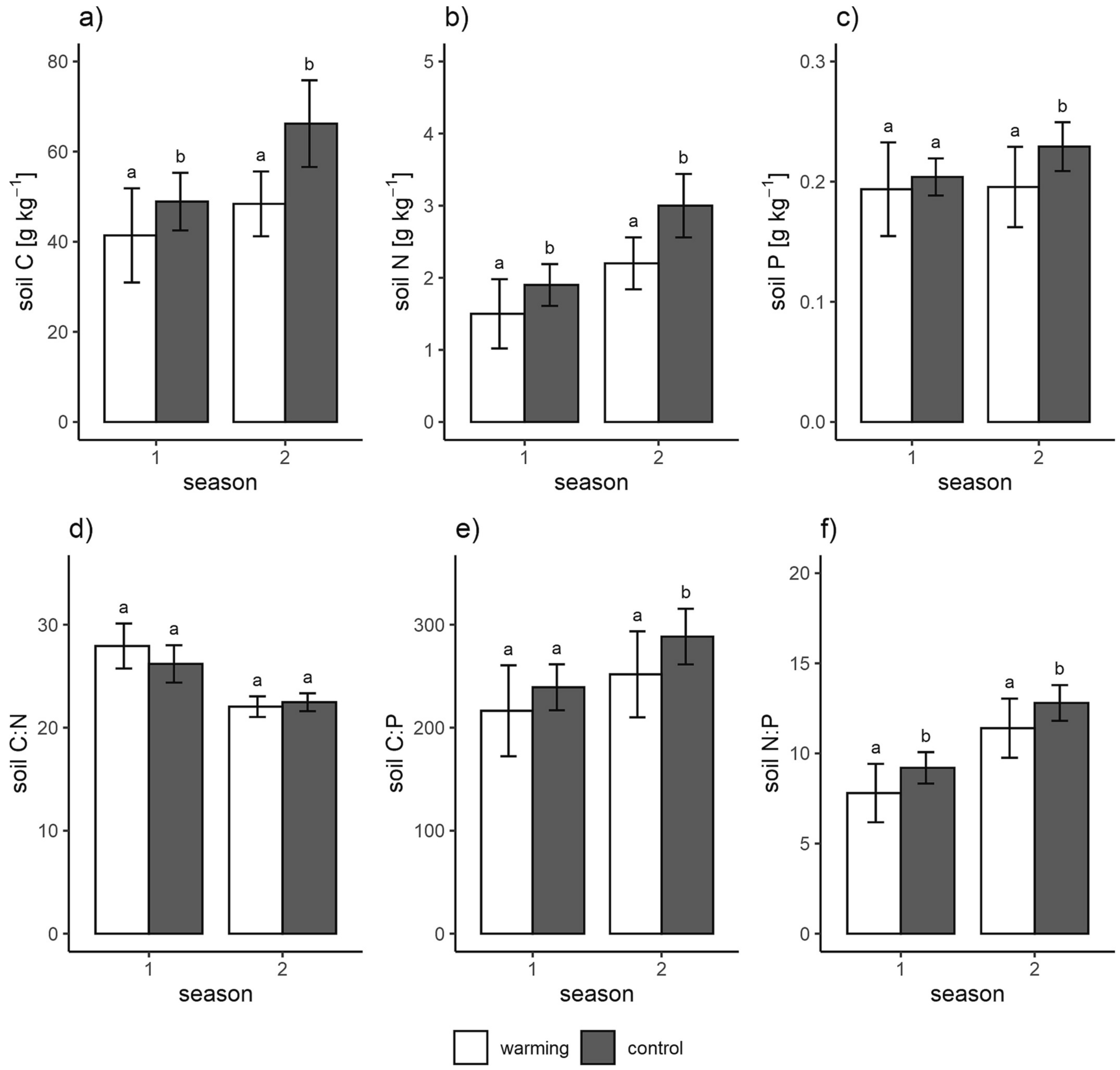

Fig. 4. Mean (with standard deviation) content of C, N, P, stoichiometric ratios C:N, C:P and N:P in soil, after first and second warming season. Different letters indicate significant differences, according to t-test ($p < 0.05$). Season 1 – September 2020, after 10 months of simulated warming; season 2 – September 2021, after 22 months of simulated warming.

4.1. Effect of warming on C, N, and P

The present results revealed significant correlations between warming and C, N, and P concentrations, either in European blueberry plants or in the soil. After the first warming season, the concentration of C in leaves was significantly higher in comparison with the control group (Fig. 2). In addition, the PCA revealed that warming was positively correlated with plant C and N contents (Fig. 7 b). The understory has the capacity to modify the operation of temperate forests by directly changing the fluxes of C and nutrients (Landuyt et al., 2019). These results complement other findings, indicating that, under warming conditions, plants increase the photosynthesis rate (mostly through higher carboxylation efficiency) (Contran et al., 2013; Hao et al., 2019). An alternative explanation of an elevated C concentration in plant tissues may be enhanced C uptake (Newton et al., 1995; Reich et al., 2006; Sardans and Peñuelas, 2012). Melillo et al. (2011) suggested that soil warming in a temperate zone forest caused an increase in C content in plant tissues (resulting from increased N availability) with a concomitant decrease in C concentration in the soil (resulting from increased microbial respiration), which is consistent with the results obtained in the current study. Nevertheless, it is important to note that the observed increase in C concentration in warmed leaves was still within the range of natural C foliar concentrations, so the changes, at least as an initial response, were not large.

Also noteworthy is the significant decrease in C concentration in warmed soil, after the first (by 15 %) and second (by 27 %) warming seasons. This trend was also demonstrated by PCA, in which the warming and C vectors exerted opposite influences (Fig. 7d). C loss from

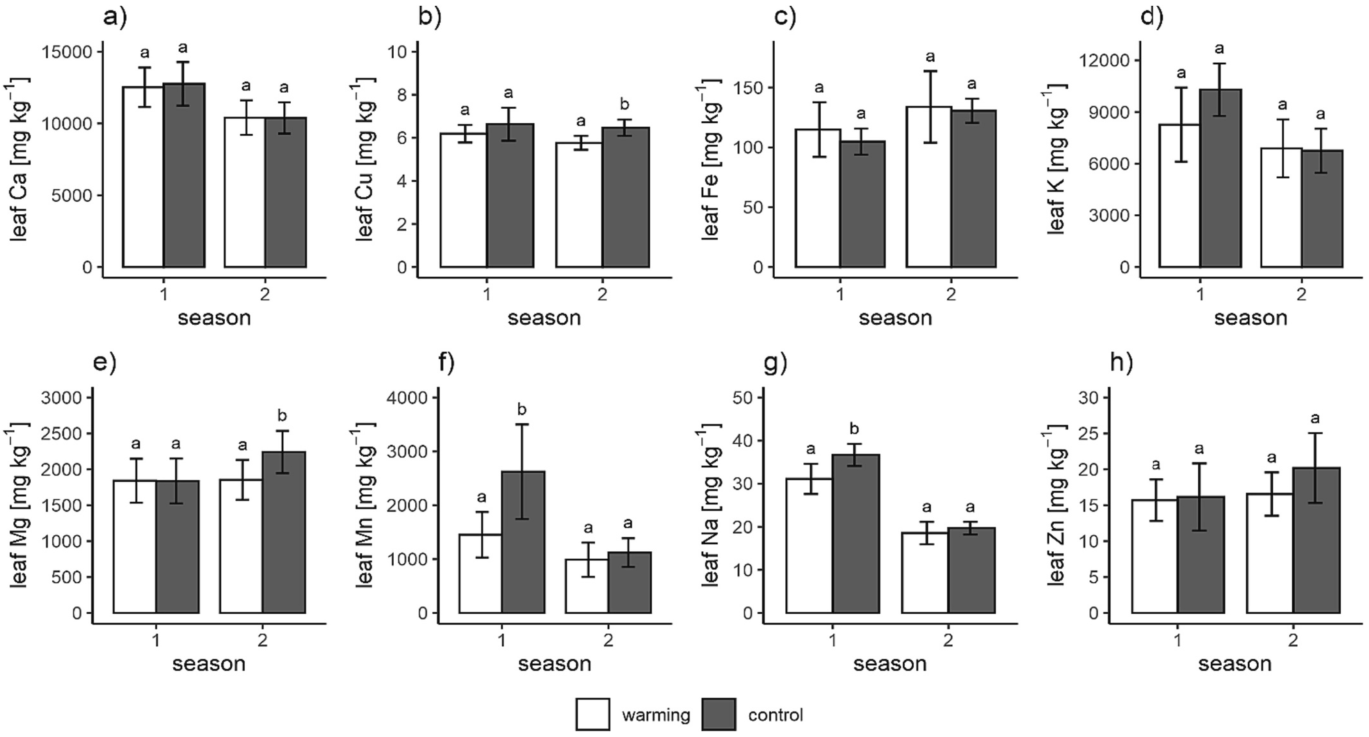

Fig. 5. Concentrations of investigated metals in the leaves of European blueberry. Different letters indicate significant differences, according to t-test ($p < 0.05$). Season 1 – September 2020, after 10 months of simulated warming; season 2 – September 2021, after 22 months of simulated warming.

