Rozprawa doktorska

Uniwersytet Rolniczy im. Hugona Kołłątaja w Krakowie
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Rola korzeni w akumulacji węgla oraz w kształtowaniu aktywności mikrobiologicznej gleb leśnych

The role of roots in carbon accumulation and in shaping the microbiological activity of forest soils

Praca wykonana pod kierunkiem
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za ogromną cierpliwość, zrozumienie, ciągłą motywację do działania, obecność i wiarę

.

Streszczenie

Efektem zmian klimatu są coraz częściej występujące zaburzenia w postaci anomalii pogodowych, które mają wpływ na ekosystem leśny oraz środowisko glebowe. W prowadzonych badaniach coraz większą uwagę zwraca się na wpływ warunków termicznych i uwilgotnienia na stabilność drzewostanów i kształtowanie właściwości gleb, zwłaszcza na obieg węgla, azotu i fosforu. Gleba jest jednym z największych rezerwuarów węgla na Ziemi, a na jego zasoby wpływają takie czynniki środowiskowe, takie jak: klimat, skała macierzysta i roślinność. Gleby leśne charakteryzują się wysoką akumulacją węgla organicznego w powierzchniowych poziomach, co jest efektem oddziaływania roślinności poprzez dostarczaną biomasę nadziemną, systemy korzeniowe i ich wydzieliny. Celem niniejszych badań było określenie roli systemu korzeniowego wybranych gatunków drzew leśnych w kształtowaniu właściwości gleb, zwłaszcza stabilizacji glebowej materii organicznej oraz kształtowaniu aktywności mikrobiologicznej gleb. Zbadano wpływ symulowanej suszy oraz nadmiernej depozycji azotu na wydzieliny i cechy morfologiczne systemu korzeniowego wybranych gatunków liściastych i iglastych. Przeprowadzono cztery eksperymenty polowe, których efektem było potwierdzenie wpływu systemu korzeniowego na fizykochemiczne i biologiczne właściwości gleby leśnej. Systemy korzeniowe drzew oraz ich wydzieliny miały istotny wpływ na kształtowanie ilości i jakości glebowej materii organicznej wyrażonej składem frakcyjnym. Uzyskane wyniki wykazały ścisły związek między morfologią systemu korzeniowego, a aktywnością enzymatyczną oraz ilością bakterii i grzybów. Wykazano różnicę w składzie bakterii i grzybów dla gatunków iglastych, takich jak sosna i modrzew. Gleba w drzewostanach jesionowych charakteryzowała się dużą różnorodnością mikroorganizmów w porównaniu do pozostałych gatunków. W badaniach wykazano, że wyższa dawka azotu wpływała na zwiększenie ilości węgla uwalnianego wraz z wydzielinami drobnych korzeni oraz na ogólną morfologię korzeni. Nadmierna depozycja azotu miała istotny wpływ na ogólny stan odżywienia sadzonek buka zwyczajnego. Eksperyment z symulowaną suszą wykazał, że ograniczenie uwilgotnienia wpływało na większy przyrost korzeni w porównaniu do wariantu kontrolnego. Susza wpłynęła na ilość wydzielanego węgla wraz z wydzielinami korzeniowymi, co miało bezpośredni wpływ na zmianę aktywności enzymatycznej. Podsumowując, przeprowadzone badania wskazują na bardzo dużą rolę systemów korzeniowych i ich wydzielin w kształtowaniu właściwości gleb leśnych. Uzyskane wyniki mogą zostać praktycznie wykorzystane w planowaniu składu gatunkowego drzewostanu, co w konsekwencji może przełożyć się na poprawę stabilności ekosystemów leśnych.

Słowa kluczowe: aktywność enzymatyczna, cechy morfologiczne korzeni, glebowa materia organiczna, mikroorganizmy glebowe, właściwości gleb leśnych, wydzieliny korzeniowe

Summary

The effect of climate change is the increasing occurrence of disturbances in the form of weather anomalies, which affect the forest ecosystem and soil environment. In conducted research, increasing attention is being paid to the impact of thermal and moisture conditions on the stability of forest stands and the formation of soil properties, especially on the cycling of carbon, nitrogen and phosphorus. Soil is one of the largest carbon reservoirs on Earth, and its reserves are influenced by such environmental factors as climate, bedrock and vegetation. Forest soils are characterized by a high accumulation of organic carbon in the surface horizons as a result of the influence of vegetation through the aboveground biomass provided, root systems and their secretions. The purpose of the present study was to determine the role of the root system of selected forest tree species in shaping soil properties, especially stabilization of soil organic matter and shaping microbial activity of soils. The effects of simulated drought and excessive nitrogen deposition on the secretions and morphological characteristics of the root system of selected deciduous and coniferous species were investigated. Four field experiments were conducted, resulting in confirmation of the influence of the root system on the physicochemical and biological properties of the forest soil. The root systems of trees and their secretions had a significant impact on shaping the quantity and quality of soil organic matter expressed by fractional composition. The results showed a close relationship between the morphology of the root system and the enzymatic activity and amount of bacteria and fungi. A difference in the composition of bacteria and fungi was shown for coniferous species such as pine and larch. Soil in ash stands had a high diversity of microorganisms compared to other species. The study showed that a higher nitrogen rate increased the amount of carbon released with the secretions of fine roots and the overall root morphology. Excessive nitrogen deposition had a significant effect on the overall nutritional status of beech seedlings. An experiment with simulated drought showed that moisture limitation influenced greater root growth compared to the control variant. Drought affected the amount of carbon secreted with root exudates, which had a direct effect on the change in enzymatic activity. In conclusion, the conducted research indicates a very important role of root systems and their secretions in shaping the properties of forest soils. The results obtained can be practically used in planning the species composition of the forest stand, which can consequently translate into improved stability of forest ecosystems.

Keywords: Enzyme activity, root morphological characteristics, soil organic matter, soil microorganisms, properties of forest soils, root exudates

Spis treści

1. Struktura pracy

Przygotowana rozprawa doktorska ma formę spójnego tematycznie zbioru pięciu prac opublikowanych w różnych czasopismach naukowych:

Publikacja 1

Staszel K., Błońska E., Lasota J. 2022a. Fine root morphology and soil properties under influence of different tree stands along an altitudinal climosequence in the Carpathian mountains. Forest Ecosystems, 9, 100066. doi.org/10.1016/j.fecs.2022.100066
(MNiSW=140; IF:4.3)

Publikacja 2

Staszel K., Lasota J., Błońska E. 2022b. Soil organic matter fractions in relation to root characteristics of different tree species in altitude gradient of temperate forest in Carpathian Mountains. Forests, 13(10), 1656. doi.org/10.3390/f13101656
(MNiSW=100; IF:3.3)

Publikacja 3

Staszel-Szlachta K., Lasota J., Szlachta A. Błońska E. 2024. The impact of root systems and their exudates in different tree species on soil properties and microorganisms in a temperate forest ecosystem. BMC Plant Biology 24, 45. doi.org/10.1186/s12870-024-04724-2
(MNiSW=140; IF:5.3)

Publikacja 4

Staszel K., Lasota J., Błońska E. 2022c. Effect of drought on root exudates from *Quercus petraea* and enzymatic activity of soil. Scientific Reports, 12(1), 7635. doi.org/10.1038/s41598-022-11754-z
(MNiSW=140; IF:4.6)

Publikacja 5

Staszel-Szlachta K., Lasota J., Kempf M., Błońska, E. 2022d. Effect of nitrogen deposition on root systems and exudates of seedlings of beech *Fagus sylvatica* L. in a temperate climate. Sylwan, 166(12). doi.org/10.26202/sylwan.2023004
(MNiSW=70; IF:0.7)

2. Wprowadzenie

W efekcie zmian klimatu coraz częściej doświadczamy zaburzeń naturalnych w postaci długotrwałych susz, pożarów, gradacji owadów czy wichur, które mają wpływ na ekosystem leśny oraz środowisko glebowe. Coraz więcej uwagi poświęca się właściwościom gleb, zwłaszcza obiegowi węgla (Bowles i in. 2014). Gleba stanowi jeden z największych rezerwuarów węgla na Ziemi i szacuje się, że ilość węgla zawarta w glebie jest 2-3 krotnie wyższa, niż jego zawartość w nadziemnej biomasie roślinnej (Tolunay 2011, Fornara i in. 2011). Na zasób węgla (C) w glebie wpływa kilka czynników takich jak klimat, skała macierzysta oraz roślinność (Zhang i in. 2012; Bardelli i in. 2017; Błońska i in. 2017). Gleby leśne charakteryzują się wysoką akumulacją węgla organicznego w powierzchniowych poziomach, co jest efektem oddziaływania roślinności poprzez biomasę nadziemną oraz podziemną, tj. systemy korzeniowe i ich wydzieliny. Drzewostan poprzez zróżnicowany skład gatunkowy może modyfikować właściwości fizyczne, chemiczne i biochemiczne gleby leśnej (Staszel i in. 2022). Wyniki wcześniejszych badań wskazują, że zróżnicowanie gatunkowe drzewostanów wpływa na społeczność mikroorganizmów, prowadząc do zmian tempa rozkładu materii organicznej, zmieniając dostępność składników odżywczych i zdolność obszarów leśnych do magazynowania węgla (Zhang i in. 2019). Drzewa mogą oddziaływać na właściwości gleb poprzez dostarczaną do gleby substancję organiczną w efekcie oddziaływania systemów korzeniowych oraz ich wydzielin. Korzenie są kluczowym składnikiem podziemnego systemu, który stanowi podstawowe źródło glebowej materii organicznej i wpływa na aktywność mikrobiologiczną (Phillips i in. 2008; Keiluweit i in. 2015; Meier i in. 2020). Korzenie są jednym z ważniejszych elementów biomasy roślinnej, dzięki której mogą kontrolować pobór wody i składników odżywczych oraz mogą wpływać na cykle biogeochemiczne. Pomimo niewielkich rozmiarów, drobne korzenie stanowią główne źródło węgla w glebie, ze względu na coroczne zamieranie korzeni (Jackson i in. 1997; Jones i in. 2004). Ponadto ilość składników odżywczych uwalnianych w efekcie obumierania tej frakcji korzeni jest znacznie większa, niż w wyniku rozkładu resztek roślinnych (Yuan i Chen 2010). Kierunek wzrostu systemu korzeniowego jest uzależniony od rozmieszczenia składników pokarmowych i dostępności wody. W odpowiedzi na ilość składników pokarmowych zmianie ulega jego architektura oraz ilość substancji wydzielanych do gleby (Richardson i in. 2009; Freschet i in. 2017; Addo-Danso i in. 2018; Borden i in. 2020). Wydzieliny korzeniowe obejmują nielotne ryzodepozyty i rozpuszczalne związki organiczne, takie jak cukry, aminokwasy i kwasy organiczne (Dennis i in. 2010). Wydzieliny korzeniowe mogą być wykorzystywane przez drobnoustroje jako źródło węgla i są uważane za kluczowy wyznacznik struktury drobnoustrojów w ryzosferze (Kuzyakov i in. 2001; Kuzyakov 2006; Panchal i in. 2022). Wydzieliny odgrywają także istotną rolę w kształtowaniu aktywności glebowych enzymów zewnątrzkomórkowych,

w tym na ich ekspresję i ograniczenie aktywności w ryzosferze (Gianfreda 2015). Enzymy glebowe stanowią wskaźnik zapotrzebowania drobnoustrojów na składniki pokarmowe i intensywność procesów metabolicznych, odgrywają też ważną rolę w obiegu węgla organicznego (C), azotu (N), fosforu (P) oraz siarki (S) w glebie (Małek i in. 2021). Enzymy glebowe reagują na zachodzące zmiany klimatu, a badanie aktywności enzymatycznej może mieć znaczenie dla przewidywania kierunku tych zmian (Zuccarini i in. 2019). Aktywność mikroorganizmów wyrażona aktywnością enzymatyczną, może być wykorzystywana do oceny jakości i żyzności gleb, poprawności obiegu składników odżywczych i zmian jakie zachodzą w środowisku glebowym (Piaszczyk i in. 2019; Błońska i in. 2021). W wierzchniej warstwie gleby korzenie dostarczają mikroorganizmom substratów bogatych w C, które napędzają procesy rozkładu, w konsekwencji cykle biogeochemiczne są znacznie szybsze w ryzosferze, niż w otaczającej ją glebie (Finzi i in. 2015).

Korzenie wykazują wysoką aktywność fizjologiczną reagując na zmiany środowiskowe, takie jak susza lub nadmierne zakwaszenie gleb (Guo i in.. 2004; Hirano i in. 2007). Badania Trachsel i in. (2013) wskazują, że wskutek suszy lub niskiej depozycji azotu wytwarzają się drobne i długie korzenie, a także zmienia się ich rozmieszczenie w glebie, co ma znaczenie w transportowaniu składników pokarmowych. Na rozwój korzeni ma wpływ forma azotu, która przy udziale mikroorganizmów zostaje przekształcona w zredukowaną formę ułatwiającą jego przyswajalność (Philippot i in. 2007). Zhao i in. (2022) podkreślają zróżnicowane reakcje cech drobnych korzeni na depozycję N. Globalny wzrost depozycji azotu wpływa na rozbudowanie systemów korzeniowych i symbiontów związanych z ryzosferą, a także na dostępność składników odżywczych w glebie (Ma i in. 2021). W zależności od dostępności składników pokarmowych zawartych w glebie, aktywność mikroorganizmów może być zróżnicowana (Razaq i in. 2017). Wcześniej prowadzone badania wskazują, że nadmierne nawożenie azotem wpływa na różnorodność i biomasę mikroorganizmów, między innymi poprzez zmianę ich strategii życiowych (Zhou i in. 2015). Mikrobiota glebowa kształtowana jest głównie przez właściwości gleby, takie jak dostępność składników odżywczych, pH, zasolenie i wilgotność gleby, w wyniku czego mikroorganizmy te, mogą szybko reagować na zmianę warunków środowiskowych (Zhao i in. 2014; Li i in.. 2014).

Procesy zachodzące w ryzosferze odgrywają ważną rolę w globalnym cyklu C (Cheng i Kuzyakov 2005). Systemy korzeniowe wraz z wydzielinami są najważniejszym źródłem glebowej materii organicznej (SOM). SOM składa się z frakcji labilnej (fLF – lekka frakcja niezwiązana), która podlega szybkim przemianom i jest źródłem substratów dla mikroorganizmów co przekłada się na ich aktywność, a dodatkowo poprawia właściwości gleby w zakresie zdolności retencyjnych i dostępności mikroelementów (Guimarães i in. 2013). Kolejne to frakcja lekka związana (oLF) i frakcja ciężka (MAF), która jest silnie związana z cząstkami mineralnymi.