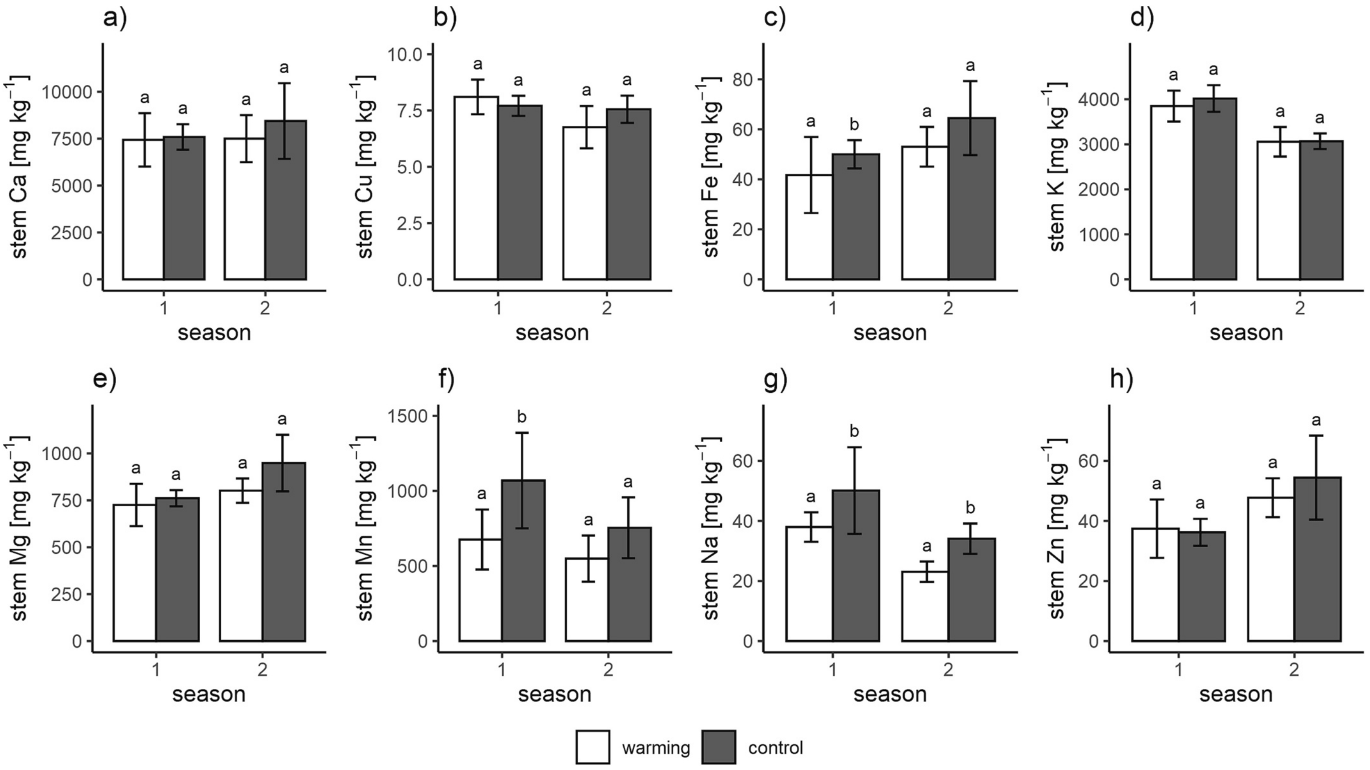

Fig. 6. Concentrations of investigated metals in the stems of European blueberry. Different letters indicate significant differences, according to t-test ($p < 0.05$). Season 1 – September 2020, after 10 months of simulated warming; season 2 – September 2021, after 22 months of simulated warming.

the soil in temperate forests as a result of warming has been reported by other authors (e.g., Melillo et al., 2011; Hopkins et al., 2012; Marek et al., 2020) and results mainly from increase in the soil respiration rate, strongly associated with the activity of soil microbial communities (Hopkins et al., 2012). The production of CO_2 associated with the increase in soil temperature and the respiratory activity of soil microorganisms, and the accumulation of CO_2 in the lowermost layer of the air, also contribute to the increase in intercellular CO_2 concentration and

Table 1
Pearson correlation coefficients between carbon (C), nitrogen (N), and phosphorus (P) concentrations, C:N:P stoichiometry, and the metals investigated.

Group			Ca	Cu	Fe	K	Mg	Mn	Na	Ni	Zn
Leaves	Warming	C	0.257	0.339	0.068	0.339	−0.206	0.112	**0.604**	**0.601**	−0.084
		N	−0.280	**0.637**	−0.200	0.338	**−0.538**	−0.258	0.374	0.269	−0.072
		P	**−0.565**	0.418	−0.173	0.307	**−0.482**	**−0.509**	−0.057	−0.095	0.091
		C:N	0.365	**−0.552**	0.206	−0.238	**0.538**	0.340	−0.219	−0.100	0.019
		C:P	**0.620**	−0.284	0.167	−0.185	0.405	**0.613**	0.197	0.275	−0.167
		N:P	**0.603**	−0.042	0.087	−0.120	0.211	**0.592**	0.394	0.408	−0.223
	Control	C	−0.213	0.339	0.581	−0.437	0.380	−0.333	−0.267	−0.115	0.212
		N	−0.085	0.302	0.091	0.423	−0.540	0.121	0.089	−0.010	−0.087
		P	−0.012	0.159	−0.071	0.422	−0.524	0.265	−0.111	−0.125	0.391
		C:N	0.052	−0.231	−0.009	−0.468	0.563	−0.148	−0.079	0.015	0.060
		C:P	0.061	−0.123	0.080	−0.387	0.487	−0.151	0.168	0.161	−0.341
		N:P	0.006	0.051	0.157	−0.157	0.218	−0.148	0.307	0.216	−0.598
Stems	Warming	C	0.364	−0.050	0.388	−0.270	0.107	**−0.580**	−0.243	−0.085	**0.631**
		N	0.124	**−0.611**	0.368	**−0.708**	0.357	−0.402	**−0.887**	**−0.686**	**0.491**
		P	−0.085	**0.533**	**−0.584**	**0.852**	**−0.542**	0.282	**0.610**	**0.505**	**−0.621**
		C:N	−0.052	**0.627**	−0.386	**0.726**	−0.360	0.308	**0.899**	**0.726**	**−0.470**
		C:P	0.097	**−0.515**	**0.574**	**−0.846**	**0.508**	−0.330	**−0.657**	**−0.549**	**0.641**
		N:P	0.115	**−0.615**	0.444	**−0.796**	0.438	−0.380	**−0.864**	**−0.693**	**0.550**
	Control	C	−0.142	−0.194	0.150	−0.406	0.014	0.229	0.143	−0.565	0.176
		N	0.337	−0.272	0.552	**−0.891**	**0.635**	−0.437	−0.628	−0.447	**0.700**
		P	−0.286	−0.102	−0.551	**0.868**	−0.587	**0.650**	0.439	0.153	**−0.650**
		C:N	−0.324	0.308	−0.513	**0.853**	**−0.635**	0.395	**0.695**	0.474	**−0.657**
		C:P	0.252	0.026	0.525	**−0.934**	0.560	−0.585	−0.467	−0.310	**0.660**
		N:P	0.332	−0.154	0.553	**−0.931**	0.626	−0.531	−0.603	−0.384	**0.712**
Soil	Warming	C	0.360	**0.533**	0.023	−0.285	−0.017	−0.107	0.023	0.191	0.210
		N	**0.487**	**0.598**	0.078	−0.176	0.071	−0.044	0.112	0.451	0.334
		P	**0.588**	**0.765**	**0.724**	0.417	**0.678**	**0.554**	**0.516**	0.406	**0.754**
		C:N	**−0.559**	**−0.579**	−0.213	−0.061	−0.236	−0.110	−0.212	**−0.735**	**−0.491**
		C:P	−0.239	−0.172	**−0.727**	**−0.743**	**−0.725**	**−0.705**	**−0.521**	−0.211	**−0.542**
		N:P	0.105	0.138	−0.456	**−0.531**	−0.438	**−0.478**	−0.275	0.214	−0.171
	Control	C	**0.800**	**0.879**	0.047	−0.335	0.106	0.404	0.402	0.565	**0.765**
		N	**0.845**	**0.849**	0.120	−0.194	0.265	0.416	0.446	0.558	**0.848**
		P	0.607	**0.803**	0.368	−0.018	0.524	0.323	0.622	0.300	**0.699**
		C:N	**−0.703**	−0.525	−0.220	−0.228	−0.570	−0.294	−0.416	−0.322	**−0.799**
		C:P	**0.793**	**0.789**	−0.253	−0.505	−0.229	0.366	0.201	**0.679**	**0.668**
		N:P	**0.902**	**0.813**	−0.060	−0.257	0.113	0.412	0.343	**0.650**	**0.858**

Significant correlations ($p < 0.05$) are shown in bold.

leads to an increase in the intensity of photosynthesis. However, C destabilization processes are complex, being strongly related to warming-sensitive physiochemical and biological conditions. At the present research site we observed significantly increased total C and dissolved organic C concentrations in the warmed O horizon (Kupka et al., 2023). We assumed that these increases were due to increase in the C concentration in the litterfall of European blueberry subjected to warming, as well as from enhanced biomass production.

Warming significantly decreased the concentration of N in the soil after the two simulated warming seasons by 27 %, which was also supported by PCA (Fig. 7d). We conclude that the reduced N concentration, as with C in the soil, is the result of increased mineralization under the influence of warming. Our assumptions correspond to previous findings of warming-induced increase in N mineralization (Du et al., 2019; Feng et al., 2015; Melillo et al., 2011). The present experiment did not result in an enhanced total N concentration in European blueberry plants, either in the stems or leaves (Figs. 2, 3). Similar results were obtained by Dawes et al. (2013), who observed an increase in *V. myrtillus* growth with no increase in N concentration in plant tissues under simulated climate-change conditions. Hence, we assume that no increase in N concentration may be associated with the individual ecological and physiological strategies of *V. myrtillus*.

After the second season of warming simulation, a significant reduction (by ca. 15 %) in soil P concentration in the warmed soil was observed. We assume that this finding reflects more intense mineralization of the SOM under warming, resulting in greater P mobilization (Elbasiouny et al., 2022; Wrage et al., 2010). Given that P in the soil is crucial for plant metabolism and sugar synthesis (Wrage et al., 2010), increased P availability in the soil is considered to be a beneficial phenomenon. However, we did not detect increased concentrations in aboveground tissues of plants subjected to simulated warming. Conversely, after the first warming season, a significant reduction in P concentration in leaves was observed (Fig. 2), which would indicate the absence of increased plant P uptake despite its higher availability in the soil. Hence, we hypothesize that the reduced P concentration in the soil, without an increase in P in *V. myrtillus* aboveground tissues, under warming may be the result of intensified P runoff. Increased mineralization and outflow of P, which is a limiting element for plants productivity, could pose a serious problem for the future stability of biogeochemical cycles and plant nutrition in the study forest (for both trees and ground vegetation). It should be noted that plant P uptake is not only associated with soil P mobility, but is a function of additional factors, such as water, light, and other major plant nutrients (Wrage et al., 2010).