Frakcja MAF glebowej materii organicznej jest bardziej odporna na rozkład i przemiany mikrobiologiczne (Grunenberg i in. 2013; Pulido-Moncada i in. 2018). Węgiel związany w lekkiej frakcji materii organicznej może występować w glebie przez kilka lat, natomiast w ciężkiej frakcji może pozostać w glebie przez wielolecia (Lasota i in. 2020). Analiza składu frakcyjnego glebowej materii organicznej jest wykorzystywana jako wskaźnik zmian zachodzących w środowisku glebowym (Leifeld i Kögel-Knabner 2005). Gleby leśne zawierają więcej lekkiej frakcji glebowej materii organicznej, co jest efektem oddziaływania szaty roślinnej (Błońska i in. 2017). Skład frakcyjny glebowej materii organicznej uzależniony jest od wpływu składników uwalnianych w wyniku rozkładu ściółki różnych gatunków drzew. Odziaływanie gatunków iglastych i liściastych na skład frakcyjny materii organicznej jest różne. Gatunki iglaste (np. jodła) wpływają na wyższą średnią zawartość węgla lekkiej frakcji glebowej materii organicznej, podczas gdy liściaste (np. jesion) wpływają pozytywnie na zawartość C frakcji ciężkiej (Błońska i in. 2021).

3. Uzasadnienie wyboru tematu badawczego

Przeprowadzone badania są próbą uzupełnienia wiedzy dotyczącej wpływu systemu korzeniowego różnych gatunków drzew leśnych na jakość i ilość glebowej materii organicznej oraz na aktywność biologiczną gleby. Do tej pory nie prowadzono szczegółowych badań dotyczących charakterystyki systemów korzeniowych drzew leśnych i ich roli w kształtowaniu właściwości fizycznych, chemicznych i biologicznych gleb leśnych. W dotychczas przeprowadzonych badaniach więcej uwagi poświęca się glebie rolniczej, która istotnie różni się od gleby leśnej, co wynika z odmiennego użytkowania oraz wpływu roślinności. Nowatorskim elementem przeprowadzonych badań jest określenie roli wydzielin korzeniowych w kształtowaniu właściwości gleb leśnych. Dodatkowo w badaniach podjęto próbę określenia jak zaburzenia związane ze zmianami klimatu i antropopresją wpływają na cechy morfologiczne korzeni oraz na ich wydzieliny. Dotychczas nie wyjaśniono, jak obumierająca biomasa korzeni i wydzieliny różnych gatunków drzewiastych kształtują akumulację węgla organicznego i procesy mikrobiologiczne w glebie leśnej. Przypuszcza się, że wydzieliny korzeniowe mogą wpływać na stabilizację glebowego węgla organicznego. Korzenie drobne są jednymi z najbardziej aktywnych części systemu korzeniowego drzew i odgrywają kluczową rolę w cyklach biogeochemicznych w glebie. Korzenie mogą również stymulować aktywność drobnoustrojów, wpływając na aktywność enzymów glebowych, które aktywnie uczestniczą w obiegu podstawowych składników odżywczych, czy przetwarzaniu materii organicznej. Poznanie mechanizmów, które zachodzą w środowisku glebowym i czynników kontrolujących wydzielanie C jest niezwykle ważnym zagadnieniem w prawidłowym zarządzaniu lasami. Niewłaściwy dobór składu gatunkowego drzewostanu może prowadzić do zmian podstawowych właściwości gleb,

a w konsekwencji do zmian w aktywności i różnorodności zbiorowiska mikroorganizmów wpływając na obieg węgla.

4. Cel pracy

Celem badań było określenie roli systemu korzeniowego wybranych gatunków drzew leśnych oraz wydzielin korzeniowych w stabilizacji glebowej materii organicznej i kształtowaniu aktywności mikrobiologicznej gleb leśnych. W badaniach została określona relacja między biomasą korzeni, ich wydzielinami a składem frakcyjnym glebowej materii organicznej oraz liczebnością i zróżnicowaniem bakterii i grzybów w glebie leśnej. W badaniach szczególną uwagę zwrócono na rolę wydzielin korzeniowych w dostarczaniu substratów węglowych jako źródła energii dla mikroorganizmów. W badaniach testowano następujące hipotezy: 1) system korzeniowy, zwłaszcza biomasa drobnych korzeni wpływa na ilość i jakość glebowej materii organicznej; 2) biomasa korzeni oraz ich wydzieliny stymulują aktywność biochemiczną gleb leśnych wyrażoną aktywnością enzymatyczną; 3) ilość i zróżnicowanie bioty glebowej jest silnie skorelowane z cechami morfologicznymi korzeni; 4) w warunkach stresu spowodowanego suszą system korzeniowy dostarcza do gleby mniejszą ilość substratu węglowego, co przekłada się na aktywność enzymatyczną gleby; 5) depozycja azotu wpływa na wzrost i morfologię korzeni, a w konsekwencji na skład wydzielanych substancji dostarczanych do gleby.

Do sprawdzenia poprawności postawionych hipotez i weryfikacji określonego celu wykonano kilka doświadczeń, których rezultaty opublikowano w publikacjach naukowych przedstawionych poniżej.

Publikacja nr 1. Celem doświadczenia było określenie biomasy i morfologii korzeni buka zwyczajnego *(Fagus sylvatica* L.*)* i jodły pospolitej *(Abies alba* Mill.*)* rosnących w różnych warunkach klimatycznych, a także określenie tempa przyrostu systemów korzeniowych i ich tempa dekompozycji.

Publikacja nr 2. Celem doświadczenia było określenie wpływu systemów korzeniowych buka zwyczajnego *(Fagus sylvatica* L.*)* i jodły pospolitej *(Abies alba* Mill.*)* na skład frakcyjny materii organicznej gleb.

Publikacja nr 3. Celem doświadczenia było określenie roli systemów korzeniowych sosny zwyczajnej (*Pinus sylvestris* L.), modrzewia europejskiego (*Larix decidua* Mill.), dębu szypułkowego (*Quercus robur* L.), jesionu wyniosłego (*Fraxinus excelsior* L.), buka zwyczajnego (*Fagus sylvatica* L.) i grabu pospolitego (*Carpinus betulus* L.) w kształtowaniu ilości i różnorodności mikroorganizmów glebowych.

Publikacja nr 4. Celem doświadczenia było określenie zmian morfologii korzeni sadzonek dębu bezszypułkowego (*Quercus petraea* (Matt.) Liebl) wzrastających w warunkach symulowanej

suszy. Dodatkowo przeanalizowano zmiany aktywności enzymów glebowych oraz wydzielin korzeniowych w odpowiedzi na suszę.

Publikacja nr 5. Celem doświadczenia było określenie wpływu różnych dawek azotu na morfologię korzeni buka zwyczajnego *(Fagus sylvatica* L) i jego wydzieliny. Dodatkowo określono stan odżywienia sadzonek buka w efekcie zastosowania różnych dawek azotu.

5. Charakterystyka terenu badań

Badania, których wyniki przedstawiono w publikacji nr 1 (Staszel i in. 2022a) oraz publikacji nr 2 (Staszel i in. 2022b) przeprowadzano na terenie Nadleśnictwa Jeleśnia w Masywie Jałowca w Beskidzie Żywieckim na południu Polski (Ryc. 1) na trzech poletkach doświadczalnych zlokalizowanych na różnej wysokości: 600, 800 i 1000 m n.p.m. Na tym obszarze występowały gleby brunatne o podobnym składzie granulometrycznym określonym na podstawie analiz laboratoryjnych – średnia zawartość piasku wynosiła 54%, iłu 42% i gliny 3%. Średnia temperatura sezonu wegetacyjnego zmniejszała się wraz ze wzrostem wysokości n.p.m., na której lokalizowano powierzchnie badawcze, wynosząc odpowiednio 12.4, 11.3 i 10.2 ˚C (Plan Urządzania Lasu Nadleśnictwa Jeleśnia na lata 2015/2024), natomiast średnia wilgotność gleby określona w momencie pobierania próbek gleby zwiększała się wynosząc odpowiednio 22.74, 29.60 i 34.10%. W każdym wariancie wysokości n.p.m. poletka doświadczalne zostały założone w 60-letnim drzewostanie bukowym (*Fagus sylvatica* L.) i jodłowym (*Abies alba* Mill.).

Ryc. 1 Lokalizacja powierzchni badawczych na tle podziału administracyjnego Lasów Państwowych

Badania opisane w publikacji nr 3 (Staszel-Szlachta i in. 2024) prowadzono na powierzchniach doświadczalnych Katedry Ekologii i Hodowli Lasu w Krakowie. Powierzchnie zlokalizowane były 25 km na północ od Krakowa, w zwartym kompleksie leśnym na terenie Nadleśnictwa Miechów (Leśnictwo Goszcza) (Ryc. 1). Badaniami objęto sześć gatunków drzew – sosnę zwyczajną (*Pinus sylvestris* L.), modrzewia europejskiego (*Larix decidua* Mill.), dęba szypułkowego (*Quercus robur* L.), jesiona wyniosłego (*Fraxinus excelsior* L.), buka zwyczajnego (*Fagus sylvatica* L.) i graba pospolitego (*Carpinus betulus* L.). Powierzchnie doświadczalne zakładano w jednogatunkowych drzewostanach o zbliżonym wieku (70–80 lat), bez domieszek innych gatunków. Teren badań charakteryzował się występowaniem gleb płowych wykształconych z jednorodnych pokryw lessowych.

Doświadczenie, którego wyniki przedstawiono w publikacji nr 4 (Staszel i in. 2022c) zostało przeprowadzone w Laboratorium Geochemii Środowiska Leśnego i Terenów Przeznaczonych do Rekultywacji na Wydziale Leśnym Uniwersytetu Rolniczego im. Hugona Kołłątaja w Krakowie. W doświadczeniu wykorzystano 3-miesięczne sadzonki dębu bezszypułkowego (*Quercus petreae* (Matt.) Liebl.). W doświadczeniu hodowane sadzonki poddano oddziaływaniu symulowanej suszy, wykorzystując substraty o różnym stopniu uwilgotnienia (25 i 55%).

Doświadczenie prezentowane w publikacji nr 5 (Staszel-Szachta i in. 2022d) przeprowadzono na terenie Leśnego Zakładu Doświadczalnego Uniwersytetu Rolniczego w Krakowie na szkółce w Kopciowej (Ryc. 1). Średni okres wegetacyjny na tym terenie wynosi 164 dni. Badania przeprowadzono na sadzonkach buka zwyczajnego (*Fagus sylvatica* L.) nawożonych trzema różnymi dawkami azotu 0,75, 2,25 oraz 4,5 kg·ha^{-1}. Dodatkowo w badaniach uwzględniono wariant kontrolny, tj. bez zastosowania nawożenia. Jako podłoże wykorzystano standardową mieszaninę trocin jodłowo-świerkowych i torfu wysokiego w stosunku 1:1.

6. Metodyka badań i wyniki

6.1. Morfologia systemów korzeniowych wybranych gatunków drzew wzrastajacych w zróżnicowanych warunkach klimatycznych

W pracy *Staszel K., Błońska E., Lasota J. 2022a. Fine root morphology and soil properties under influence of different tree stands along an altitudinal climosequence in the Carpathian mountains,* została przeanalizowana szczegółowa morfologia korzeni buka zwyczajnego *(Fagus sylvatica* L.*)* i jodły pospolitej *(Abies alba* Mill.*)* w odniesieniu do podstawowych właściwości gleb, zlokalizowanych w gradiencie wysokości. Powierzchnie badawcze zostały zlokalizowane w trzech różnych lokalizacjach tj. na 600, 800 i 1000 m n.p.m. W każdym wariancie wysokości wytypowano trzy powierzchnie dla każdego gatunku w trzech powtórzeniach. Próbki gleby pobierano po usunięciu poziomu ścioły do głębokości 15 cm. W terenie próbki zostały zaetykietowane, zapakowane do worków foliowych i przetransportowane do laboratorium. Próbki

gleb zostały wysuszone i przesiane przez sito o średnicy oczek 2 mm. W przesuszonych próbkach gleby oznaczono pH metodą potencjometryczną w wodzie i 1M KCl. Kwasowość hydrolityczną (Y) oznaczono metodą Kappena. Całkowitą zawartość azotu i węgla oznaczono za pomocą analizatora LECO CNS True Mac Analyzer (Leco, St. Joseph, MI, USA). Do oznaczenia ilości kationów zasadowych (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) wykorzystano analizator ICP-OES (iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK). Dodatkowo z każdego poletka badawczego pobrano próbki gleb o znanej objętości (15x15x15 cm) w trzech powtórzeniach w celu określenia biomasy korzeni. Z próbek wyodrębniono korzenie o średnicy >2 mm i korzenie drobne o średnicy <2 mm. Wyekstrahowane fragmenty systemu korzeniowego skanowano w rozdzielczości 800 dpi, a następnie analizowano za pomocą skanera i programu WinRhizo™ Pro 2003b (Regent Instruments Inc., Ville de Québec, QC, Kanada). W trakcie analizy określono podstawowe cechy morfologiczne korzeni, tj. długość korzeni, średnicę, powierzchnię korzeni. Korzenie suszono w temperaturze 70°C przez 24 godziny, a następnie ważono. Na podstawie uzyskanych wyników obliczono wskaźniki takie jak: wskaźnik zagęszczenia (root tissue density - RTD) ($kg \cdot m^{-3}$), wskaźnik powierzchni korzeni (specific root area - SRA) ($m^2 kg^{-1}$) i wskaźnik długości korzenia (specific root length - SRL) ($m\ g^{-1}$) (Ostonen i in. 1999). Dodatkowo obliczono biomasę korzeni drobnych. W dalszym etapie przeanalizowane korzenie wykorzystano do określenia tempa ich dekompozycji. Worki (15 x 20 cm) z siatki o drobnych oczkach zawierające 10 gramów korzeni, zakopano na głębokości 10 cm na każdym poletku badawczym. Metodą rdzeniową określano roczny przyrost biomasy drobnych korzeni (o średnicy <2,0 mm) (Böhm 1979). W celu określenia wzajemnych zależności pomiędzy poszczególnymi parametrami korzeni, a właściwościami gleby obliczono współczynniki korelacji Spearmana. Do potwierdzenia zależności między właściwościami gleb i korzeni wykorzystano analizę głównych składowych (PCA). Do zbadania wpływu wysokości i różnych gatunków drzew oraz interakcji tych dwóch czynników na biomasę i morfologię drobnych korzeni wykorzystano ogólny model liniowy (GLM). Do oceny różnic pomiędzy średnimi wartościami cech użyto testu Tukeya. Wyniki uznano za istotne statystycznie przy $\alpha < 0.05$.