Although no significant differences were observed in the magnitude of the C:N ratio in all groups studied, a significant effect of warming on the stoichiometry of C:P (in stems and soil) and N:P (in soil) was observed (Figs. 3, 4). Previous studies on C:N:P stoichiometry under simulated climate change have shown that the impact of climate change varies on different parts of plants (Du et al., 2019; Sardans et al., 2017; Yang et al., 2011). It is also noteworthy that changes in C:N:P stoichiometry are not only linked to warming, but also to successional stage or ecological strategies (Sardans and Peñuelas, 2012). A significantly reduced C:P ratio in warmed soil is indicative of a higher SOM decomposition rate, according to Zechmeister-Boltenstern et al. (2015), which is in line with significant decrease in the concentrations of C, N, and P in the soil. An increase in C:P ratio in warmed stems of *V. myrtillus*, according to numerous studies summarized by Zechmeister-Boltenstern et al. (2015), is related to increased water and nutrient use efficiency. The present results showed that warming visibly disrupted the C:N:P

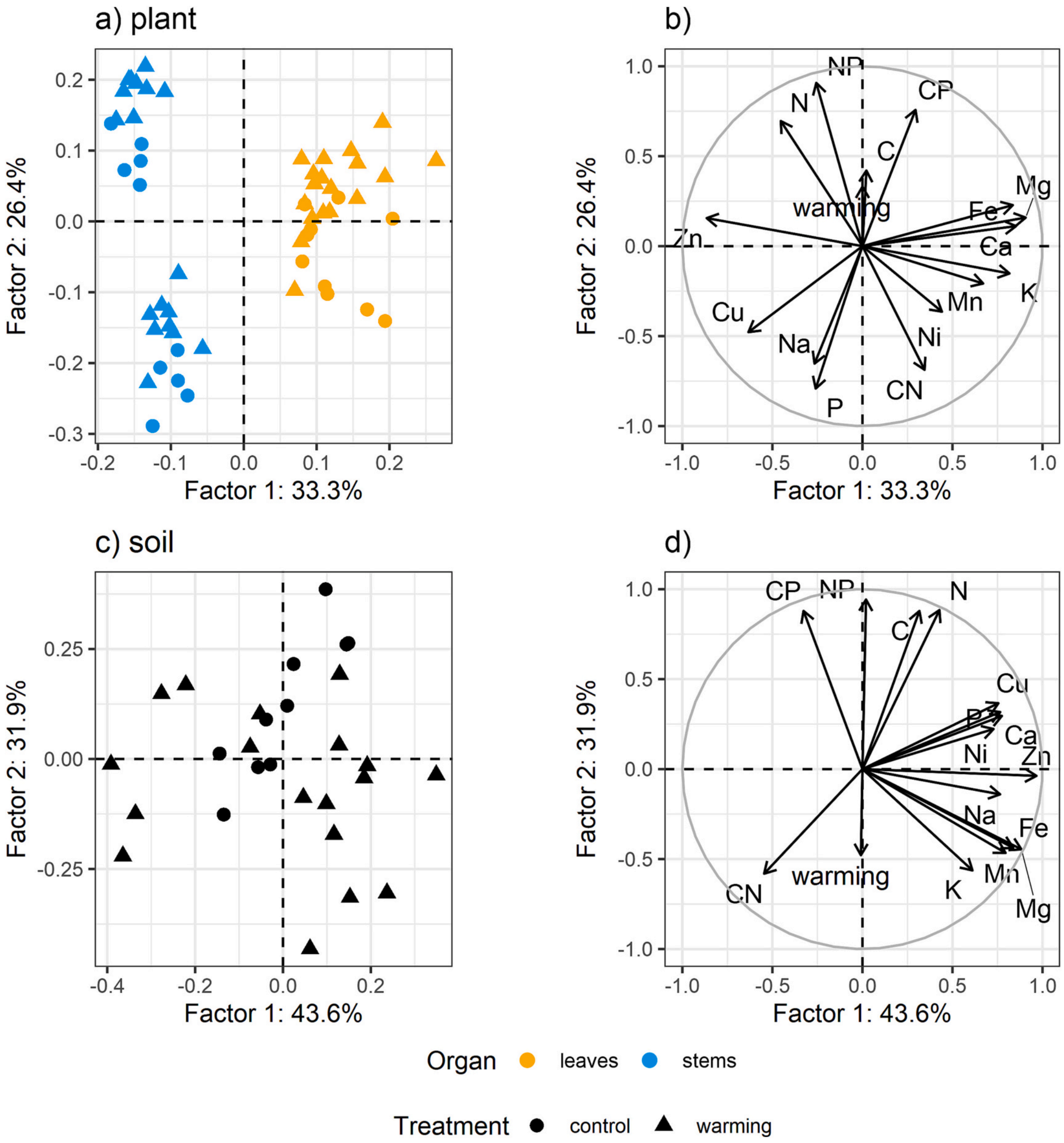

Fig. 7. Principal component analysis (PCA) of nutrient and metal contents and C:N:P stoichiometry in leaves and stems of European blueberry (*Vaccinium myrtillus*) (a, b) and in the soil (c, d).

stoichiometry. As an initial response after two warming seasons, the soil showed greater sensitivity (as demonstrated by significant changes in the C:P and N:P ratios) compared with the aboveground plant parts (exhibited by significant change only in the C:P ratio).

Particularly noteworthy is the observation of a significant decrease in the soil N:P ratio under the influence of warming (Fig. 4f). Also, the PCA showed a negative correlation of warming and N:P in soil (Fig. 7d). The N:P ratio has been proposed as a potential indicator of either N or P limitation (Liu et al., 2019) . However, it should be noted that N:P ratios serve as a numerical variable to highlight the fact that N and P limitations frequently occur in a gradient with varied degrees of shortage (Güsewell, 2004).

We assume that the decrease in this ratio is mainly due to a significant decrease in soil N content under the influence of warming (Fig. 4b). A plausible explanation of this phenomenon may be the acceleration of SOM mineralization processes under warming conditions. The mineralization process releases N in the form of ammonium (NH_4-N), which increases the amount of available N in the soil. Previous studies by the authors (Kupka et al., 2023) have shown a significant increase in NH_4-N in soil under warming, suggesting faster ammonification of SOM. Since an N:P value above 16 indicates P limitation and below 11 N limitation (Garrish et al., 2010; Koerselman and Meuleman, 1996), it can be concluded that in temperate zone spruce forest soils (already limited by N availability), there is an even greater N limitation in the soil due to warming.

4.2. *Effect of warming on metals*

Warming caused a significant decrease in the contents of Cu, Mg, Mn, and Na in the leaves, and of Fe, Mn, and Na in the stems of European blueberry (Figs. 5, 6). It is worth noting that no significant increase in the content of any of the tested metals was detected in the warmed plants. We hypothesize that this may be due to the “dilution effect”. An increase in plant C acquisition (evidenced by increased leaf C concentration in warmed plants, as well as by PCA analysis; Figs. 2a, 7b) under elevated temperature may lead to nutrient dilution in plant tissue, as nutrient uptake cannot keep up with the same pace of increase (Landuyt et al., 2019). As a consequence, a decreased mineral concentration may be linked to the dilution resulting from increased biomass production (Mcgrath and Lobell, 2013; Sardans et al., 2017). Given that many metals are essential for the proper functioning of plants, providing substances needed for metabolic processes, reduced contents caused by warming could disrupt future growth (Elbasiouny et al., 2022). Pearson correlation analysis demonstrated that stronger negative correlations between metals and C, N, and P (as well as with the stoichiometric ratios) were observed under warming, mostly in stems (Table 1). More in-depth research in the area of plant physiology is necessary to describe the most plausible mechanisms causing these alterations. It is also worth highlighting the PCA results for the tested plants (Fig. 7a, b), which suggested that leaves showed greater affinity for binding Ca, Fe, K, Mg, Mn, and Ni, whereas stems showed an affinity for Cu, Na, and Zn. This division is most likely due to individual allocation mechanisms of *V. myrtillus* (Dawes et al., 2013).

Although most of the studied nutrients were significantly correlated with the C, N, and P concentrations (and the stoichiometric ratios) in soil subjected to warming, no significant differences in their concentrations between studied groups were observed. A possible explanation for this phenomenon is that the total forms of these metals were studied, rather than solely the labile forms. Decrease in organic C concentration reduces the rate of immobilization of metals, mostly by binding metals into the SOM structure (Ayangbenro and Babalola, 2017; Grobelak and Kowalska, 2020).

4.3. Seasonality

No clear pattern in seasonality was observed in the present study, other than the Na concentrations in stems were significantly decreased during both warming seasons, as well as the C and N concentrations and N:P ratio in the soil. ANCOVA only provided significant results in some cases, hence we conclude, a weak correlation between elemental concentration after the first season with concentration after the second season. Thus, we conclude that the effect of warming on plant and soil chemistry is not direct and depends on a larger number of factors. Warming may influence either the overall or seasonal nutrient content, hence observation of similar changes over the same intervals may not be possible (Elbasiouny et al., 2022; Koller and Phoenix, 2017).

5. Conclusions

This research indicates that climate warming in Western Carpathian spruce forests is affecting the chemistry of both the soil and the aboveground tissues of *V. myrtillus*. The simulated warming caused an apparent decrease in the total contents of C, N, and P in the mineral topsoil, which is most likely related to more intense mineralization of SOM and mobilization of these elements. Using stoichiometric C:P and N:P ratios yielded information on the effects of warming on plants and topsoil. Significantly, the soil N:P ratio decreases under warming conditions, indicating a shift towards N-limitation. A substantially decreased C:P ratio observed in the warmed soil suggests an elevated rate of SOM decomposition. Decrease in soil P concentration under warming conditions may be an important threat for the supply of P to plants in the forest ecosystem. The increase in C concentration in the leaves of *V. myrtillus* may be a response to warming, resulting from more intensive C allocation. The increase in C:P ratio in plant tissues under warming represents a response to increased water and nutrient use efficiencies. Reduced nutrient content in aboveground tissues as a result of warming can lead to disorders in physiological processes. We conclude that ongoing climate change will significantly affect the plant and soil chemistry of Carpathian spruce forests, thereby posing a major future challenge for forest managers and policy makers.

CRediT authorship contribution statement

Dawid Kupka: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft. **Kaiwen Pan:** Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – review & editing. **Marcin Pietrzykowski:** Supervision, Writing – review & editing. **Wojciech Kraj:** Validation, Writing – review & editing. **Piotr Gruba:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The dataset generated and analyzed during the current study is available from the corresponding author on reasonable request.