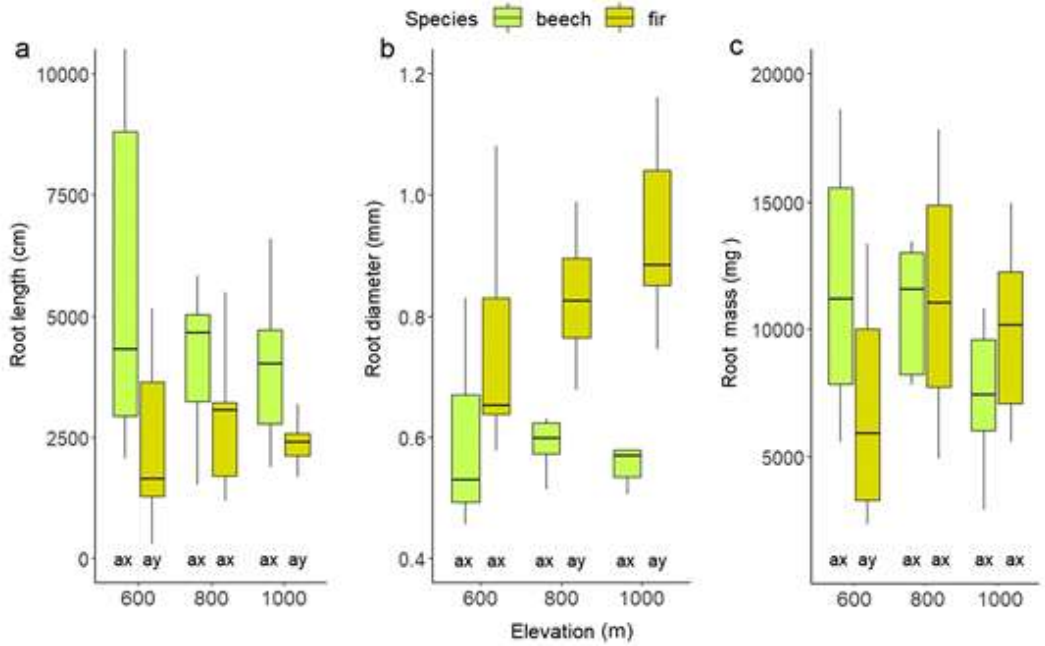
Analiza GLM potwierdziła znaczenie lokalizacji w gradiencie wysokości na biomasę korzeni i przyrost korzeni badanych gatunków drzew. Gatunek drzewa miał istotne znaczenie dla kształtowania się długości i średnicy korzeni, SRL oraz przyrostu korzeni. Dla biomasy korzeni odnotowano jednoczesne znaczenie gatunku drzewa i położenia w gradiencie wysokości (Tab. 1).

Tabela 1. Podsumowanie analizy GLM dla parametrów korzeni, na które wpływają różne gatunki drzew w gradiencie wysokości (m n.p.m.)

Parametr	Źródło zmienności - test F (poziom istotności *p*)		
	Wysokość n.p.m. (W)	Gatunek (G)	Interakcja W x G
Sumaryczna długość korzeni [cm]	0,8431 (0,437)	8,8972 (***0,005***)	2,0804 (0,136)
Średnica korzeni [mm]	0,0211 (0,307)	0,9214 (***<0,001***)	0,0345 (0,148)
Sucha masa korzeni [mg]	1,6969 (0,194)	0,4913 (0,487)	4,2219 (***0,021***)
Wskaźnik powierzchni korzeni (SRA) [m^2kg^{-1}]	1,2995 (0,282)	3,0327 (0,088)	2,1182 (0,131)
Wskaźnik długości korzeni (SRL) [$m\ g^{-1}$]	0,6782 (0,512)	13,5058 (***<0,001***)	1,3166 (0,278)
Wskaźnik zagęszczenia korzeni (RTD) [$kg \cdot m^{-3}$]	1,3633 (0,266)	0,2692 (0,606)	1,9940(0,147)
Biomasa korzeni w określonej próbce gleby [$g \cdot dm^{-3}$]	5,1241 (***0,010***)	0,0559 (0,814)	3,1443 (0,052)
Tempo rozkładu korzeni [%]	2,3459 (0,107)	0,3806 (0,540)	0,3560 (0,702)
Przyrost korzeni [$g \cdot m^{2} \cdot rok^{1}$]	3,7390 (***0,031***)	11,2197 (***0,002***)	0,6031 (0,551)

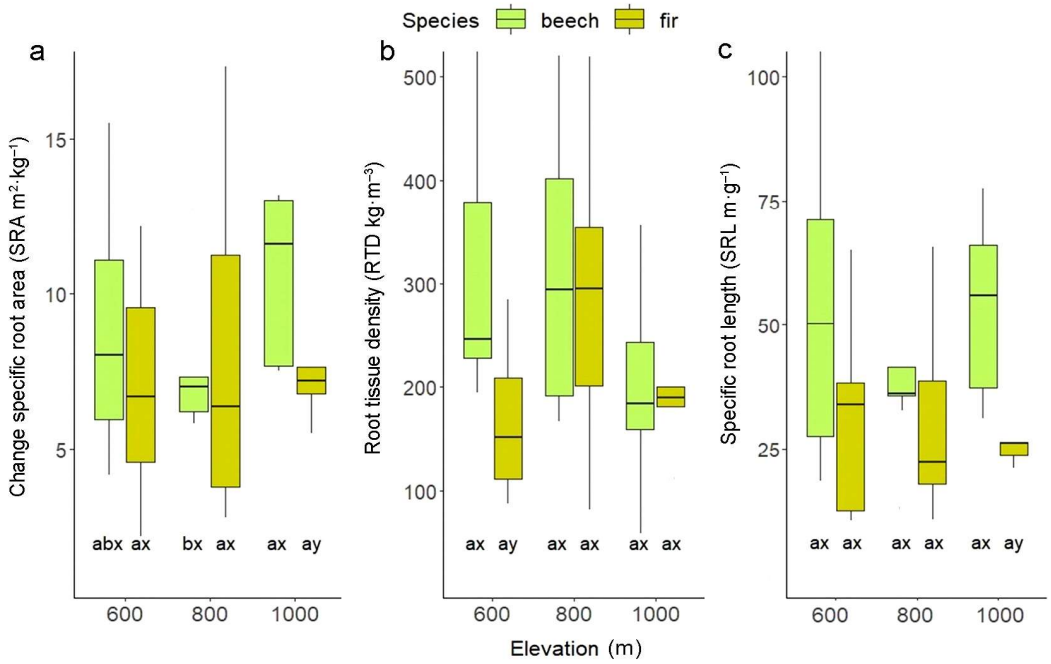
Istotne różnice ***(p < 0.05)***

Badane gatunki drzew różniły się istotnie sumaryczną długością korzeni, która dla buka wahała się od 6452,58 do 3957,80 cm, a dla jodły od 2269,63 do 2475,62 cm na wysokości 600 i 1000 m n.p.m. U obu gatunków zaobserwowano tendencję do zmniejszania się sumarycznej długości korzeni wraz ze wzrostem wysokości n.p.m. (Ryc.2a). Z kolei średnica korzeni jodły zwiększała się wraz z wysokością n.p.m. od 0,77 do 0,93 mm, jednak nie była to różnica istotna (Ryc.2b). Statystycznie istotną różnicę między gatunkami odnotowano dla średnicy korzeni na wysokościach 800 i 1000 m n.p.m., natomiast dla masy korzeni tylko na wysokości 600 m n.p.m. (Ryc.2c).



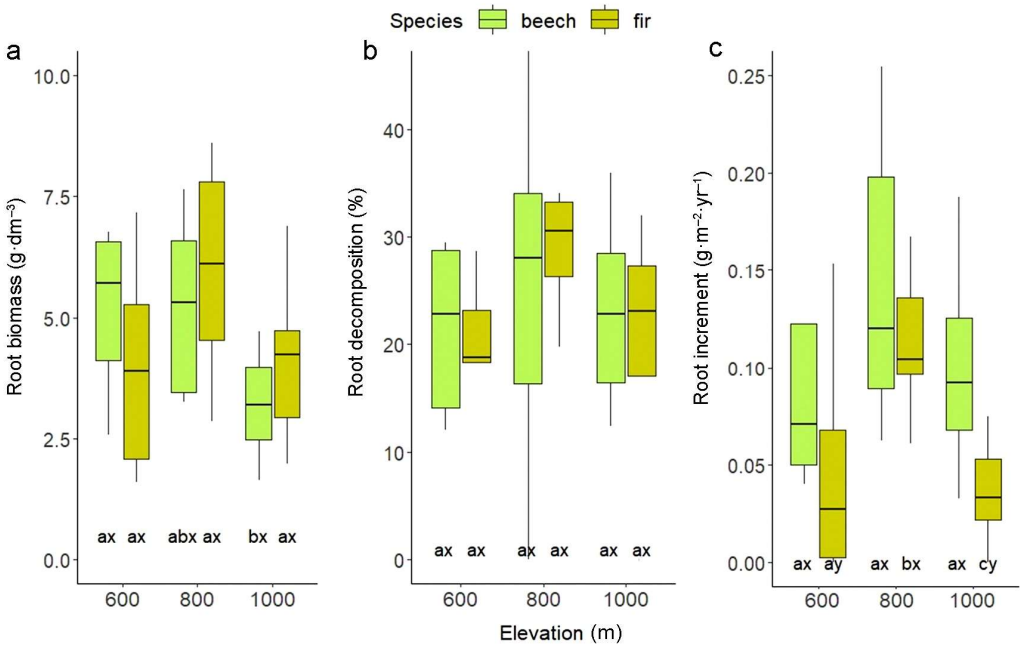
Ryc. 2 a-Długość korzeni (cm), b-średnica korzeni (mm) i c- sucha masa korzeni (mg) w różnym gradiencie wysokości. Różne małe litery (a, b, c) wskazują na istotne różnice parametrów pomiędzy różnymi wysokościami, (x, y) wskazują na istotne różnice parametrów pomiędzy gatunkami; TukeyHSD p < 0,05 (wykresy wąsów pudełkowych z medianą, 25- i 75- percentyle).

W przypadku SRA istotną różnicę między badanymi gatunkami odnotowano na poletku zlokalizowanym na wysokości 1000 m n.p.m. (średnio dla jodły 7,34 i buka 11,70 $m^2 kg^{-1}$). SRA korzeni jodły nie uległa istotnym zmianom wraz ze wzrostem wysokości n.p.m. (Ryc.3a). Istotnie najwyższą wartość SRA dla buka uzyskano na wysokości 1000 m n.p.m., natomiast wartości RTD i SRL dla obu gatunków nie zmieniały się znacząco w gradiencie wysokości, z wyjątkiem w RTD w drzewostanach jodłowych na wysokości 600 i 1000 m n.p.m. (Ryc.3b). Stwierdzono istotne różnice w wartościach SRL między bukiem a jodłą, niezależnie od lokalizacji n.p.m. poletka doświadczalnego (Ryc. 3c).



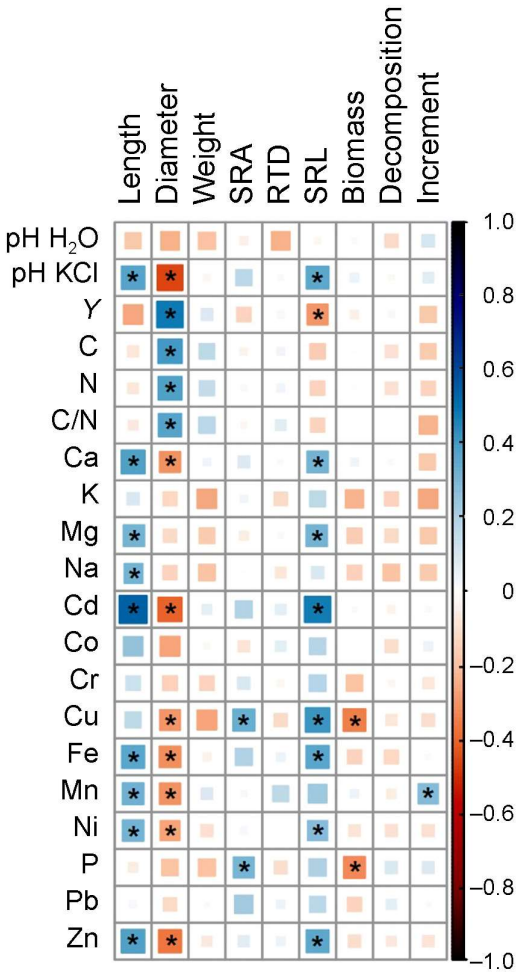
Ryc. 3 a-Zmiana wskaźnika powierzchni korzeni (SRA), wskaźnik gęstości tkanki korzeni (RTD) i wskaźnik długości korzeni (SRL) w gradiencie wysokości. Różne małe litery (a, b, c) wskazują na istotne różnice parametrów pomiędzy różnymi wysokościami, (x, y) wskazują na istotne różnice parametrów pomiędzy gatunkami; Tukey HSD $p < 0,05$.

Badane gatunki nie różniły się istotnie biomasą korzeni, niezależnie od położenia w gradiencie wysokości, podobnie jak jodła rozpatrywana indywidualnie. Wyraźnie najniższą biomasę korzeni u buka odnotowano na najwyżej położonych stanowiskach (średnia od 5,66 do 3,27 $g \cdot dm^{-3}$) (Ryc.4a). Nie stwierdzono znaczącej różnicy wynikającej z wpływu gatunku i wysokości n.p.m. na szybkość rozkładu korzeni, która oscylowała od 24,38 do 29,24% dla buka, oraz od 19,48 do 31,35% dla jodły, rosnącej na wysokości 800 m n.p.m. (Ryc.4b). W przypadku przyrostu korzeni istotną statystycznie różnicą międzygatunkową odnotowano na poletkach zlokalizowanych na wysokościach 600 i 1000 m n.p.m. (Ryc. 4c).



Ryc. 4 a- Biomasa korzeni, b-rozkład korzeni, c-przyrost korzeni w gradiencie wysokości. Różne małe litery (a, b, c) wskazują na istotne różnice parametrów pomiędzy różnymi wysokościami, (x, y) wskazują na istotne różnice parametrów pomiędzy gatunkami; TukeyHSD $p < 0.05$.

Przeprowadzone analizy wskazują na różnicę we właściwościach fizykochemicznych gleby, wynikające z oddziaływania różnych gatunków drzew i ich systemów korzeniowych oraz położenia w gradiencie wysokości. Między próbkami gleby pobranymi z poletek z badanymi gatunkami drzew odnotowano istotną statystycznie różnicę w zawartości azotu. Na wysokości 600 m n.p.m. gleba pod wpływem oddziaływania buka charakteryzowała się wyższą zawartością N w porównaniu z glebą pod wpływem oddziaływania jodły. Na wysokości 800 m n.p.m. odnotowano różnicę między badanymi gatunkami w parametrach gleby w odniesieniu do pH w KCl, zawartości C i N, stosunku C/N oraz zawartości Mg, K i Na. W przypadku mikroelementów odnotowano mniej wyraźne znaczenie lokalizacji w gradiencie wysokości. Stwierdzono natomiast związek między cechami korzeni a właściwościami gleby w gradiencie wysokości. Długość korzeni korelowała dodatnio z pH oraz zawartością Ca, Mg, Na, Cd, Fe, Mn, Ni i Zn. Średnica korzeni korelowała dodatnio z kwasowością hydrolityczną, zawartością C, N i stosunkiem C/N, a ujemnie z pH, zawartością Ca, Cd, Cu, Fe, Mn, Ni i Zn. Zawartość Cu i Pb korelowała dodatnio z SRA i ujemnie z biomasą korzeni. SRL wykazywało dodatnią korelację z pH, zawartością Ca, Mg, Cd, Cu, Fe, Ni i Zn (Ryc.5). Przeprowadzona analiza PCA potwierdziła zależność między parametrami opisującymi morfologię korzeni, a właściwościami gleby. Ponadto wynik PCA potwierdził różnicę między drzewostanami bukowymi i jodłowymi, a czynniki 1 i 2 wyjaśniły 46.8% wariancji badanych cech.