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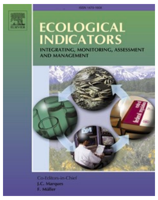

Effect of pH on the sorption of dissolved organic carbon derived from six tree species in forest soils

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Litter-derived dissolved organic carbon

ABSTRACT

Dissolved organic carbon (DOC) is an important component of the total carbon (C) pool in temperate forest soils. The ability of forest soils to sorb DOC is an essential feature in the context of ecosystem changes (such as climate change) and the resulting changes in the C cycle. The aim of this study was to investigate the ability of forest soils with different pH levels to sorb DOC from litter of different forest tree species. To accomplish this, we conducted two laboratory experiments in which the soil differed in the depth from which they were sampled (topsoil vs. subsoil) and the initial DOC content. We modified the soil pH to obtain a range covering most soils in temperate forests (approximately 3.5–8). Soil samples were leached with extracts of similar DOC content obtained from the litter of six main forest-forming tree species in temperate zone. Increased DOC sorption was observed at approximate soil pH levels of 3.5 and 7.5, with the highest sorption capacity occurring at low pH. The DOC sorption capacity was decreasing with consecutive leaching with litter extract. The capacity of DOC sorption also depended on the litter species. In addition, the initial concentration of C in soil had no significant relationship with sorption capacity. Litter species were related to the sorption of released DOC, which was probably linked to the Al content in the litter. The increased DOC sorption at low pH was likely because of dissolved organic matter protonation (saturation of functional groups with H^+ ions resulting in proton bridging and van der Waals forces), while Ca^{2+} ions causing cation bridging, flocculation or non-columbic interactions were likely responsible for the increased sorption at high pH. Our research shed light on the significant effects of soil pH on the stabilization of DOC, as well as the important role and positive influence of Al on DOC sorption by forest soils. Our findings provide new, significant insight by presenting that litter-derived DOC sorption is dependent both on soil pH and litter chemical composition.

1. Introduction

Forest soils are particularly important in the carbon (C) cycles of forest ecosystems. These C cycles are influenced by many factors, including forest stand species composition and canopy structure, which are currently subject to frequent changes under the influence of external factors (Senf et al., 2021). For example, in Central Europe, coniferous (e.g., Norway spruce or Scots pine) species are predicted to decline as a result of climate change (Dyderski et al., 2018).

One of the most important elements of C cycle is the decomposition of organic matter and the release of dissolved organic matter (DOM) (Bolan et al., 2011; Kaiser and Kalbitz, 2012; Thieme et al., 2019). The large diversity in DOM chemistry and associated different physiochemical properties, which is related to the intrinsic chemical properties of DOM originating from different tree species, are characteristic of forest soils (Thieme et al., 2019). DOM is recognized as the most mobile labile fraction of soil organic matter (SOM) (Kalbitz and Kaiser, 2008). Although DOM represents only a fraction of SOM, it has a considerable influence on biogeochemical relationships in soil, particularly those related to environmental cycles of C, nitrogen (N) and other nutrients (Bolan et al., 2011; Fujii et al., 2009; Thieme et al., 2019; Veum et al., 2009). DOM also plays essential roles in the pedogenesis process, as well as in the transport of pollutants in soil (Kalbitz et al., 2000). In temperate forests soils, a major source of DOM is leaching from the forest floor (Michalzik et al., 2001). DOM primarily originates from plant residues, root litter, microbial biomass, rainfall and the stable

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fraction of SOM (Gmach et al., 2020). The chemical composition of DOM is non-specific because of the variety of materials it contains, which range from small molecular particles to polymeric humic acids (Michalzik et al., 2001). Sorption of DOM to minerals is a significant process in organic matter stabilization in mineral soil horizons (Guggenberger and Kaiser, 2003; Kalbitz et al., 2005).

Given that quantification of DOM is often based on its C content, DOM is often reported as dissolved organic carbon (DOC) (Bolan et al., 2011). It is estimated that 40–370 kg ha^{-1} $year^{-1}$ of DOC is sequestered via sorption processes (Guggenberger and Kaiser, 2003; Michalzik et al., 2001; Mikutta et al., 2007), while DOC leached from the forest organic (O) horizon is considered a major C input to mineral layers of forests soils (Cronan and Aiken, 1985; Hansson et al., 2010). Thus, the O horizon is considered a DOC source, while the mineral soil is considered a sink (Kalbitz and Kaiser, 2008; Michalzik et al., 2001; Qualls et al., 2000). Some authors have suggested that DOC is one of major contributors to the accumulation of total C in soils, contributing up to 1440 kg ha^{-1} $year^{-1}$ (Schulze et al., 2000). Both sorption and SOM stabilization processes depend, among others, on the chemical DOM composition (Yano et al., 2004).

DOM binding to the mineral phase occurs through different organic-mineral associations, mainly via ion or ligand exchange, hydrogen bonding, cation bridges and van der Waals interactions (McBride, 1994; Mikutta et al., 2007; Neff and Asner, 2001). In forest soils, DOC primarily interacts with Al and Fe to form stable complexes. Al minerals are considered especially efficient DOC sorbents because of their large surface areas (Kalbitz and Kaiser, 2008; Schwesig et al., 2003). In forest ecosystems, DOC fluxes from the organic horizon and topsoil into deeper parts of soil decrease because of different types of sorption to the mineral phase (Kalbitz and Kaiser, 2008; Wang et al., 2019). One result of DOC binding to the soil mineral phase is reduced biodegradability. Specifically, DOC molecules are able to strongly sorb to minerals or to polar carboxyl or proteinaceous compounds as an another layer (Sollins et al., 2006), resulting in greater stability and resistance to mineralization (Hansson et al., 2010; Mikutta et al., 2007).

An understanding of the factors regulating sorption of DOM from the organic horizons of forest soils is crucial to forecasting soil C pools under different environmental conditions and potential changes in forest species compositions. Research conducted to date has mainly focused on DOM sorption related to forest soils texture (e.g., Seely et al., 1998), structure (e.g., Asano et al., 2006), mineral composition (e.g., Yano et al., 2000) and C content (e.g., Lilienfein et al., 2004). DOM sorption studies have also focused on soil acidity and the characteristics of cation exchange capacity (CEC), particularly the proportions of cations in soil sorption complexes (Gruba and Mulder, 2015; Mueller et al., 2012; Mulder et al., 2001; Ross et al., 2008).

Despite these studies, the effects of soil pH and CEC on DOM sorption and their final effects on the total soil C budget are still uncertain (Gmach et al., 2020). Moreover, there is a need to investigate the connections between DOC sorption and soil chemical and mineral properties (Yeasmin, 2020). Recent research conducted by Gruba and Socha (2019) using a large database of the Polish forests inventory revealed a significant increase in C accumulation in topsoil at pH levels <4.0 and >6.0. That study also indicated the lowest accumulation occurred at topsoil of pH 4–6. One hypothesis stemming from these findings is that the difference in C increase under different soil pH thresholds is due to differences in DOM sorption. In this study we aim to test this hypothesis. Therefore, we established two laboratory experiments to examine the ability of forest soils of different pH levels and cation composition to sorb DOC. In addition, we used soil taken from different depths of the soil profile to investigate the effects of initial C concentration on DOC sorption capacity. This work is a synthesis of two independently conducted experiments investigating the sorption of DOC derived from the litter of six main forest-forming species in temperate zone by forest soil. In the first experiment, the experimental design was adjusted to examine the DOC sorption from three consecutive DOC leachings. The second experiment was conducted to investigate the sorption of DOC by soil with a pH varying from acidic to alkaline.

Specifically, we investigated whether (1) the soil pH and relationships between cations significantly influence the ability of soil to sorb litter-derived DOC; (2) the sorption of litter-derived DOC by soil is also dependent on the forest tree species from which the litter is derived.

2. Materials and methods

2.1. *Overall assumptions of the research*

In both experiments, litter was collected from the litter overburden layer of organic (O) horizon of soils under six mature and homogenous forest stands: Norway spruce (*Picea abies* (L.) H. Karst), Scots pine (*Pinus sylvestris* L.), silver fir (*Abies alba* Mill.), European beech (*Fagus sylvatica* L.), European hornbeam (*Carpinus betulus* L.) and pedunculate oak (*Quercus robur* L.). The material was collected in May so that litter derived during the previous fall would undergo initial decomposition. All stands were of similar age (approximately 70 years old). Both experiments are briefly summarized in Table 1.

The soil used for the experiment I was taken from the parent material horizon (C, 80–100 cm). Soil for the experiment II was taken from the mineral horizon (A, 0–10 cm) after removing the overlaying organic layer. Samples were collected using a sampling core. The litter and soil samples used for the experiments conducted in this study were taken from a forest complex located between the cities of Kielce and Skarzysko-Kamienna in central Poland (51°02′54.1″N 20°43′45.5″E), located on the Mesozoic shield of Holy Cross Mountains massif. The soil at the sampling area was a Dystric Cambisol based on the World Reference Base (FAO, 2014). The Dystric Cambisol is developed from the weathering material, washed from parent material - Quaternary sandstones and claystones. The main clay mineral in the region's soils is kaolinite (Gruba et al., 2015; Krajewski, 1955). The sampling was performed in May.

2.2. *Experimental design*

2.2.1. *Experiment I*

Soil preparation. The soil was dried in air at ambient temperature for five days, then sieved through a 2-mm mesh, after which it was divided into three parts (300 g each). To decrease the pH and increase the share of hydrogen (H) in cation exchange capacity (CEC), one subsample was acidified by leaching with 400 mL of 0.5 M hydrochloric acid (HCl) solution. The second subsample was not treated. The third subsample

Table 1
The main experimental assumptions of experiments.

	Experiment	
	I	II
Soil horizon	C (80–100 cm)	A (0–10 cm)
Tree litter species	Norway Spruce (Picea abies (L.) H. Karst), Scots Pine (Pinus sylvestris L.), Silver fir (Abies alba Mill.), European beech (Fagus sylvatica L.), European hornbeam (Carpinus betulus L.), Pedunculate oak (Quercus robur L.)	
Initial C concentration in soil [g kg^{-1}]	~0	~30
Number of soil pH variants	3	5
pH range of soil	4.38–5.52	3.52–7.89
Number of consecutive leachings of one soil sample	3	1
Analysis of soil chemical properties	Yes	Yes
Analysis of chemical properties of litters	Yes	Yes

was alkalized using 400 mL of 0.5 M calcium hydroxide ($Ca(OH)_2$) to increase the pH and the share of exchangeable Ca in CEC. Samples were put in the filter paper and leached by acid/base solution, respectively. The liquid was extracted using gravity. For each soil variant, we determined the pH, content of exchangeable cations, and the total concentrations of carbon (C) and nitrogen (N).