Ryc. 5. Korelacja pomiędzy parametrami korzeni a właściwościami gleby; * wartości istotne dla $p \leq 0{,}05$

Przeprowadzone badania potwierdzają różnice w cechach morfologicznych korzeni jodły i buka oraz wpływ warunków klimatycznych w kształtowaniu biomasy i pozostałych parametrów korzeni. Dodatkowo odnotowano efekt gradientu wysokościowego w kształtowaniu się podstawowych właściwości gleby. Wraz ze spadkiem temperatury i skróceniem okresu wegetacyjnego w wyższych położeniach górskich, natężenie procesu dekompozycji korzeni ulegało ograniczeniu, obniżało się pH gleby oraz pogarszały warunki dla rozwoju systemu korzeniowego. Na najwyżej położonych stanowiskach badawczych, charakteryzujących się bardziej surowym klimatem, uzyskano niższą biomasę korzeni i mniejszy ich przyrost. Ograniczenie przyrostu korzeni dotyczyło obu badanych gatunków, natomiast w przypadku buka zaobserwowano większe możliwości adaptacyjne do zmieniających się warunków środowiskowych. Na podstawie przeprowadzonej szczegółowej charakterystyki cech morfologicznych korzeni oraz przyrostu korzeni można przypuszczać, że w przypadku ocieplenia klimatu buk będzie zdolny do zwiększania swojego zasięgu występowania.

6.2. Skład frakcyjny glebowej materii organicznej w odniesieniu do cech korzeni różnych gatunków drzew w gradiencie wysokości

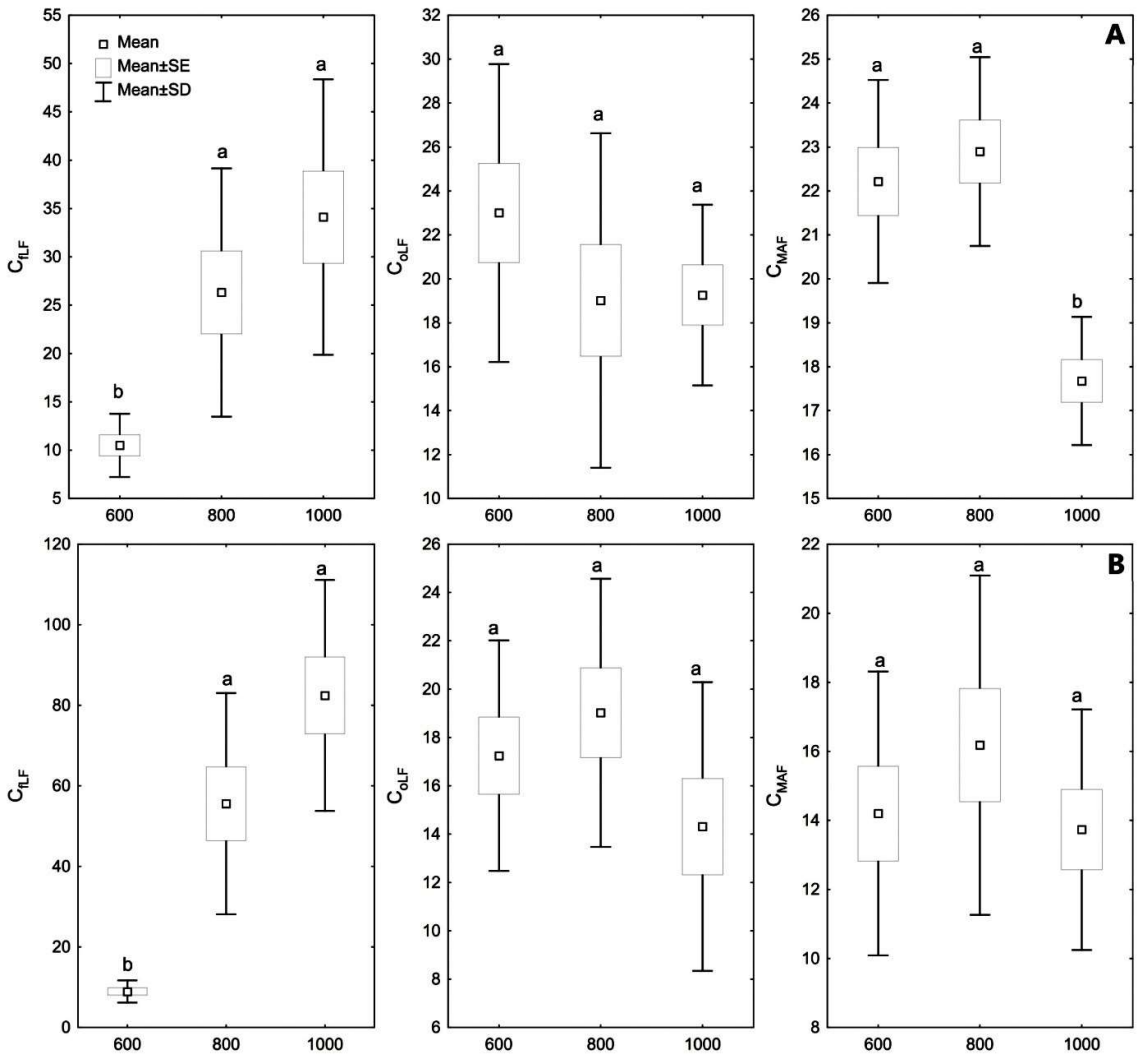
Zmiana składu frakcyjnego glebowej materii organicznej w zależności od cech morfologicznych korzeni wybranych gatunków drzew w gradiencie wysokości została przedstawiona w pracy: *Staszel K., Lasota J., Błońska E. 2022b. Soil organic matter fractions in relation to root characteristics of different tree species in altitude gradient of temperate forest in Carpathian Mountains.* Analizę gleby i systemów korzeniowych przeprowadzono zgodnie z metodyką opisaną w podrozdziale *6.1.* Dodatkowo w próbkach gleby pobranych z różnych drzewostanów w gradiencie wysokości przeprowadzono analizę fizycznego frakcjonowania glebowej materii organicznej według metody opisanej przez Sohi i in. (2001). Próbkę gleby (15 g) umieszczono w 200 ml probówce wirówkowej i dodano 90 ml NaI (1,7 g cm^{-3}). Każdą probówkę delikatnie wytrząsano początkowo przez 1 minutę a następnie wirowano przez 30 minut. Wolną frakcję lekką (fLF) zebrano na filtrze z włókna szklanego. Pozostałą glebę na dnie probówek wirówkowych zmieszano z kolejną 90 ml porcją NaI i poddano sonikacji (60 watów przez 200 s) w celu zniszczenia agregatów. Po odwirowaniu, uwolnioną z agregatów frakcję lekką związaną (oLF) – zbierano na filtrze z włókna szklanego. Założono, że pozostałą frakcję stanowi frakcja ciężka związana z minerałami (MAF). Po wysuszeniu (50°C) podpróbki różnych frakcji materii organicznej zważono i analizowano pod kątem zawartości węgla (C_{fLF}, C_{oLF} i C_{MAF}) oraz azotu (N_{fLF}, N_{oLF} i N_{MAF}) za pomocą analizatora LECO CNS True Mac Analyzer (Leco, St. Joseph, MI, USA). Uzyskane wyniki frakcjonowania glebowej materii organicznej, właściwości gleb oraz cech systemów korzeniowych przeanalizowano z wykorzystaniem narzędzi statystycznych. Do oceny istotności różnic między parametrami gleby wykorzystano analizę ANOVA. Obliczono korelację *r*-Pearsona, pomiędzy składem frakcyjnym glebowej materii organicznej a badanymi właściwościami gleby oraz cechami korzeni. Do zbadania wpływu gatunku drzewa i wysokości n.p.m. na zawartość C i N w różnych frakcjach glebowej materii organicznej wykorzystano ogólny model liniowy (GLM). Do interpretacji zależności między gatunkiem drzewa i wysokością n.p.m. a składem frakcyjnym i cechami korzeni wykorzystano analizę głównych składowych (PCA). Różnice przy $p \leq 0{,}05$ uznawano za istotne statystycznie. Gleba na wysokości 600 m n.p.m. charakteryzowała się wyższym pH w porównaniu do gleby zlokalizowanej na wysokości 800 i 1000 m n.p.m. Zawartość C i N wzrastała wraz ze wzrostem wysokości n.p.m. Istotnie wyższą zawartość C i N stwierdzono w glebach położonych na wysokości 1000 m n.p.m. Zawartość kationów zasadowych zmniejszała się wraz ze wzrostem wysokości n.p.m. Istotnie wyższą zawartość kationów odnotowano w glebach położonych najniżej w gradiencie wysokości (Tab. 2).

Tabela 2. Właściwości chemiczne badanych gleb będących pod wpływem różnych gatunków w gradiencie wysokości

Gatunek	Wysokość	pH		Y	C	N	C/N	Ca	Mg	K	Na
		H_2O	KCl								
Buk	600	4,84± 1,04[a]	4,18± 0,40[a]	2,42± 0,53[a]	5,23± 0,91[b]	0,37± 0,05[b]	14,12± 1,58[a]	142,46± 70,39[a]	17,47± 8,41[a]	11,35± 2,49[a]	1,13± 0,71[a]
	800	4,09± 0,19[b]	3,45± 0,12[b]	4,90± 1,02[b]	6,72± 1,65[ab]	0,43± 0,09[ab]	15,65± 0,81[ab]	19,49± 7,62[b]	4,17± 0,92[b]	6,56± 1,64[b]	0,65± 0,11[b]
	1000	4,04± 0,25[b]	3,4± 0,19[b]	5,32± 0,98[b]	8,80± 2,02[a]	0,49± 0,09[a]	17,89± 1,76[b]	9,99± 4,14[b]	3,64± 0,64[a]	7,44± 2,45[b]	0,70± 0,09[b]
Jodła	600	4,91± 0,20[a]	3,91± 0,18[a]	2,61± 0,37[a]	4,51± 0,62[b]	0,32± 0,04[b]	14,01± 0,61[a]	93,76± 28,64[a]	12,89± 4,19[a]	11,63± 3,25[b]	0,83± 0,07[b]
	800	3,94± 0,15[b]	3,27± 0,14[b]	5,83± 1,04[b]	10,44± 2,92[b]	0,55± 0,10[a]	18,66± 1,81[b]	14,57± 5,72[b]	4,18± 1,09[b]	7,15± 3,68[a]	0,93± 0,15[a]
	1000	3,65± 0,08[b]	3,02± 0,11[b]	8,69± 1,71[b]	14,81± 5,60[a]	0,75± 0,23[a]	19,64± 1,31[b]	17,71± 16,92[b]	5,60± 2,09[ab]	8,43± 4,33[b]	1,24± 0,32[ab]

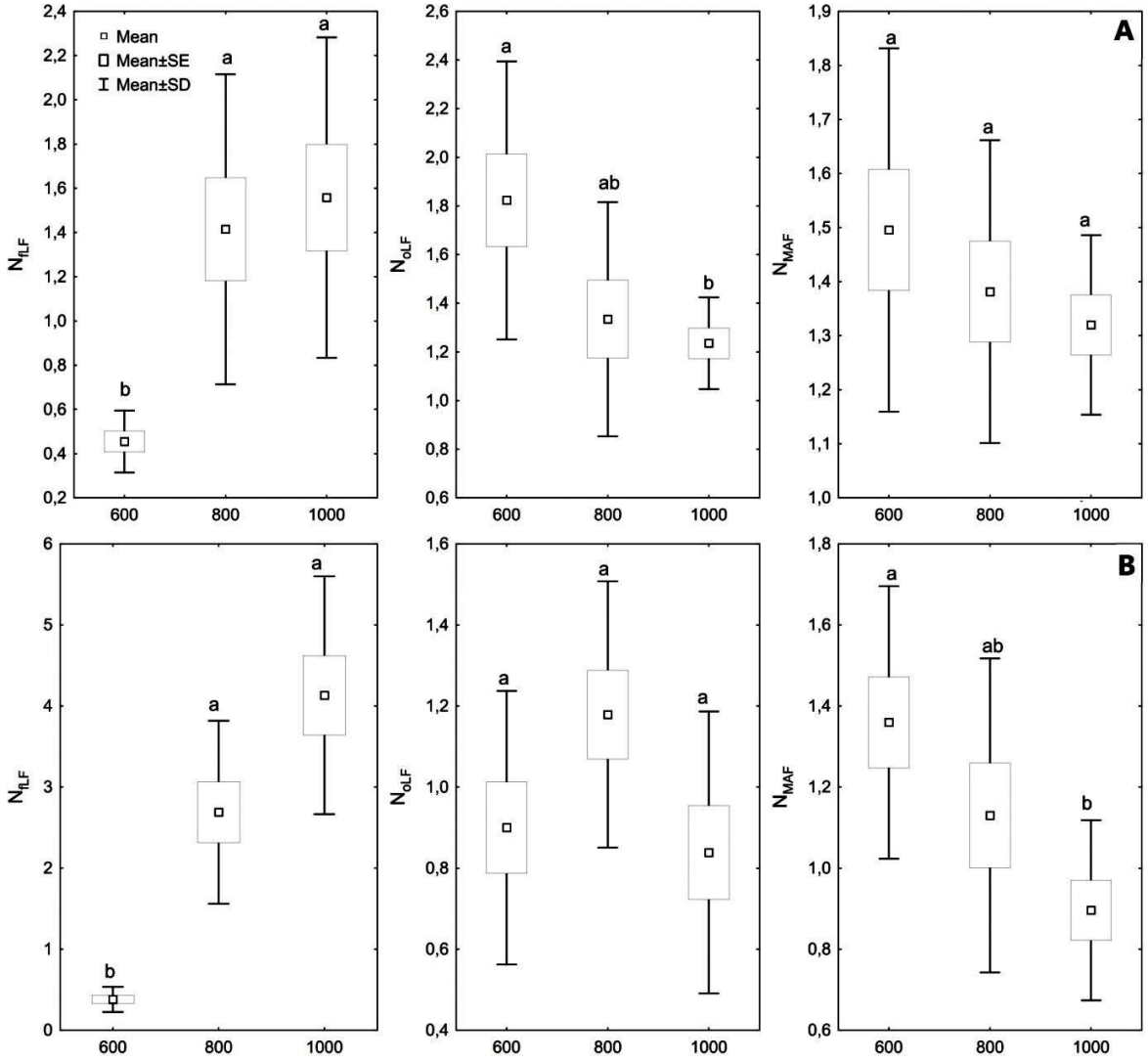
Średnia±błąd stan.; C, N (%); Ca, K, Mg, Na (cmol(+)·kg^{-1}); Y – kwasowość hydrolityczna (cmol(+)·kg^{-1}); małe litery w górnym indeksie wartości średnich oznaczają istotne różnice w gradiencie wysokości (a, b); TukeyHSD<0.05

W przeprowadzonym doświadczeniu stwierdzono różnice w składzie frakcyjnym materii organicznej w glebie pozostającej pod wpływam oddziaływania różnych gatunków drzew rosnących na różnych wysokościach n.p.m. Niezależnie od gatunku drzewa zawartość C_{fLF} wzrastała wraz z wysokością n.p.m. Istotnie niższą zawartość C_{fLF} stwierdzono w glebie na najniżej położonych poletkach doświadczalnych. Nie odnotowano znaczących różnic w zawartości C_{oLF} wzdłuż gradientu wysokości, chociaż zawartość C_{oLF} wykazywała tendencję spadkową. Dla buka odnotowano istotnie niższą zawartość C_{MAF} w glebie na poletkach zlokalizowanych najwyżej, natomiast dla jodły występowała tendencja spadkowa zawartości C_{MAF} w glebie wzdłuż gradientu wysokości. Obecność buka wpływała na wyższą zawartość C_{MAF} w glebie w porównaniu z jodłą (Ryc. 6).