Leachate preparation. The freshly collected O-horizon samples were placed in plastic bags and immediately transported to the laboratory. Deionized water (3 l) was added to beakers containing 1 l of each O-horizon and stirred for 5 min using a glass rod. After 24 h of equilibration, the suspensions were filtered through a 2 mm sieve (using gravity), then through a 0.45 μm Millipore membrane filter (using vacuum). The litter was then collected and dried, while the DOC was measured in the obtained extracts. Finally, the solutions were diluted to obtain equal DOC concentrations (150 mg L^{-1}). Following dilution, the DOC was measured again.

Soil leaching. 50 g each of the previously prepared soil variants were mixed with 100 mL of litter extract and shaken for 1 h. The obtained suspensions were then poured into centrifuge tubes and gently shaken for 1 min, after which they were centrifuged for 30 min (3000 *g*). Following centrifugation, supernatants were collected and another 100 mL of prepared litter extract (with DOC concentration 150 mg L^{-1}) was added to the sediment remaining following decanting after the first leaching. After that, one hour of shaking and centrifugation were then performed again. After collecting second supernatants, third doses of 100 mL of litter extracts were added to the sediments, after which shaking and centrifuging were performed the last time. This operation was conducted for each type of soil (natural, alkalized and acidified) and each litter extract. After each of three rounds of mixing and centrifuging, supernatants were collected and filtered through a 0.45 μm Millipore membrane filter (using vacuum) to determine the DOC concentration.

2.2.2. Experiment II

Soil preparation. We modified the pH and the share of exchangeable Al, Ca, and H in the CEC of the soil. To accomplish this, soil was dried at room temperature for five days, then sieved through a 2 mm mesh. The soil was then divided into five subsamples (300 g each). Two subsamples were acidified using 1 M HCl solution (one sample with 400 mL as strongly acidified, one with 200 mL as slightly acidified), while one subsample was untreated. Additionally, two subsamples were alkalized using 1 M $Ca(OH)_2$ solution (one with 200 mL as slightly alkalized, one with 400 mL as strongly alkalized). Samples were put in the filter paper and leached by acid/base solution, respectively. The liquid was extracted using gravity. We then measured the pH, concentrations of exchangeable base cations, exchangeable aluminum (Al^{3+}) and hydrogen (H^+), total acidity (TA) and cation exchange capacity (CEC) of each variant.

Leachate preparation. Deionized water (3 l) was added to beakers containing 1 l of each fresh O-horizon material and stirred for 5 min using a glass rod. After 24 h of equilibration, suspensions were filtered through a 2 mm sieve, then through a 0.45 μm Millipore membrane filter (using vacuum). The litter was then collected and dried, after which it was milled in ball mill (Fritsch) to obtain heterogeneity. The pH, exchangeable base cations (Ca^{2+}, K^+, Mg^{2+}, Na^+) concentration and Al^{3+}, organic matter (OM), total C and N concentrations of the milled material were then determined. Additionally, the DOC of the obtained extracts was measured. Solutions were subsequently diluted to give similar concentrations of DOC (approximately 150 mg L^{-1}).

Soil leaching. Duplicate 15 g samples of each variant of the soil were placed in 50 mL centrifuge tubes and amended with 30 mL of each type of litter solution. Additionally, one duplicated part of the soil was mixed with distilled water as a comparative sample. In total, 70 samples were prepared. Tubes were gently shaken for 1 min, then centrifuged for 30 min (3000 *g*), after which the supernatants were filtered through a 0.45 μm Millipore membrane filter (using vacuum) to determine the pH and DOC concentration.

2.3. Laboratory analyses

The particle size composition of the soil was determined using laser diffraction (Analysette 22, Fritsch, Idar-Oberstein, Germany).

The pH was measured using a potentiometric method, with a combined electrode in a suspension of soil in distilled water (1:5 mass:volume) after 24 h of equilibration (Buurman et al., 1996). The concentration of DOC was determined using a high temperature platinum-catalyzed combustion analyzer (Shimadzu TOC-5000). The content of OM in litter samples was determined using the loss on ignition (LOI) method. Briefly, samples were ashed in a muffle furnace at 400 °C for 4 h, after which the OM content was calculated as the difference in the sample weight before and after combustion. Total C and N was determined using a LECO CNS TrueMac Analyzer (Leco, St. Joseph, MI, USA). Exchangeable base cations (Ca^{2+}, K^+, Mg^{2+}, Na^+) were extracted with 1 mol L^{-1} ammonium acetate (CH_3COONH_4) at pH 7, during which samples were mixed with the extractant (10 g in 30 mL), shaken for 1 h and then allowed to equilibrate for 24 h. Suspensions were subsequently filtered, after which soil samples were mixed with a supplementary extractant to a total volume of 100 mL. The content of cations and the Al^{3+} content were then measured using an ICP-OES (ICP-OES Thermo iCAP 6500 Duo, Thermo Fisher Scientific, Cambridge, UK). The exchangeable aluminum (Al^{3+}) was measured in filtrate obtained after extracting 3 g of soil by shaking in 30 mL of 0.5 M copper (II) chloride ($CuCl_2$) for 3 h. The total acidity (TA) of the soil samples was measured in filtrate obtained after extracting 10 g of soil by shaking in 30 mL 1 mol L^{-1} calcium acetate ($(CH_3COO)_2Ca$) for 1 h. Soil on the filter was flushed several times with extractant solution to give a final volume of 100 mL. Next, 25 mL of the obtained solution was subjected to potentiometric titration with 0.5 mol L^{-1} NaOH using an automatic titrator (Mettler Toledo, Inc. Columbus, Ohio) to give a final pH of 8.2. Exchangeable hydrogen was calculated as the difference between TA and Al^{3+}. The CEC was calculated as the sum of BC and TA.

2.4. Calculation of sorbed DOC

In performed laboratory experiments, it was necessary to consider that soil that contains organic carbon releases DOC. To determine this dose, each variant of the soil was mixed with distilled water (DOC_{soil}), after which the DOC sorbed by the substrates was calculated using the formula (1):

$$DOC_{sorbed} = DOC_{litter} - (DOC_{mixed} - DOC_{soil}) \tag{1}$$

where: DOC_{litter} was the DOC content in aqueous litter extract, DOC_{mixed} was the DOC content in solution after rinsing soil with litter extract, DOC_{soil} was the DOC content in soil.

The soil samples in the experiment II contained trace amounts of DOC; therefore, we assumed that it was absent (DOC_{soil}=0). Hence, the formula was as follows (2):

$$DOC_{sorbed} = DOC_{litter} - DOC_{mixed} \tag{2}$$

DOC_{sorbed} was expressed in [mg L^{-1}] and in % of initial DOC concentration in extracts.

2.5. Statistical analyses

Mean values were calculated to estimate the chemical properties of soil samples, litter and litter extracts. Standard deviations, analysis of variance (ANOVA) and Tukey's HSD (honestly significant difference) were calculated in experiment II for the values of DOC sorbed by particular soil type and for particular litter species. Parametric comparisons of chemical properties of two groups of litters (coniferous vs. deciduous) were performed using t tests, while the Kolmogorov-Smirnov (K-S) test was used for non-parametric comparisons of these two groups. The normality of distributions was tested using Shapiro-Wilk test.

Non-linear regression analysis was performed to estimate parameters describing the relationship between the sorbed DOC and pH of soil. Nonlinear regression model was generated for all observations ($n = 70$) from experiment II to evaluate the relationship between soil pH and sorbed DOC. The least squares regression method with Gauss-Newton estimation was applied, and the best fit was a quadratic function given by the following formula:

$$y = b_1(x - b_2)^2 + b_3 \quad (3)$$

where y was the sorbed DOC and x was the soil pH. All statistical analyses were conducted using the R statistical software, using packages “ggplot2” and “factoextra” (Kassambara and Mundt, 2020; R Core Team, 2022; Wickham, 2016).

3. Results

3.1. Chemical properties of litter types

The relationship between the OM content and total C concentration was very clear in the investigated litter samples ($r = 0.99$, $p < 0.05$). Overall, the pH of coniferous species was lower than that of broadleaved species (Table 2). Moreover, the concentration of base cations was lower for deciduous species (mean ± standard error: coniferous 20.2±6.7 $\text{cmol}_{(+)}\ \text{kg}^{-1}$; deciduous 41.0±6.6 $\text{cmol}_{(+)}\ \text{kg}^{-1}$; $p < 0.05$ according to *t* test).

Fir litter had the lowest pH, as well as the lowest C and OM content (Table 2). This species also had the poorest exchangeable Ca, K and Mg concentrations, but the highest exchangeable Na concentration. However, litter from this species had a high concentration of exchangeable Al, which was likely responsible for the TA. Overall, TA of coniferous species was higher than that of deciduous species (mean ± standard error: coniferous 62.4±11.2 $\text{cmol}_{(+)}\ \text{kg}^{-1}$; deciduous 36.2±11.4 $\text{cmol}_{(+)}\ \text{kg}^{-1}$; $p<0.05$ according to *t* test).

3.2. Experiment I

Soil samples. The soil used consisted primarily of sand (76% sand, 21% silt, 3% clay). The native soil pH was 5.02. The pH of the acidified part of the soil was 4.38, while that of the alkalized part was 5.52. All three pH variants of the soil were characterized by negligible total C concentration (natural: 0.11%; acidified: 0.10%; alkalized: 0.11%), as well as negligible total N (0.009%, 0.008%, 0.009%, respectively). Analysis of the soil DOC concentrations also revealed trace amounts; therefore, we assumed it was absent.

Leachates. Following dilution with deionized water, all litter extracts had DOC concentrations of 150 mg L^{-1}. Based on the DOC concentrations determined in the litter extracts after each of the three soil leachings, the DOC sorption by the soil was calculated and presented in Fig. 1. The table data is presented in Appendix A.

Evaluation of all litter species revealed a noticeable overall decrease in DOC sorption with increasing soil pH. The sample error was <5% (with 95% confidence interval). In all cases, DOC sorption was greatest during the first leaching, which was most pronounced for the acidified soil. With subsequent leachings, the ability of the soil to sorb DOC decreased. When leached with oak (Fig. 1c), fir (Fig. 1d) or spruce (Fig. 1f) litter extract, the alkalized soil variant sorbed almost the same amount of DOC. For the fir and spruce litter extract, the alkalized soil sorbed small amounts of DOC (after the third leaching with the spruce litter extract, the alkalized soil did not sorb any DOC).

3.3. Experiment II

3.3.1. Relationship between soil pH and DOC sorption

Soil samples. The soil was sandy (72% sand, 23% silt, 5% clay). As expected, variants of the soil differed in chemical properties (Table 3). There were no large differences in total C and N concentrations between soil types. Additionally, the pH of the soil was strictly related to the type and the volume of added solution (acid vs. base). The concentrations of exchangeable Ca, K and Mg increased with pH, and the concentration of exchangeable Ca increased significantly at > pH 6. A similar relationship with exchangeable Na was observed, except for the variant with distilled water. Exchangeable Al reached the highest concentration in soils that were strongly acidified and slightly alkalized (soil pH about 3.5 and 6.5, respectively). Exchangeable H and TA decreased with increasing pH. Overall, the lowest CEC was observed at pH 4.5, whereas it increased in soils that were strongly alkalized and acidified.

DOC in leachates. The concentration of $\text{DOC}_{\text{litter}}$ (DOC content in aqueous litter extracts) in investigated litter solutions after dilution with water was similar for all species (beech, 114.2 mg L^{-1}; fir, 119.9 mg L^{-1}; pine, 122.5 mg L^{-1}; spruce, 126.7 mg L^{-1}; hornbeam, 128.8 mg L^{-1}; oak 130.6 mg L^{-1}).

The DOC content in soil (DOC_{soil}) varied clearly between soil samples as follows: natural (37.18 mg L^{-1}) < slightly acidified (45.73 mg L^{-1}) < strongly acidified (52.25 mg L^{-1}) < slightly alkalized (61.82 mg L^{-1}) < strongly alkalized (113.6 mg L^{-1}).

DOC sorption. Soil with the lowest pH had the greatest ability to sorb the DOC released from the litter extracts (Fig. 2, Appendix B). The HSD test revealed that the highest sorption was in the strongly acidified variant, and this was significantly higher than the sorption of the slightly acidified, natural and slightly alkalized soils. Although the DOC sorption by the strongly alkalized soil was not significantly different from that of the other variants, its mean value was slightly increased. The mean values of the DOC sorbed by the soil depended on the litter extract species, regardless of the pH variant (Appendix B). We detected no clear pattern between litter type (coniferous vs. deciduous) and the amount of

Table 2
Chemical properties of the litter.

Parameter	Unit	Litter species					
		Beech	Hornbeam	Oak	Fir	Pine	Spruce
C	g kg^{-1}	453.9	449.5	387.8	355.9	475.2	474.1
N		12.5	16.6	15.5	16.8	15.9	17.4
OM		882.0	878.8	741.2	672.4	893.4	893.1
pH	–	5.33	4.8	4.54	3.81	4.22	3.83
Ca^{2+}	$\text{cmol}_{(+)}\ \text{kg}^{-1}$	28.73	26.39	21.44	9.70	18.52	20.50
K^{+}		8.76	10.42	6.52	1.04	2.23	2.50
Mg^{2+}		6.04	8.58	5.34	1.34	2.34	1.86
Na^{+}		0.26	0.39	0.20	0.49	0.11	0.10
Al^{3+}		0.66	2.11	2.92	27.86	3.34	2.50
H^{+}		22.39	41.41	38.95	46.09	48.34	59.13
BC		43.80	45.78	33.50	12.57	23.20	24.96
TA		23.06	43.52	41.87	73.95	51.68	61.63
CEC		66.86	89.30	75.37	86.53	74.88	86.59

BC – base cations, CEC – cation exchange capacity, OM – organic matter content, TA – total acidity.

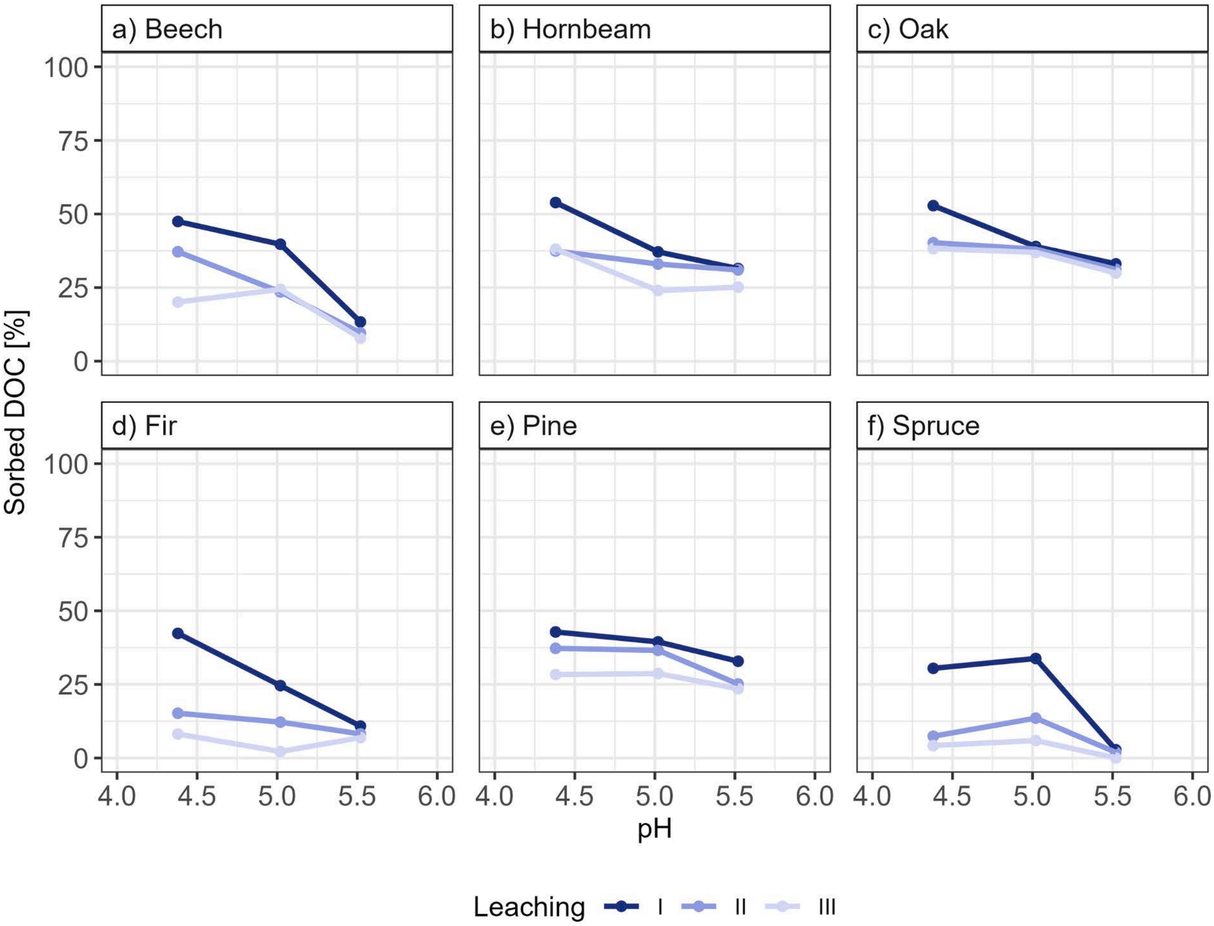

Fig. 1. Relationship between soil pH and DOC sorbed from different litter extracts.

Table 3
Chemical properties of the soil.

Parameter	Unit	Soil type				
		Strongly acidified (pH = 3.52)	Slightly acidified (pH = 4.01)	Natural (pH = 4.65)	Slightly alkalized (pH = 6.41)	Strongly alkalized (pH = 7.89)
C	g kg^{-1}	30.68	30.81	30.60	28.22	33.33
N		1.44	1.45	1.38	1.34	1.50
Ca^{2+}	$cmol_{(+)}$ kg^{-1}	0.19	0.55	1.20	6.11	14.20
K^{+}		0.02	0.03	0.05	0.06	0.07
Mg^{2+}		0.02	0.06	0.13	0.19	0.22
Na^{+}		0.01	0.03	0.02	0.04	0.06
Al^{3+}		1.76	1.47	1.22	1.75	1.25
H^{+}		5.91	5.51	3.99	1.10	0.04
TA		7.67	6.98	5.21	2.85	1.29
CEC		7.92	7.65	6.61	9.25	15.84

TA – total acidity, CEC – cation exchange capacity.

DOC sorbed (sorption ± std. deviation 36.2±15.4 and 29.1±15.4 mg L^{-1} for litters from coniferous and deciduous species, respectively; K-S test: p = 0.141). The HSD test indicated that the fir DOC sorbed by soil was significantly higher than that of DOC from spruce, oak and beech litter.

The parameters of estimated non-linear regression model is presented in Appendix C. The model including all analyzed litter types explained 24% of the variance, and all parameters were significant. The sorbed DOC and the estimated model results are plotted against the soil pH in Fig. 2. To make the plot clearer, data were divided into two sections according to litter type (deciduous vs. coniferous).

3.3.2. *Relationship between the sorption of litter-derived DOC and chemical properties of litters*

The amount of DOC sorbed by the soil can also be related to the chemical properties of the litter. Therefore, principal component analysis (PCA) was performed to examine the relationship between DOC sorbed by soil and the chemical parameters of the litter. Prior to this, a dataset was prepared from Table 2 and the mean concentration of sorbed DOC of each litter type (Appendix B). Factors 1 and 2 used for the analysis explained 85.8% of the variance (59.4% and 26.4%, respectively). The projection of individuals (Fig. 3a) showed three different groups of litters. Deciduous species (beech, hornbeam and oak) occupied the upper left quadrant of the graph, while pine and spruce were located

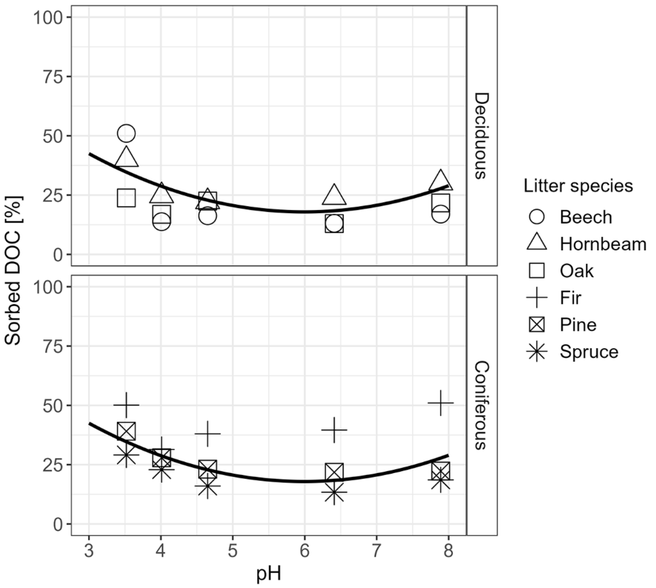

Fig. 2. Relationship between sorbed DOC and soil pH, divided into litter types. Black lines denote the nonlinear regression model with parameters from Appendix C.

in the lower right quadrant and fir remained a separate group in the upper right quadrant. The graph of investigated variables (Fig. 3b) revealed a strong relationship between DOC sorbed by soil and exchangeable Al in the litter. A positive relationship between sorbed DOC and exchangeable Na was also observed. Additionally, a negative correlation was observed between sorbed DOC and C concentration in litter. N, pH, exchangeable Ca, K and Mg were not correlated with sorbed DOC.