Ryc. 6 Zmiana zawartości węgla w frakcji glebowej materii organicznej w glebach w gradiencie wysokości i w różnych drzewostanach (C_{fLF} – węgiel wolnej frakcji lekkiej ($g \cdot kg^{1}$), C_{oLF} – węgiel okludowanej frakcji lekkiej ($g \cdot kg^{-1}$), C_{MAF} – węgiel frakcji związanej z minerałami ($g \cdot kg^{-1}$); (A) – gleby pod wpływem drzewostanów bukowych, (B) – gleby pod wpływem drzewostanów jodłowych; małe litery oznaczają istotne różnice w gradiencie wysokości (a, b)).

Zawartość N_{fLF} w glebie wzrastała wraz z gradientem wysokości niezależnie od gatunku drzewa. Istotnie niższą zawartość N_{fLF} odnotowano na poletkach doświadczalnych położonych najniżej (600 m n.p.m.). Dla buka istotnie niższą zawartość N_{oLF} stwierdzono w glebie na poletkach położonych najwyżej (1000 m n.p.m.). Nie stwierdzono istotnych różnic w zawartości N_{oLF} w glebie z jodłą wzdłuż gradientu wysokości. Istotnie niższą zawartość N_{MAF} odnotowano w glebie z jodłą dla najwyżej położonych poletek doświadczalnych (Ryc. 7).



Ryc. 7. Zmiana zawartości azotu w frakcji glebowej materii organicznej w glebach w gradiencie wysokości i w różnych drzewostanach (N_{fLF} – azot wolnej frakcji lekkiej ($g \cdot kg^{1}$), N_{oLF} – azot okludowanej frakcji lekkiej ($g \cdot kg^{-1}$), N_{MAF} – azot frakcji związanej z minerałami ($g \cdot kg^{-1}$); (A) – gleby pod wpływem drzewostanów bukowych, (B) – gleby pod wpływem drzewostanów jodłowych; małe litery oznaczają istotne różnice w gradiencie wysokości (a, b)).

Skład frakcji glebowej materii organicznej był silnie skorelowany z właściwościami badanych gleb. Zawartość C_{fLF} i N_{fLF} była ujemnie skorelowana z pH gleby i zawartością kationów zasadowych. Zawartość C_{fLF} i N_{fLF} była istotnie dodatnio skorelowana z kwasowością hydrolityczną oraz zawartością C i N. Zawartość N_{oLF} była ujemnie skorelowana z kwasowością hydrolityczną i zawartością C oraz dodatnio z pH oraz zawartością Ca i Mg. Zawartość C_{MAF} i N_{MAF} korelowała z zakwaszeniem gleby oraz zawartością C i N. Skład frakcyjny glebowej materii organicznej skorelowano z właściwościami systemów korzeniowych badanych gatunków drzew. Zawartość C_{fLF} i N_{fLF} była dodatnio skorelowana ze średnicą korzeni, ich biomasą i tempem rozkładu. Zawartość C_{oLF} i N_{oLF} była istotnie dodatnio skorelowana z długością korzeni. Zawartość C_{MAF} i N_{MAF} była ujemnie skorelowana ze średnicą korzeni, a zawartość C_{MAF} była dodatnio

skorelowana z przyrostem rocznym korzeni. Analiza GLM potwierdziła znaczenie lokalizacji w gradiencie wysokości oraz gatunków drzew w kształtowaniu składu frakcyjnego glebowej materii organicznej (Tab. 3). Gatunek drzewa istotnie wpływał na kształtowanie się zawartości N i C we wszystkich frakcjach materii organicznej. Wysokość n.p.m. wpłynęła na zawartość C_{fLF}, N_{fLF}, C_{MAF} i N_{MAF}. Dla zawartości C_{fLF}, N_{fLF} i N_{oLF} odnotowano jednoczesne znaczenie gatunku drzew i wysokości nad poziomem morza.

Tabela 3. Podsumowanie analizy GLM dla frakcji glebowej materii organicznej

	C_{fLF}		N_{fLF}		C_{oLF}		N_{oLF}		C_{MAF}		N_{MAF}	
	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*	F	*p*
Gatunek	26.51	**0.0000**	28.62	**0.0000**	4.89	**0.0318**	20.94	**0.0000**	48.07	**0.0000**	11.16	**0.0016**
Wysokość	33.33	**0.0000**	36.92	**0.0000**	1.48	0.2370	3.16	0.0512	6.27	**0.0037**	5.20	**0.0090**
Gatunek*Wysokość	8.71	**0.0005**	10.57	**0.0000**	1.25	0.2952	4.44	**0.0169**	1.79	0.1778	1.07	0.3525

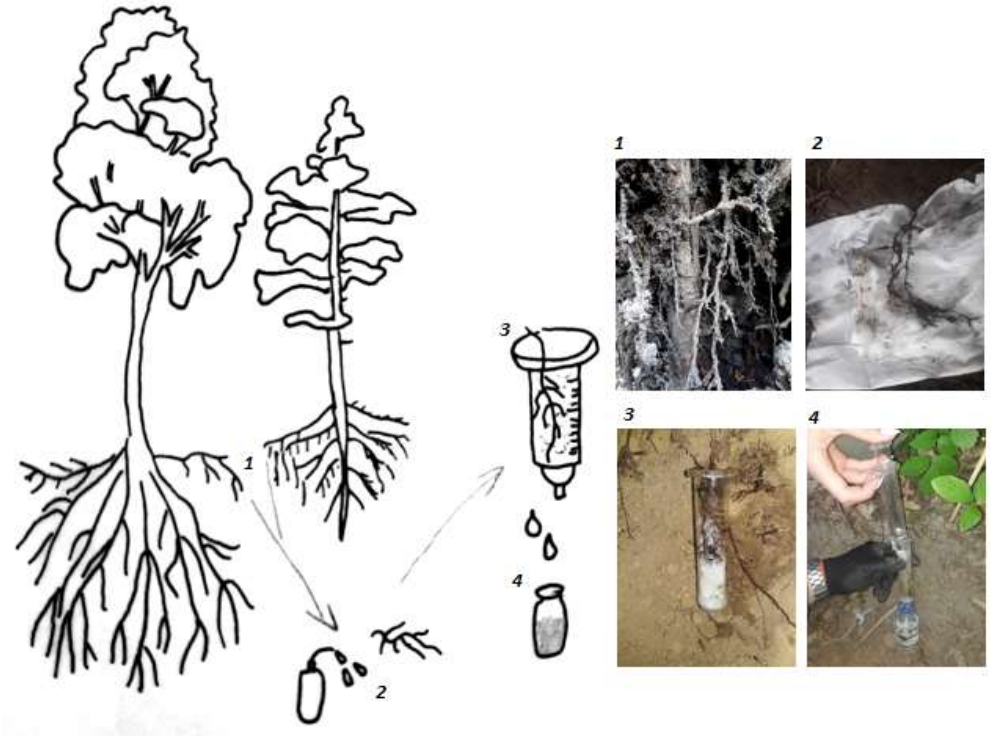
C_{fLF} – C wolnej frakcji lekkiej, N_{fLF} – N wolnej frakcji lekkiej, C_{oLF} – C zokludowanej frakcji lekkiej, N_{oLF} – N zokludowanej frakcji lekkiej, C_{MAF} – C frakcji związanej z minerałami, N_{MAF} – N frakcji związanej z minerałami

Przeprowadzona analiza PCA potwierdziła zależność pomiędzy składem frakcyjnym glebowej materii organicznej a gatunkiem drzewa i położeniem poletka doświadczalnego w gradiencie wysokości. Ponadto PCA potwierdziło odrębność składu frakcyjnego materii organicznej gleby pod drzewostanami bukowymi i jodłowymi. Czynniki 1 i 2 łącznie wyjaśniły 61,5% wariancji badanych cech, w tym czynnik 1 - 42,5%, a czynnik 2 - 18,9% wariancji. Czynnik 1 był powiązany z frakcjami glebowej materii organicznej i położeniem w gradiencie wysokości. Czynnik 2 był związany z gatunkiem drzew. Zatem analiza PCA potwierdziła znaczenie gatunku i położenia w gradiencie wysokości w kształtowaniu charakterystyki systemów korzeniowych i frakcji glebowej materii organicznej.

Przeprowadzone badania potwierdzają zależność między cechami systemu korzeniowego, a składem fakcyjnym glebowej materii organicznej. Dodatkowo uzyskane wyniki wskazały na znaczący efekt gradientu wysokości i związanej z tym czynnikiem zmiany temperatury i wilgotności, które wpływają na akumulację węgla i azotu, a także tempo rozkładu oraz udział lekkiej i ciężkiej frakcji materii organicznej. W glebie w wyższych lokalizacjach n.p.m. dominowała lekka frakcja materii organicznej, której duża ilość wynikała z wolniejszych procesów rozkładu. Bez względu na położenie w gradiencie wysokościowym, gleba w drzewostanach bukowych charakteryzowała się mniejszym udziałem frakcji lekkiej i większym udziałem frakcji ciężkiej materii organicznej w porównaniu z glebą w drzewostanach jodłowych. Gatunki iglaste takie jak jodła, poprzez dostarczaną biomasę, między innymi poprzez obumieranie części korzeni systemu korzeniowego, obniżają pH gleby, spowalniając procesy dekompozycji, co przekłada się na skład frakcyjny glebowej materii organicznej.

6.3. Wpływ systemów korzeniowych różnych gatunków drzew i ich wysięków korzeniowych na właściwości gleb i mikroorganizmy

W pracy *Staszel-Szlachta K., Lasota J., Szlachta A. Błońska E. 2024. The impact of root systems and their exudates in different tree species on soil properties and microorganisms in a temperate forest ecosystem* analizowano wpływ systemów korzeniowych na kształtowanie właściwości chemicznych gleb oraz na ilość i zróżnicowanie mikroorganizmów glebowych. Badaniami objęto jednogatunkowe drzewostany z sześcioma gatunkami, tj. sosną zwyczajną (*Pinus sylvestris* L.), modrzewiem europejskim (*Larix decidua* Mill.), dębem szypułkowym (*Quercus robur* L.), jesionem wyniosłym (*Fraxinus excelsior* L.), bukiem zwyczajnym (*Fagus sylvatica* L.) oraz grabem pospolitym (*Carpinus betulus* L.). Łącznie wyznaczono 30 powierzchni badawczych o wielkości 0.1 ha (sześć typów drzewostanu × pięć powtórzeń = 30 powierzchni badawczych). Na każdej powierzchni badawczej wyznaczono pięć punktów poboru próbek do szczegółowej analizy systemów korzeniowych i właściwości gleby. Próbki gleby do analizy systemu korzeniowego pobierano w odległości 100 cm od pnia drzewa. Próbki gleby do pozostałych analiz pobierano do głębokości 15 cm, po usunięciu warstwy ściółki i podzielono je na dwie części. Jedna część była przeznaczona do oznaczenia podstawowych właściwości gleb, druga część została wykorzystana do oznaczenia aktywności enzymatycznej. W laboratorium, próbki gleby wysuszono i przesiano przez sito o wielkości oczek 2 mm. W przesuszonych próbkach gleby oznaczono pH metodą potencjometryczną w wodzie i 1M KCl. Kwasowość hydrolityczną (Y) oznaczono metodą Kappena. Całkowitą zawartość azotu i węgla oznaczono za pomocą analizatora LECO CNS True Mac Analyzer (Leco, St. Joseph, MI, USA). Do oznaczenia ilości kationów zasadowych (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) wykorzystano analizator ICP-OES (iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK). Świeże próbki gleby o naturalnej wilgotności przesiano przez sito o średnicy 2 mm i przechowywano w temperaturze +4°C, w celu oznaczenia aktywności enzymatycznej. Aktywność enzymów zewnątrzkomórkowych β-D-celobiozydazy (CB), β-glukozydazy (BG), ksylanazy (XYL), N-acetylo-β-D-glukozaminidazy (NAG), fosfatazy (PH) i arylsulphataze (SP) oznaczano przy użyciu substratów znakowanych fluorogenicznie. Fluorescencję mierzono na czytniku płytek, przy długości fali wzbudzenia 355 nm i długości fali emisji 460 nm. Aktywność dehydrogenaz (DH) oznaczono metodą Lenharda zgodnie z procedurą Casidy (1964). Wydzieliny korzeniowe pobierano zgodnie z metodyką zaproponowaną przez Philips i in. (2008).

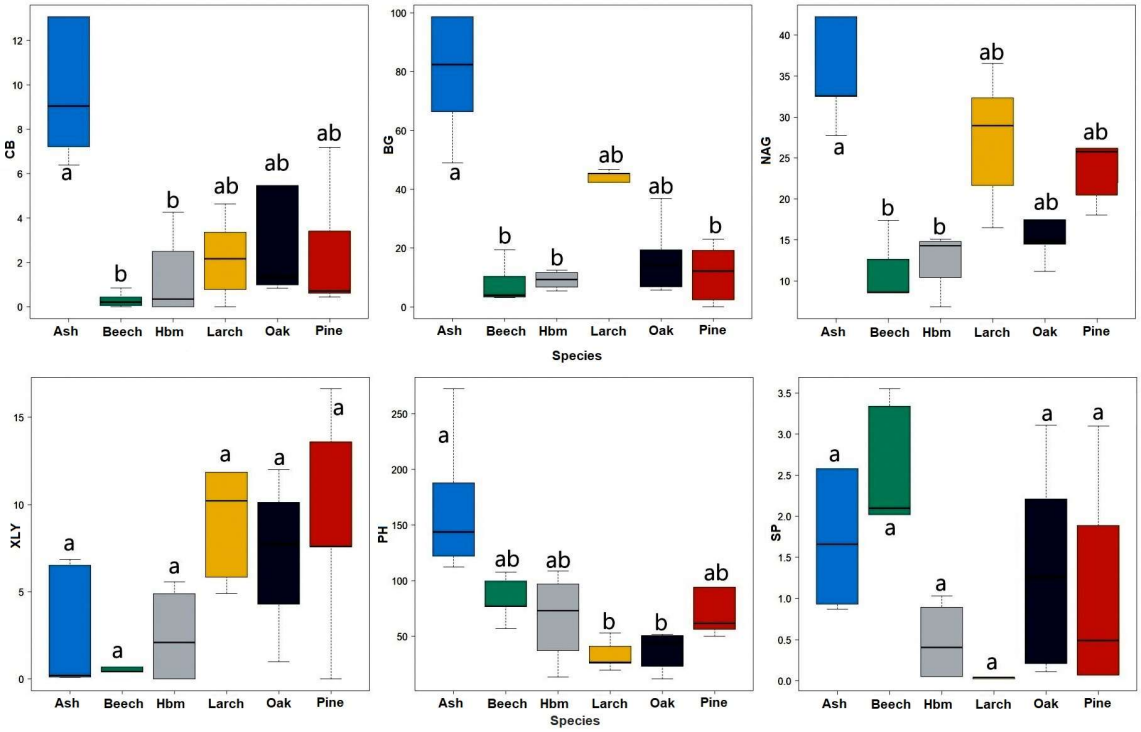


Ryc. 8 Schemat poboru wydzielin korzeniowych (1-pozyskanie z gleby fragmentu korzenia; 2-czyszczenie; 3-napełnienie strzykawki fragmentem korzenia wraz z pożywką i szklanymi kulkami; 4-pobór wydzielin korzeniowych)

Wydzieliny korzeniowe pobierano dwukrotnie, w czerwcu i wrześniu 2022 r. z jednego fragmentu systemu korzeniowego o podobnej długości i rozgałęzieniu (Ryc. 8). Każdy system korzeniowy był starannie oczyszczony z gleby przy użyciu wody dejonizowanej i cienkiej pęsety, w odpowiedniej kolejności aby zachować integralność systemu korzeniowego. Żywe fragmenty korzeni umieszczono w sterylnych, szklanych strzykawkach zawierających sterylne, szklane kulki, zwilżone roztworem niezawierającym węgla (0.5 mM azotan amonu/NH_4NO_3, 0.1 mM diwodorofosforan potasu/KH_2PO_4, 0.2 mM siarczan potasu/K_2SO_4, 0.15 mM siarczan magnezu/$MgSO_4$ i 0.3 mM chlorek wapnia/$CaCl_2$). Po 24 h stabilizacji w strzykawce korzenie były przepłukiwane trzykrotnie świeżym roztworem, niezawierającym węgla, w celu usunięcia węgla organicznego pochodzącego z wydzielin. Wydzieliny pobierano do 50 ml szklanych fiolek uszczelnionych silikonowymi zakrętkami i przechowywano je w temperaturze +4°C do momentu wykonania analizy. W uzyskanych próbkach wydzielin korzeniowych oznaczono zawartość całkowitego węgla organicznego (TOC). Zebrane roztwory przefiltrowano przez filtr twardy Munktell 390, a przesącze przeanalizowano wykorzystując analizator Shimadzu Total Organic Carbon (Shimadzu, Japan). Na każdej powierzchni badawczej pobrano próbki gleby o znanej objętości 15 × 15 × 15 cm, w celu określenia biomasy korzeni. Grube korzenie (średnica > 2 mm) zostały oddzielone od drobnych korzeni (średnica < 2 mm), następnie były skanowane w rozdzielczości 800 dpi i analizowane przy użyciu skanera z oprogramowaniem WinRhizo Pro

2003b (Regent Instruments Inc., Ville de Québec, QC, Kanada). Określono średnicę, długość i powierzchnię korzenia. Korzenie wysuszono w temperaturze 70°C przez 24 godziny, a następnie zważono. Na podstawie uzyskanych wyników obliczono wskaźniki takie jak: wskaźnik zagęszczenia (root tissue density - RTD) ($kg \cdot m^{-3}$), wskaźnik powierzchni korzeni (specific root area - SRA) ($m^2 kg^{-1}$) i wskaźnik długości korzeni (specific root length - SRL) ($m\ g^{-1}$) (Ostonen i in. 1999). DNA wyizolowano z 1 g gleby przy użyciu zestawu Genomic Mini AX Bacteria+. Lizę mechaniczną przeprowadzono w homogenizatorze FastPrep-24. Lizę ścian komórkowych dokonano przy użyciu enzymu Litykazy (A&A Biotechnology, Polska). Analiza metagenomiczna populacji grzybów została przeprowadzona na bazie hiperzmiennego regionu ITS1. Do amplifikacji wybranego regionu i przygotowania biblioteki zostały użyte specyficzne sekwencje primerów ITS1FI2 oraz 5.8S (analiza ITS1) (Gardes and Bruns, 1993). Reakcję PCR przeprowadzono z użyciem termocyklera Q5 Hot Start High-Fidelity 2X Master Mix. Sekwencjonowanie odbyło się na sekwenatorze MiSeq, w technologii paired-end (PE). Analiza metagenomiczna populacji bakterii została przeprowadzona na bazie hiperzmiennego regionu V3-V4 genu 16S rRNA. Do amplifikacji wybranego regionu i przygotowania biblioteki zostały użyte specyficzne sekwencje primerów 341F oraz 785R (analiza 16S) (Ferris i in. 1996). Reakcję PCR przeprowadzono również z użyciem termocyklera Q5 Hot Start High-Fidelity 2X Master Mix. Sekwencjonowanie odbyło sie na aparacie MiSeq, w technologii paired-end (PE), 2x300nt, z użyciem zestawu v3 Illuminy. Oznaczenie populacji grzybów oraz bakterii zostało wykonane przez firmę Genomed. Analizy statystyczne przeprowadzono z wykorzystaniem oprogramowania R (R Core Team 2020) i R Studio (RStudio Team 2020). Siła zależności pomiędzy właściwościami gleb, a parametrami korzeni była określona z wykorzystaniem współczynnika korelacji Spearmana. Dodatkowo do potwierdzenia zależności między właściwościami gleby a właściwościami korzeni wykorzystano analizę głównych składowych (PCA). Test Kruskala-Wallisa został wykorzystany do oceny różnic właściwości gleby i korzeni pomiędzy badanymi wariantami drzewostanów.

Przeprowadzone analizy wykazały, że gleba na której wzrastały poszczególne gatunki drzew różniła się pod względem właściwości fizykochemicznych. Istotnie wyższe wartości pH (średnie pH w H_2O = 4,86) stwierdzono w glebie z jesionem, a najniższe w glebie pod drzewostanem modrzewiowym (średnie pH w H_2O = 3,71). Gleba spod jesionów różniła się istotnie pod względem zawartości kationów zasadowych, zwłaszcza wapnia. Wyższe zawartości węgla odnotowano w glebie pobranej w drzewostanach bukowych, modrzewiowych i sosnowych (średnia zawartość węgla wynosiła odpowiednio 5,64, 5,19 i 4,75%). Pod względem tego parametru drzewostany z tymi gatunkami różniły się istotnie od pozostałych. Zaobserwowano istotnie wyższy stosunek węgla do azotu w glebie spod drzewostanu modrzewiowego – 18,6, bukowego – 18,3 i sosnowego - 15,4.



Ryc. 9 Aktywność enzymatyczna (nmol MUB $g^{-1} \cdot C \cdot h^{-1}$) gleb pod wpływem różnych gatunków drzew (Ash-jesion, Beech-buk, Hbm–grab, Larch-modrzew, Oak-dąb, Pine-sosna; CB-β-D-celobiozydaza,, BG - β-glucozydaza, NAG - N-acetyl-β-D-glukozaminidaza, XYL - β-xylozydaza, PH – fosfataza, SP – arylsulfataza; małe litery (a, b) oznaczają istotne różnice pomiędzy gatunkami drzew

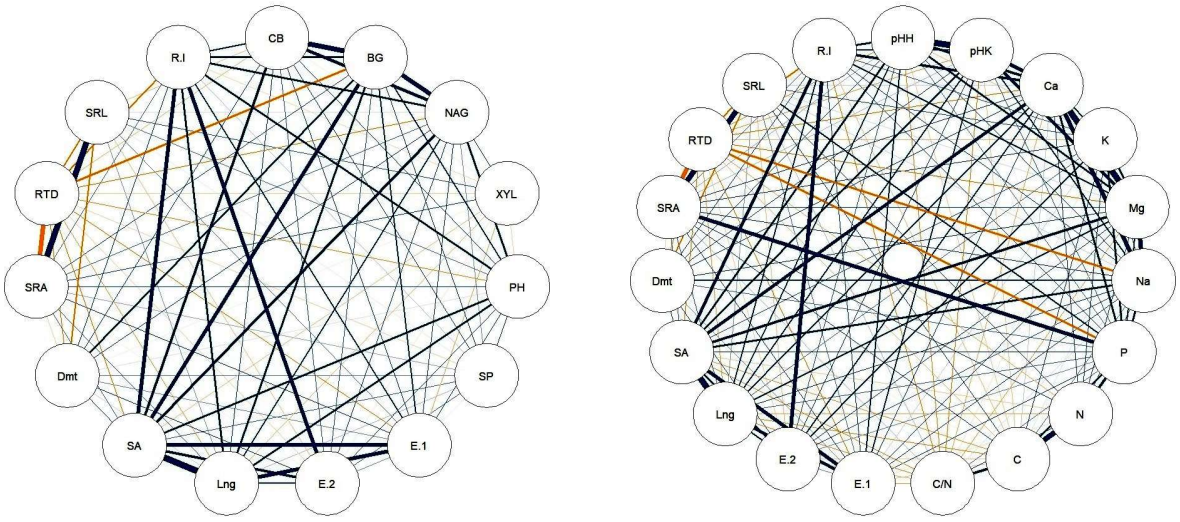
Gleba, na której wzrastały różne gatunki drzew charakteryzowała się odmienną aktywnością enzymatyczną. Istotnie wyższą aktywność CB, BG, NAG i PH zarejestrowano w glebie pobranej w drzewostanach jesionowych. Pod względem aktywności XYL i SP nie stwierdzono istotnej różnicy między badanymi gatunkami drzew (Ryc. 9). Dla drzewostanów jesionowych wykazano statystycznie istotną różnicę w ilości węgla uwalnianego do gleby wraz z wydzielinami korzeniowymi. System korzeniowy drzew w drzewostanach jesionowych różnił się istotnie w porównaniu do innych gatunków pod względem sumarycznej długości korzeni, ich przeciętnej średnicy i powierzchni. Nie stwierdzono istotnej różnicy między gatunkami w odniesieniu do SRL i SRA, natomiast istotnie niższą wartość RTD odnotowano dla jesionu. Biomasa korzeni u jesiona była istotnie wyższa niż u pozostałych badanych gatunków (buk, dąb i sosna) (Tab. 4).

Tabela 4. Analizy systemu korzeniowego różnych gatunków drzew (średnia ± SE)

Gatunek	Analizowany parametr *							
	ER	**LR [cm]**	**SA cm²**	**Φ [mm]**	**V cm³**	**SRA**	**RTD**	**SRL**
Jesion	14,67±15,92^{a}	351,18±175,81^{a}	54,06±24,92^{a}	0,50±0,08^{a}	0,67±0,29^{a}	19,63±5,01^{ab}	42,67±6,81^{b}	131,05±54,05^{a}
Modrzew	6,74±4,07^{ab}	227,96±165,36^{ab}	30,70±21,00^{b}	0,45±0,10^{a}	0,34±0,24^{b}	20,84±7,81^{a}	48,62±12,44^{b}	164,89±78,97^{a}
Dąb	4,49±1,16^{b}	257,94±192,00^{ab}	30,50±30,81^{b}	0,41±0,07^{a}	0,29±0,19^{b}	14,58±5,77^{bc}	74,03±15,05^{a}	123,55±74,61^{a}
Sosna	5,49±2,56^{b}	205,19±128,76^{ab}	25,01±12,62^{b}	0,43±0,13^{a}	0,26±0,13^{b}	13,99±3,12^{c}	71,53±15,09^{a}	115,19±54,33^{a}
Grab	4,20±1,40^{b}	178,08±84,15^{b}	23,45±10,27^{b}	0,43±0,07^{a}	0,25±0,12^{b}	12,74±3,69^{c}	78,92±14,76^{a}	100,63±40,17^{a}
Buk	5,56±2,96^{ab}	174,39±92,18^{b}	23,07±14,22^{b}	0,41±0,09^{a}	0,26±0,21^{b}	14,49±3,99^{bc}	73,03±16,09^{a}	120,96±49,17^{a}

** ER- węgiel wydzielany przez korzenie mg C g^{-1} day^{-1}, LR- długość korzeni, SA – powierzchnia korzeni, Φ- średnica, RTD – wskaźnik zagęszczenia [kg m^{-3}], SRA – wskaźnik powierzchni [m^2 kg^{-1}] i SRL – wskaźnik długości korzeni [m kg^{-1}]; TukeyHSD<0.05*

Analiza statystyczna potwierdziła związek między cechami korzeni i właściwościami badanej gleby. Powierzchnia i długość korzeni były silnie, dodatnio skorelowane z zawartością kationów zasadowych. Węgiel uwalniany wraz z wydzielinami korzeniowymi był dodatnio skorelowany z pH i zawartością kationów, zwłaszcza wapniem. Współczynniki SRA i SRL były dodatnio skorelowane z zawartością fosforu i węgla. Zaobserwowano ujemną korelację między RTD oraz zawartością sodu i fosforu. Dodatkowo, zaobserwowano silny związek między parametrami korzeni a aktywnością enzymatyczną gleby. Aktywność CB, BG, NAG i PH była dodatnio skorelowana z powierzchnią i długością korzeni, natomiast słabiej z ich średnicą. Dodatkowo przyrost korzeni był silnie, dodatnio skorelowany z ilością wydzielin korzeniowych. Wskaźnik RTD ujemnie korelował z aktywnością BG i w mniejszym stopniu z aktywnością NAG (Ryc.10).