4. Discussion

Two independent experiments confirmed the significant effect of soil pH and (or) cation exchange relationships on the ability of soil to sorb litter-derived DOC. Application of a homogenous mineral soil in these experiments allowed exclusion of additional variability associated with other soil properties. Both acidification and alkalization of the soil resulted in a wide range of soil pH, from strongly acidic to slightly alkaline. The obtained pH ranges of soil represent the majority of temperate forest soils, which is particularly important since only a small percentage of soils in previous studies were strongly alkaline (Gruba and Socha, 2019). In addition, the use of soil differing in initial C concentration confirmed that DOC sorption is independent of native C concentration.

Experiment I provided information regarding DOC sorption by carbonless soil with acidic pH values. The acidified variant of the soil (pH = 4.38) in each leaching sorbed the largest amount of initial litter-derived DOC (on average: first leaching 45%; second 29%, third 22%, for all litter species). With some exceptions, DOC sorption decreased with increasing soil pH. Moreover, soil with the highest pH in experiment I (5.52) sorbed smaller amounts of DOC with each successive leaching with another portion of leachate containing litter-derived DOC than lower pH variants (pH 5.02 and 4.38) (Fig. 1). Krettek and Rennert (2021), as well as Avneri-Katz et al. (2017) demonstrated that the availability of mineral surfaces for DOC sorption is limited, which is consistent with our results. We hypothesize that the DOC sorption capacity decreased with the leaching number because of saturation of the soil capacity to sorb DOC, as well as with increasing pH because of decreasing protonation of functional groups.

The results of experiment II were corresponding with those of the experiment I with respect to the relationship between soil pH and DOC sorption. The strongly acidified variant of the soil (pH = 3.5) had the highest DOC sorption capacity, with an average value of 39% of the initial DOC (Appendix B). Moreover, the strongly alkalized soil showed a tendency for increased DOC sorption (27%, on average). The increased DOC sorption capacity in the low and high pH ranges was also confirmed by the nonlinear regression model, (Fig. 2). The relatively low proportion of explained variance ($R^2_{adj} = 0.24$) may be related to differences in the chemical compositions among the investigated litter species. Similar relationships were observed for a large database obtained from soil with a naturally large range of pH. Gruba and Socha (2019) showed an increased accumulation of total C in the 0–10 cm layer of mineral topsoil at pH below 4.0 and above ca. pH 6.0. Our data suggest that the significant increase in DOC sorption ability was mainly related to the soil pH and corresponding saturation of soil CEC with H and Ca. We suggest that, at low pH, the main mechanism responsible for the increased DOC sorption was protonation (saturation of H^+) of the DOM exchange sites. DOM protonation was previously suggested as a major mechanism controlling stabilization of soluble organic matter (Berggren et al. 1998). Moreover, Kalbitz et al. (2000) suggested that protonation of functional groups can reduce the DOM solubility via intensive proton bridging and van der Waals forces. Numerous authors also identified Al^{3+} and $Fe^{2+/3+}$ saturation as important mechanisms positively influencing DOM sorption in acid forest soils (Greenland, 1971; McDowell and Wood, 1984; Tipping and Woof, 1991; 1990). Al and Fe oxides or hydroxides derived from parent material weathering provide significant sorption capacity and contribute to DOM stabilization (Kalbitz and Kaiser, 2008).

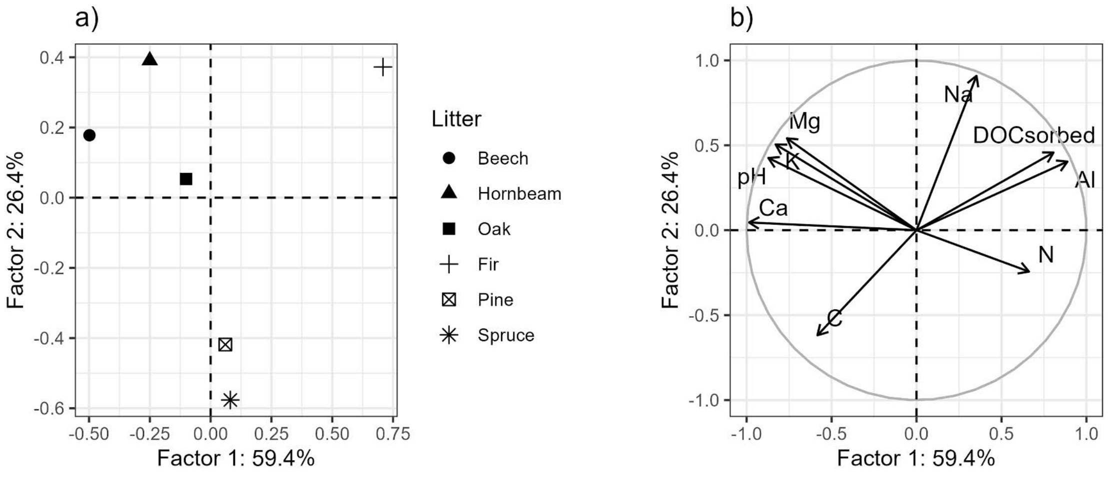

Fig. 3. Principal component analysis (PCA) of the chemical properties of litter and DOC sorbed by soil; (a) individual species and (b) variables.

However, our study did not find significantly increased Al^{3+} concentrations in acidified soil variants. Indeed, the Al^{3+} concentration in the strongly acidified variant was the same as that in the slightly alkalized variant (1.8 $cmol_{(+)}$ kg^{-1}). Hence, we believe that, in our study, the major mechanism of DOC stabilization at low pH was the considerable protonation of functional groups. Moreover, TA, which is the sum of Al^{3+} and H^{+}, could be considered a parameter affecting DOC sorption; however, in our study, H^{+} was the main component of TA (Table 3). The highest TA value was found in the strongly acidified soil (7.7 $cmol_{(+)}$ kg^{-1}), mainly because of the high level of H^{+}, indicating a relationship with the highest DOC sorption capacity. TA is positively correlated with C stock in mineral layers, indicating the high importance of H and Al to DOC stability (Hobbie et al., 2007; Mueller et al., 2012).

As previously mentioned, our data also suggest that there was increased DOC sorption in the strongly alkalized soil variant ($pH = 7.9$) (Fig. 2). We assume that this observation was related to the increased Ca^{2+} concentration in the CEC (Table 3). At $pH > 5$, the proportion of Ca in the soil CEC increases considerably (Prenzel and Schulte-Bisping, 1995; Reuss et al., 1990). These results are in-line with those of Singh et al. (2016), who found that DOM sorption was positively correlated with Ca^{2+} concentration. Mikutta et al. (2007) also reported that Ca^{2+} had a considerable impact on SOM binding to the mineral phase at higher pH. Polyvalent cations such as Ca^{2+} are able to link negatively charged DOM functional groups together, thereby reducing DOM solubility via cation bridging or flocculation processes (Kalbitz et al., 2000). Moreover, in the presence of Ca^{2+}, DOM can be stabilized via non-columbic interactions (van der Waals forces) (Mikutta et al., 2007).

Studies based on column experiments have shown that soil can sorb between 60% and 80% of litter-derived DOC (Müller et al., 2009; Scott and Rothstein, 2014; Wang et al., 2019), which is larger than the results observed for both of our experiments (Figs. 1 & 2). However, those previous studies did not consider variable pH ranges or different types of organic matter. In addition, most column experiments investigating total DOC sorption use the entire soil profile rather than a small amount of topsoil or subsoil as in our study.

Our research also showed that the chemical composition of the litter from which DOM is derived affects its sorption by soil. Principal component analysis (Fig. 3b) indicated that the main chemical parameter of the litter determining DOC sorption was the Al content. This observation corresponds with the finding that fir-derived litter had the highest Al concentration (Table 2), and that DOC from this litter was strongly sorbed by the soil (Appendix B). Similar conclusions were made by McDowell and Wood (1984), who found a positive correlation between DOC retention in soil and Al concentration in litter. Schwesig et al. (2003) also found that Al stimulated DOM stabilization, enabling incorporation of up to half of the DOC into stable fractions of organic matter. Mulder et al. (2001) found that DOC leaching from the forest floor decreased as levels of Al increased. The data shown in Fig. 3 suggest that differences in DOC sorption between tree species are a result of intrinsic DOM properties because DOM dynamics are strongly connected with the parameters of organic residues, which depend on their origins (Gmach et al., 2020; Kalbitz et al., 2000; Leinemann et al., 2018; Mcdowell and Likens, 1988). It is suggested that the differences between coniferous and deciduous DOM are mainly related to differences in the aromatic compounds these species contain (Bischoff et al., 2015). Specifically, extracts from the litter of coniferous forests contain more lignin- and protein-like molecules, as well as fewer tannin-like particles (Ide et al., 2017; Thieme et al., 2019).

Further studies should focus on the sorption of DOM derived from forest litter of different stages of decomposition and its relationship with variations in the mineral phase pH. Attention should also be paid to DOM released from root decomposition, as this fraction represents a significant component of the total DOM pool in forest soils (Hansson et al., 2010). Some insight into DOM sorption and its relationship with pH and litter species diversity can be obtained by molecular analysis and extraction of individual DOM fractions. Moreover, investigation of the relationships between DOC sorption and soil pH changes induced by external factors (e.g., anthropopressure, climate change) will provide valuable information.