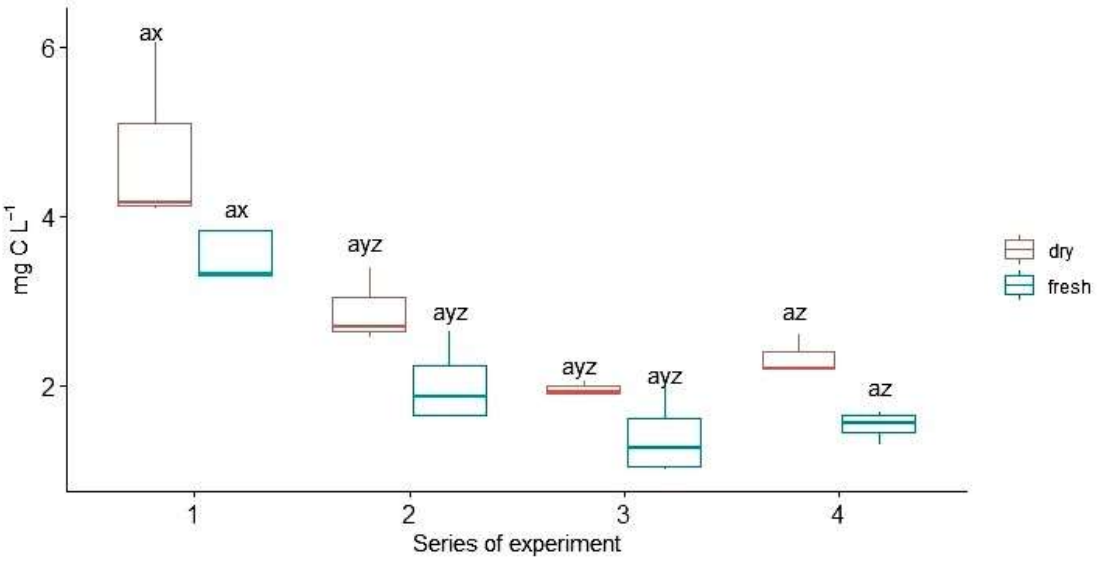
Ryc. 10 Wskaźnik korelacji między cechami korzeni, a właściwościami gleby pod wpływem różnych gatunków drzew (SRL – wskaźnik długości korzenia, RTD – wskaźnik zagęszczenia, SRA – wskaźnik powierzchni korzeni, Dmt – średnica korzeni, SA – powierzchnia korzeni, Lng – długość korzeni, E.1 - węgiel wydzielony z korzeni na początku sezonu wegetacyjnego, E.2 - węgiel wydzielony z korzeni na koniec sezonu wegetacyjnego, R.I - przyrost korzeni, PH - fosfataza, BG - β-glukozydaza, NAG - N-acetyl -β-D-glukozaminidaza, XYL – β-ksylozydaza, CB – β-D-celobiozydaza, SP – arylosulfataza, pHH – pH w H2O, pHK – pH w KCl, granatowy – korelacja dodatnia, pomarańczowy – korelacja ujemna)

W trakcie prowadzonych badań przeanalizowano ilość i różnorodność mikroorganizmów występujących w analizowanych próbkach gleby. Spośród bakterii najliczniej była reprezentowana gromada *Proteobacteria*, której średni udział we wszystkich analizowanych wariantach wynosił 35,3–37,0%. Drugą co do liczebności była gromada *Actinobacteriota* występująca w glebie pod drzewostanami dębowymi, grabowymi i modrzewiowymi (odpowiednio 30,8, 29,4, 28,8%). Analiza jednostek taksonomicznych grzybów wykazała, że najliczniej były reprezentowane gromady *Basidiomycota* i *Ascomycota*. Udział pierwszej wynosił 52,2% w glebie drzewostanów bukowych i nieco mniej w jesionowych – 44,2%, modrzewiowych – 40,3% i sosnowych – 36,3%. Przeciwne wartości stwierdzono w przypadku grzybów gromady *Ascomycota*, których liczebność różniła się istotnie w glebach, na które oddziaływały badane gatunki drzew. Grzyby tej gromady stanowiły najliczniejszą grupę (odpowiednio 52,5 i 43,0%) w glebach pod grabem i dębem, a w przypadku pozostałych gatunków od 24,5% (buk) do 32,7% (modrzew). W glebie pod drzewostanami sosnowymi najliczniejsze były grzyby z gromady *Mortierellomycota* (35,0%), które w przypadku gleb drzewostanów bukowych i jesionowych były nieco mniej liczne (odpowiednio 19,0% i 22,4%).

Badania potwierdziły znaczenie różnych gatunków drzew w kształtowaniu właściwości gleby poprzez systemy korzeniowe. Zidentyfikowano silny związek między cechami morfologicznymi korzeni i podstawowymi właściwościami fizykochemicznymi gleby i aktywnością enzymatyczną. Stwierdzono, że ilość węgla w wydzielinach korzeniowych była dodatnio skorelowana z pH, zawartością wapnia i aktywnością enzymów. Analiza morfologiczna cech korzeni i ich wydzielin z właściwościami gleby potwierdziła odrębność drzewostanów jesionowych. Zaobserwowano również różnice w składzie zespołów bakterii i grzybów w glebie gatunków liściastych w odniesieniu do gatunków iglastych, takich jak sosna i modrzew. Próbki gleby pozyskane w drzewostanach jesionowych różniły się pod względem ilości i różnorodności mikroorganizmów w porównaniu do gleby dla innych gatunków. W przeprowadzonych badaniach stwierdzono, że gatunki bakterii należące do gromady *Proteobacteria* i *Actinobacteriota* były najbardziej rozpowszechnione. W przypadku grzybów w badanych glebach dominujące były gatunki należące do gromad *Basidiomycota i Ascomycota* . W glebie pod grabem i dębem najliczniej występowały gatunki z gromady *Ascomycota*, podczas gdy w glebie z sosną stwierdzono obecność grzybów głównie z gromady *Mortierellomycota*. Uzyskane wyniki sugerują, że należy unikać jednogatunkowych drzewostanów iglastych, których hodowla prowadzi do pogorszenia właściwości gleby, zmniejszenia różnorodności mikroorganizmów, a w konsekwencji do zmniejszenia stabilności drzewostanu. W celu poprawy właściwości gleby i ich bioróżnorodności należy wprowadzać do drzewostanów gatunki liściaste, takie jak jesion, grab i dąb.

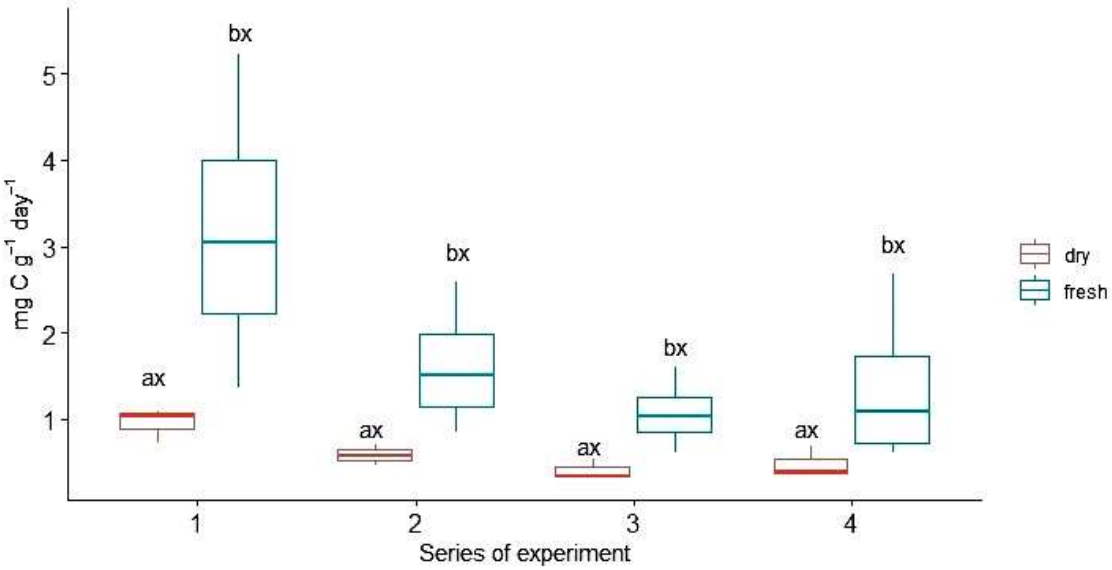
6.4. Wpływ suszy na wydzieliny korzeniowe *Quercus petraea* oraz aktywność enzymatyczną

W pracy *Staszel, K., Lasota, J., Błońska, E. 2022c. Effect of drought on root exudates from Quercus petraea and enzymatic activity of soil* przedstawiono wyniki dotyczące wpływu symulowanej suszy na morfologię korzeni i ich wydzieliny, a w konsekwencji na aktywność enzymatyczną. W doświadczeniu wykorzystano trzymiesięczne sadzonki dębu bezszypułkowego, które wzrastały na typowym podłożu wykorzystywanym w szkółkach leśnych. Sadzonki hodowano w identycznych polietylenowych doniczkach o średnicy i wysokości 15 cm. W badaniach uwzględniono dwa warianty wilgotności podłoża, podłoże o wilgotności względnej 25% stanowiło wariant suchy (symulowana susza), a podłoże o wilgotności 55% stanowiło wariant kontrolny (wariant świeży). Do monitorowania stopnia uwilgotnienia podłoża, na którym wzrastały sadzonki wykorzystano czujniki glebowe, które dokonywały pomiaru w interwale godzinnym. W przypadku odnotowania zmian wilgotności podłoża, przywracano ją do stanu początkowego. Doświadczenie przeprowadzono w okresie pomiędzy 20 marca – 20 kwietnia 2021 r. Próbki wydzielin korzeniowych oraz próbki gleb do analiz laboratoryjnych pobierano czterokrotnie w odstępie tygodniowym. W każdym wariancie wilgotności i w każdej serii doświadczenia pobierano próbki z jednej z czterech sadzonek. Łącznie przeanalizowano 32 próbki wydzielin (4 sadzonki x 2 warianty wilgotności x 4 ekstrakcje = 32). Metodyka poboru wydzielin oraz określenia aktywności enzymatycznej została szczegółowo omówiona w podrozdziale *6.3.* Obliczono współczynnik korelacji Spearmana pomiędzy parametrami korzeni a ich wydzielinami. Analiza składowych głównych (PCA) została wykorzystana do potwierdzenia zależności pomiędzy cechami korzeni, a wydzielinami korzeniowymi w odniesieniu do wariantów uwilgotnienia podłoża. Test Tukeya HSD wykorzystano do oceny różnic cech korzeni pomiędzy analizowanymi wariantami doświadczenia. Wszystkie analizy statystyczne zostały przeprowadzone przy użyciu programów statystycznych R Studio20 oraz Statistica 10 (StatSoft Inc. USA 2010). Korzenie dębu wydzielały średnio 12,64 mg C g^{-1} dzień$^{-1}$ w świeżym wariancie doświadczenia. Większą całkowitą zawartość C w wydzielinach odnotowano dla sadzonek dębu w wariancie suchym. We wszystkich seriach doświadczenia występowała tendencja do większej ilości C w eksudatach w wariancie suchym w porównaniu do wariantu świeżego (Ryc. 11), która jednak nie została potwierdzona statystycznie.



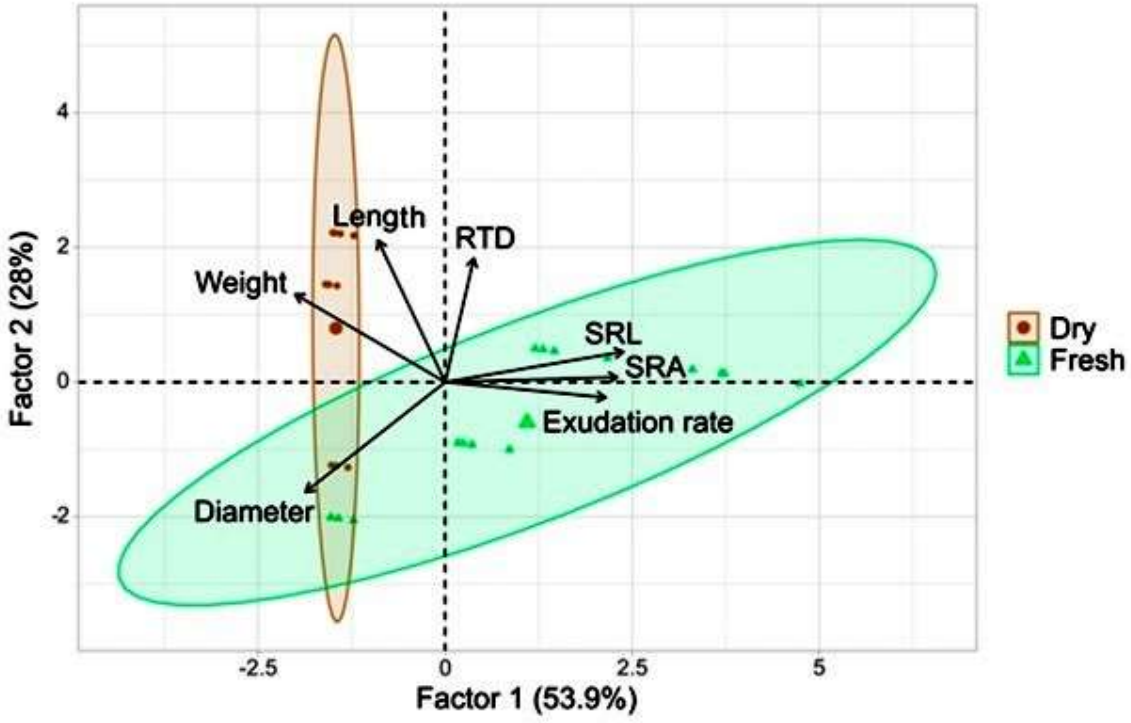
Ryc. 11 Zmiana całkowitego węgla wydzielonego z korzeni (mg C L^{-1}) w czasie, w zależności od wariantu wilgotności; różne małe litery (a, b) wskazują istotne różnice w parametrach pomiędzy różnymi wilgotnościami; różne małe litery (x,y,z) wskazują na istotne różnice parametrów pomiędzy seriami; Tukey HSD p < 0.05.

Jednak przy przeliczeniu ilości C na jednostkę biomasy korzeni, uzyskano wyższe ilości C w wydzielinach korzeniowych w wariancie świeżym. W pierwszej serii doświadczenia ilość C w wydzielinach w wariancie świeżym była trzykrotnie wyższa, niż w wariancie suchym. W kolejnych seriach doświadczenia większą ilość C z wydzielin odnotowano w przypadku wariantu świeżego, ale różnica względem wariantu suchego była mniejsza w porównaniu do pierwszej serii doświadczenia (Ryc. 12).



Ryc. 12 Zmiana wydzielanego węgla z korzeni na jednostkę biomasy w czasie, w zależności od wariantu wilgotności (mg C g^{-1} $dzień^{-1}$); różne małe litery (a, b) wskazują istotne różnice w parametrach pomiędzy różnymi wariantami wilgotności; różne małe litery (x,y,z) wskazują na istotne różnice parametrów pomiędzy seriami; Tukey HSD; p < 0,05.