5. Conclusions

Insights into DOC sorption as a function of pH and the composition of CEC based on two independent experiments shed light on two mechanisms of DOC stabilization occurring at different pH levels. Our laboratory approach confirmed the results of field studies conducted by Gruba and Socha (2019) describing the relationship between soil pH and soil C concentration as a parabolic function with upward pointing arms. Our results represent one of the few (to the best of our knowledge) works focusing on DOC sorption by soils with a relatively broad pH spectrum, representing the vast majority of temperate forest soils. Increased sorption in soil at $pH \sim 3.5$ was mainly related to DOM protonation (saturation with H^{+} ions), which stabilized the material through proton bridging and van der Waals forces. Conversely, DOC sorption at $pH > 7$ primarily increased because of the presence of Ca^{2+} ions, which led to DOC stabilization via cation bridging, flocculating or non-columbic interactions. We suggest that aluminum in litter from different tree species influences their sorption by topsoil. However, the influence of intrinsic SOM properties cannot be excluded.

CRediT authorship contribution statement

Dawid Kupka: Conceptualization, Data curation, Investigation, Visualization, Writing – original draft. **Piotr Gruba:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Values of DOC sorbed ($DOC_{sorbed} = DOC_{litter}–DOC_{mixed}$) by different kinds of soil from various litter species (mg L^{-1} and %).

Leaching	Unit	Soil pH			Soil pH		
		Acidified	Natural	Alkalized	Acidified	Natural	Alkalized
		(pH = 4.38)	(pH = 5.02)	(pH = 5.52)	(pH = 4.38)	(pH = 5.02)	(pH = 5.52)
		Deciduous			Coniferous		
		Beech			Fir		
I	mg L^{-1}	71.2	59.6	20.0	63.5	36.9	16.3
	%	47.4	39.7	13.3	42.3	24.6	10.9
II	mg L^{-1}	55.8	35.3	14.4	22.8	18.3	12.3
	%	37.2	23.5	9.6	15.2	12.2	8.2
III	mg L^{-1}	30.1	36.6	11.7	12.2	3.3	10.4
	%	20.1	24.4	7.8	8.1	2.2	6.9
		Hornbeam			Pine		
I	mg L^{-1}	80.8	55.7	47.2	64.2	59.2	49.3
	%	53.9	37.1	31.5	42.8	39.5	32.9
II	mg L^{-1}	56.2	47.5	46.5	55.9	54.9	37.7
	%	37.4	33.0	31.0	37.3	36.6	25.1
III	mg L^{-1}	57.1	36.0	37.7	42.5	43.0	35.3
	%	38.1	24.0	25.1	28.3	28.7	23.5
		Oak			Spruce		
I	mg L^{-1}	79.2	58.4	49.6	45.7	50.7	4.2
	%	52.8	38.9	33.1	30.5	33.8	2.8
II	mg L^{-1}	60.4	57.1	46.8	11.1	20.3	2.9
	%	40.2	38.1	31.2	7.4	13.5	1.9
III	mg L^{-1}	57.4	55.5	44.9	6.3	8.9	0
	%	38.2	37.0	29.9	4.2	5.9	0

Appendix B. Mean values of DOC sorbed ($DOC_{sorbed} = DOC_{litter}–DOC_{mixed}$) by different kinds of soil from various litter species. For each case, $n = 2$.

Litter species	Unit	Soil type					Mean
		Strongly acidified (pH = 3.52)	Slightly acidified (pH = 4.01)	Natural (pH = 4.65)	Slightly alkalized (pH = 6.41)	Strongly alkalized (pH = 7.89)	
Beech	mg L^{-1}	58.3	15.7	18.6	14.9	19.4	25.4 ± 22.7^{ab}
	%	51.0	13.8	16.3	13.1	17.0	22.2 ± 19.8
Hornbeam	mg L^{-1}	51.5	31.8	28.6	31.0	38.8	36.3 ± 10.0^{ab}
	%	40.0	24.7	22.2	24.0	30.1	28.2 ± 7.8
Oak	mg L^{-1}	30.8	22.1	29.4	17.0	28.4	25.5 ± 8.1^{b}
	%	23.8	16.9	22.5	13.0	21.7	19.6 ± 6.2
Fir	mg L^{-1}	60.0	37.7	45.5	47.5	61.2	50.4 ± 15.1^{a}
	%	50.1	31.4	38.0	39.6	51.0	42.0 ± 12.6
Pine	mg L^{-1}	47.8	34.2	28.3	26.7	27.4	32.9 ± 9.2^{ab}
	%	39.1	27.9	23.1	21.8	22.3	26.8 ± 7.5
Spruce	mg L^{-1}	36.9	29.0	20.2	17.0	23.6	25.3 ± 9.4^{b}
	%	29.1	22.9	16.0	13.4	18.6	20.0 ± 7.4
Mean	mg L^{-1}	47.6 ± 17.9^{a}	28.4 ± 13.2^{b}	28.4 ± 9.6^{b}	25.7 ± 12.4^{b}	33.1 ± 15.8^{ab}	
	%	38.8 ± 15.9	22.9 ± 10.9	23.0 ± 8.0	20.8 ± 10.3	26.8 ± 13.2	

Letters indicate statistical groups according to Tukey's HSD test ($p < 0.05$).

Appendix C. Parameters of estimated model describing the relationship between soil pH and DOC sorbed by soil.

Parameters	Estimate	SE	p	R^2_{adj}
b_1	2.741	1.046	0.014	0.244
b_2	5.993	0.236	0.000	
b_3	17.916	3.501	0.000	

SE – standard error.

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Oświadczenia o udziale autorów w publikacji / Declaration of author contribution

Kupka D., Pan K., Gruba P. 2023. Initial responses of soil chemical properties to simulated warming in Norway spruce (*Picea abies* (L.) H.Karst.) stands in Western Carpathians. Geoderma, 432, 116400. DOI: 10.1016/j.geoderma.2023.116400

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, przeprowadzeniu doświadczenia w terenie, poborze materiału badawczego, analizach laboratoryjnych, opracowaniu i interpretacji wyników, przygotowaniu wstępnej wersji manuskryptu i edycji finalnej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, conducting the experiment in the field, collection of research material, laboratory analyses, preparation and interpretation of the results, preparation of the preliminary version of the manuscript and editing the final version of the manuscript.

Udział / Contribution: 65%

mgr inż. Dawid Kupka ..Dawid Kupka..............

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, administrowaniu projektem oraz recenzji ostatecznej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, administration of the project, and review of the final version of the manuscript.

Udział / Contribution: 5%

Prof. Kaiwen Pan ....

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, administrowaniu projektem, pracach terenowych, interpretacji wyników, recenzji i edycji wstępnej oraz ostatecznej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, administration of the project, field work, interpretation of the results, review and editing of the preliminary and final version of the manuscript.

Udział / Contribution: 30%

Prof. dr hab. inż. Piotr Gruba ....Piotr Gruba..................

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Kupka D., Pan K., Pietrzykowski M., Gruba P. 2023. Simulated warming decreases acidity of organic horizons of forest soils under Norway spruce (*Picea abies* (L.) H.Karst.) stands in the Western Carpathians. CATENA, 235, 107669. DOI: 10.1016/j.catena.2023.107669

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Udział / Contribution: 60%

mgr inż. Dawid Kupka ………………………………………

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Udział / Contribution: 5%

Prof. Kaiwen Pan ………………………………………

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia oraz recenzji ostatecznej wersji manuskryptu.

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Udział / Contribution: 10%

Prof. dr hab. inż. Marcin Pietrzykowski ………………………………………

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, administrowaniu projektem, pracach terenowych, interpretacji wyników, recenzji i edycji wstępnej oraz ostatecznej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, administration of the project, field work, interpretation of the results, review and editing of the preliminary and final version of the manuscript.

Udział / Contribution: 25%

Prof. dr hab. inż. Piotr Gruba ………………………………………

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Kupka D., Pan K., Pietrzykowski M., Kraj W., Gruba P. 2023. Effect of warming on ground vegetation in Carpathian Norway spruce stands, exemplified by European blueberry (*Vaccinium myrtillus* L.) nutrient stoichiometry. Science of the Total Environment, 896, 166396. DOI: 10.1016/j.scitotenv.2023.166396

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, przeprowadzeniu doświadczenia w terenie, poborze materiału badawczego, analizach laboratoryjnych, opracowaniu i interpretacji wyników, przygotowaniu wstępnej wersji manuskryptu i edycji finalnej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, conducting the experiment in the field, collection of research material, laboratory analyses, preparation and interpretation of the results, preparation of the preliminary version of the manuscript and editing the final version of the manuscript.

Udział / Contribution: 60%

mgr inż. Dawid Kupka ....Dawid Kupka..........

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, administrowaniu projektem oraz recenzji ostatecznej wersji manuskryptu.

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Udział / Contribution: 5%

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Udział / Contribution: 10%

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I declare that my contribution consisted of reviewing and editing the preliminary and final versions of the manuscript.

Udział / Contribution: 5%

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I declare that my contribution consisted of conceptual design of the experiment, administration of the project, field work, interpretation of the results, review and editing of the preliminary and final version of the manuscript.

Udział / Contribution: 20%

Prof. dr hab. inż. Piotr Gruba ……………………………………

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Kupka D., Gruba P. 2022. Effect of pH on the sorption of dissolved organic carbon derived from six tree species in forest soils. Ecological Indicators 140, 108975. DOI:10.1016/j.ecolind.2022.108975

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, przeprowadzeniu doświadczenia, analizach laboratoryjnych, opracowaniu i interpretacji wyników, przygotowaniu wstępnej wersji manuskryptu i edycji finalnej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, performance of the experiment, laboratory analyses, preparation and interpretation of the results, preparation of the preliminary version of the manuscript and editing of the final version of the manuscript.

Udział / Contribution: 60%

mgr inż. Dawid Kupka ....

Oświadczam, że mój wkład polegał na opracowaniu koncepcyjnym doświadczenia, administrowaniu projektem, pracach laboratoryjnych, interpretacji wyników, recenzji i edycji wstępnej oraz ostatecznej wersji manuskryptu.

I declare that my contribution consisted of conceptual design of the experiment, administration of the project, laboratory work, interpretation of the results, review and editing of the preliminary and final version of the manuscript.

Udział / Contribution: 40%

Prof. dr hab. inż. Piotr Gruba ....