Wariant wilgotności podłoża miał istotny wpływ na morfologię korzeni sadzonek dębu. W przypadku korzeni wzrastających w wariancie suchym odnotowano większą ich średnicę w porównaniu do wariantu świeżego. Sadzonki dębu wzrastające na podłożu w wariancie suchym charakteryzowały się statystycznie istotnie większą długością korzeni. Wyższą wartość SRA (38,90 m^2 kg^{-1}) odnotowano w przypadku sadzonek tego gatunku z wariantu świeżego w porównaniu do wariantu suchego (26,90 m^2 kg^{-1}). Wskaźnik RTD nie różnił się pomiędzy badanymi wariantami. Ilość C w wydzielinach istotnie korelowała z cechami morfologicznymi korzeni. Stwierdzono istotną statystycznie, ujemną korelację pomiędzy ilością C w wydzielinach, a średnicą i długością korzeni (odpowiednio $r = –0,52$ i $r = –0,66$). Najsilniejszą dodatnią korelację odnotowano pomiędzy ilością C w wydzielinach a wskaźnikami SRA ($r = 0,69$) i SRL ($r = 0,72$). Analiza PCA wyjaśniła łącznie 81,9% zmienności badanych cech, a także potwierdziła zależność pomiędzy ilością C w wydzielinie a wskaźnikami SRA i SRL oraz większą ilość C w wydzielinie korzeniowej sadzonek hodowanych w świeżym wariancie podłoża szkółkarskiego (Ryc.13).



Ryc. 13 Analiza głównych składowych (PCA), wykres zmiennych: wskaźnik zagęszczenia (RTD), wskaźnik powierzchni korzeni (SRA) i wskaźnik długości (SRL), natężenie wydzielanego C – mg C g^{-1} dzień$^{-1}$)

Istotnie niższą aktywność enzymatyczną stwierdzono w wariancie suchym w porównaniu do wariantu świeżego. W pierwszej serii doświadczenia zaobserwowana różnica w aktywności badanych enzymów pomiędzy wariantami doświadczenia była niewielka. W czwartej serii doświadczenia, w wariancie suchym, aktywność enzymatyczna obniżyła się o 90% w przypadku CB, 50% w przypadku BG i NAG, 20% dla XYL i 10% dla SP i PH w porównaniu do wariantu

świeżego. W wariancie świeżym doświadczenia istotne różnice pomiędzy serią I i IV odnotowano jedynie w przypadku NAG. Natomiast w wariancie suchym zaobserwowano znaczny spadek aktywności wszystkich badanych enzymów (oprócz SP).

Z przeprowadzonych badań wynika, że ilość C w wydzielinach ma ścisły związek z morfologią korzeni, szczególnie ze wskaźnikiem SRA i SRL. Cechy morfologiczne korzeni są czynnikiem determinującym wydzieliny korzeniowe w warunkach suszy. Sadzonki dębu bezszypułkowego rosnące w wariancie suchym charakteryzowały się niższymi wartościami SRA i SRL, co skutkowało niższym wydzielaniem C wraz z wysiękiem korzeni. Przeprowadzone badania wykazały zmniejszenie się ilości wydzielin korzeniowych w warunkach symulowanej suszy, co doprowadziło do zmiany aktywności enzymatycznej. Wiedza na temat czynników kształtujących akumulację C w glebie oraz związek między tym procesem, a wydzielinami korzeniowymi jest niezbędny do zrozumienia obiegu C w ekosystemach leśnych. Lepsze zrozumienie mechanizmów i czynników wpływających na dynamikę C w glebach leśnych pozwoli na celowe przewidywanie tych zjawisk w przyszłości, co może być wykorzystane przy zapobieganiu niekorzystnym skutkom zmian klimatu.

6.5. Wpływ depozycji azotu na systemy korzeniowe i wydzieliny sadzonek buka *Fagus sylvatica* L.

W doświadczeniu prezentowanym w artykule *Staszel-Szlachta K., Lasota J., Kempf M., Błońska, E. 2022d. Effect of nitrogen deposition on root systems and exudates of seedlings of beech Fagus sylvatica L. in a temperate climate* przeanalizowano wpływ nadmiernej depozycji azotu na rozwój systemów korzeniowych, ilość wydzielanego C z korzeni oraz stan odżywienia sadzonek buka zwyczajnego (*Fagus sylvatica*). Badaniami objęto sadzonki tego gatunku, które potraktowano trzema różnymi dawkami azotu: 0,75, 2,25 oraz 4,5 $kg \cdot ha^{-1}$. Dodatkowo doświadczenie obejmowało wariant kontrolny bez nawożenia azotem. Każdy z czterech wariantów doświadczenia przygotowano w 5 powtórzeniach. Doświadczenie prowadzono od maja do września 2021. W maju 2021 roku do kontenerów polietylenowych wysiano nasiona buka. Jako podłoże zastosowano mieszaninę trocin jodłowo-świerkowych i torfu wysokiego w stosunku objętościowym 1:1. We wszystkich wariantach eksperymentu zastosowano to samo podłoże. W lipcu 2021 roku pobrano pierwszą serię wydzielin korzeniowych po 5 próbek z każdego wariantu doświadczenia. Wydzieliny pobierano zgodnie z metodyką przedstawioną w podrozdziale *6.3*. Drugą serię wydzielin pobierano pod koniec eksperymentu, tj. we wrześniu 2021 roku. Równocześnie pobierano próbki systemów korzeniowych. Ponadto pobrano próbki substratów w celu określenia aktywności enzymów biorących udział w obiegu C, N i P. Po zakończeniu doświadczenia zbadano morfologię korzeni wszystkich sadzonek oraz oznaczono zawartość C, N oraz makro i mikroelementów w materiale roślinnym. Wysuszone i zmielone

próbki liści i korzeni mineralizowano w mieszaninie HNO_3 i $HClO_4$ (3:1), a następnie oznaczono stężenie makro i mikroelementów z wykorzystaniem ICP (ICP-OES Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, Wielka Brytania). Zawartość węgla i azotu oznaczono za pomocą analizatora LECO CNS TrueMac Analyzer. Metodyka przeprowadzonych analiz laboratoryjnych została szczegółowo opisana w podrozdziale *6.1.* Do oceny zależności pomiędzy parametrami korzeni a wydzielinami korzeniowymi wykorzystano współczynnik korelacji Spearmana. Dodatkowo do potwierdzenia zależności pomiędzy badanymi parametrami wykorzystano analizę głównych składowych (PCA), a analizę skupień do pogrupowania analizowanych próbek. Analizę regresji wykorzystano do zbadania zależności pomiędzy wydzielinami korzeniowymi a wskaźnikiem SRL. Do oceny różnic badanych parametrów pomiędzy wariantami doświadczenia wykorzystano test *post-hoc*. Wyniki uznano za istotne statystycznie przy wartości $p<0,05$. Wszystkie analizy statystyczne przeprowadzono przy użyciu oprogramowania statystycznego R (R Core Team, 2022), R Studio (RStudio Team, 2022) i oprogramowania Statistica 13 (Tibco Software Inc., 2017). Długość korzeni różniła się istotnie w drugiej serii doświadczenia. Najdłuższe systemy korzeniowe odnotowano w wariancie z dawką azotu 0,75 kg·ha^{-1}, a najkrótsze przy dawce azotu 4,5 kg·ha^{-1}. Długość korzenia wahała się od 823,03 do 602,38 cm (Tab.5).

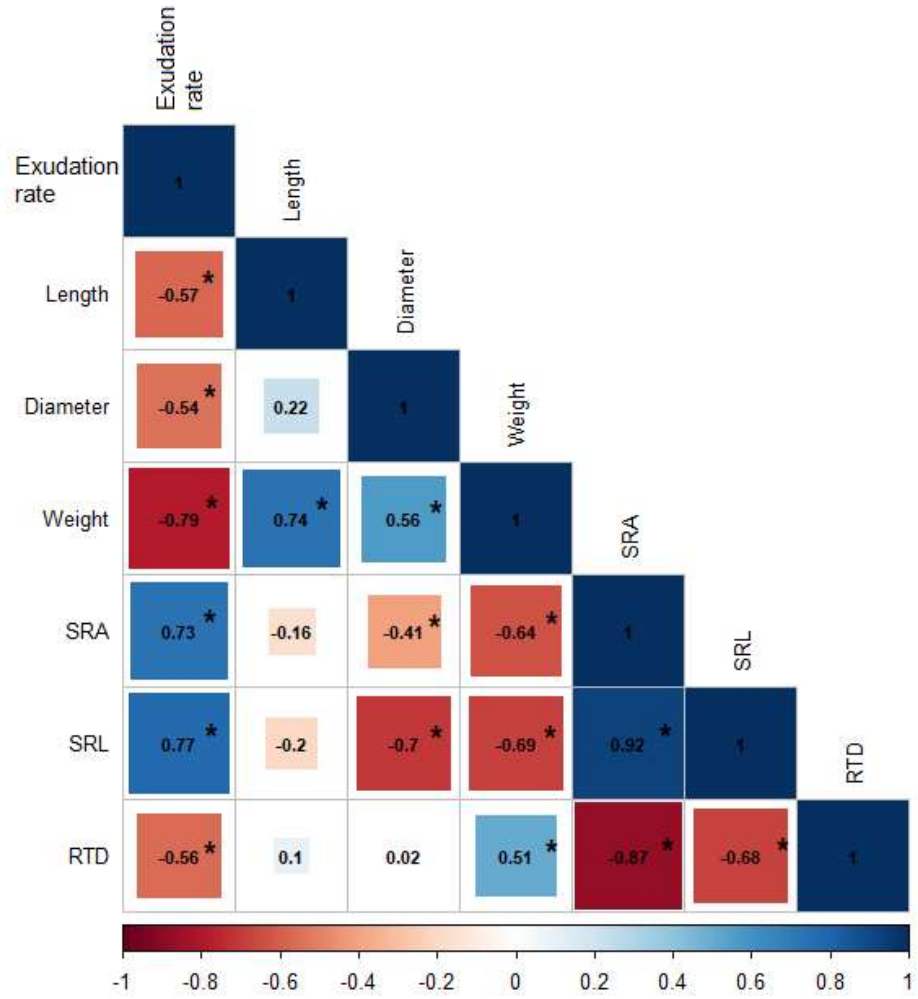
Tabela 5. Charakterystyka morfologii korzeni i szybkość wysięku (mg C g^{-1} dzień$^{-1}$) w różnych wariantach nawożenia azotem

Seria poboru wydzielin	Dawka nawozu	Ilość wydzielonego C	Długość [cm]	Średnica [mm]	Masa [mg]	SRA [m^2 kg^{-1}]	SRL [m kg^{-1}]	RTD [kg m^{-3}]
1	0	11,70± 1,92^{ax}	353,21± 60,49^{ax}	0,21± 0,03^{ax}	206,16± 36,66^{ax}	11,20± 1,19^{ax}	173,48± 28,57^{ax}	175,04± 27,53^{ax}
	0,75	12,09± 4,01^{ax}	402,44± 122,54^{ax}	0,22± 0,02^{ax}	217,72± 50,58^{ax}	12,97± 2,94^{ax}	189,22± 55,40^{ax}	145,17± 31,14^{ax}
	2,25	10,62± 3,98^{ax}	331,57± 87,36^{ax}	0,22± 0,03^{ax}	182,72± 47,86^{ax}	12,62± 2,24^{ax}	188,52± 51,13^{ax}	149,90± 28,67^{ax}
	4,50	13,42± 8,28^{ax}	389,33± 155,66^{ax}	0,21± 0,02^{ax}	198,46± 80,83^{ax}	13,90± 2,96^{ax}	201,71± 42,07^{ax}	140,48± 17,19^{ax}
2	0	13,52± 1,86^{ax}	621,73± 389,15^{ax}	0,24± 0,02^{ax}	475,24± 104,60^{abx}	9,31± 3,76^{abx}	127,87± 57,26^{ax}	201,94± 61,00^{bx}
	0,75	13,89± 1,76^{ax}	823,03± 201,80^{ay}	0,25± 0,02^{ax}	798,12± 212,92^{by}	8,19± 2,16^{aby}	107,20± 34,65^{ay}	207,48± 42,53^{by}
	2,25	14,09± 2,82^{ax}	620,64± 212,06^{ay}	0,27± 0,03^{ay}	723,72± 381,85^{aby}	7,69± 1,66^{by}	94,00± 32,61^{ay}	198,27± 18,25^{by}
	4,50	13,86± 1,74^{ax}	602,38± 97,27^{ay}	0,27± 0,04^{ay}	443,38± 156,85^{ay}	12,15± 3,34^{ax}	144,91± 35,08^{ax}	134,44± 49,10^{ax}

średnia ±błąd stan.; szybkość wysięku (mg C g^{-1} dzień$^{-1}$), wskaźnik powierzchni korzenia (SRA,) wskaźnik długości (SRL), wskaźnik zagęszczenia (RTD); różne małe litery (a, b, c) wskazują na istotne różnice parametrów pomiędzy różnymi dawkami azotu; litery (x,y,z) oznaczają istotne różnice parametrów pomiędzy seriami; TukeyHSD $p<0,05$)

Średnica i masa korzeni w pierwszej serii poboru nie wykazywały statystycznie istotnej różnicy pomiędzy zastosowanymi dawkami, natomiast w drugiej serii poboru masa korzeni różniła się w zależności od zastosowanej dawki azotu, zwłaszcza pomiędzy dawką 4,5 i 2,25 kg·ha^{-1}.

W drugiej serii doświadczenia dla wskaźnika SRA odnotowano istotną różnicę między dawką azotu 2,25 i 4,5 kg·ha^{-1}. Dla wskaźnika SRL w drugiej serii doświadczenia różnice odnotowano przy dawce azotu 0,75 i 2,25 kg·ha^{-1}. Wskaźnik RTD dla sadzonek w wariancie z dawką azotu 4,5 kg·ha^{-1} różnił się istotnie w porównaniu do pozostałych dawek (Tab. 5). Analiza statystyczna potwierdziła związek ilości C z wydzielin z parametrami korzeni. Uzyskano silną, dodatnią korelację pomiędzy ilością C z wydzielin a wskaźnikami SRL i SRA. Stwierdzono ujemną, istotną statystycznie korelację pomiędzy długością, średnicą, biomasą, wskaźnikiem RTD a ilością C z wydzielin. Zaobserwowano silną, dodatnią korelację pomiędzy wskaźnikami SRA i SRL (Ryc.14).



*Ryc. 14 Wskaźnik korelacji pomiędzy różnymi parametrami korzeni i szybkością wysięku (wskaźnik zagęszczenia korzeni (RTD), wskaźnik zagęszczenia korzeni (SRA) i wskaźnik długości korzeni (SRL); * $p < 0,05$)*

W analizie skupień, w której uwzględniano ilość wydzielin korzeniowych, wskaźnik RTD i wskaźnik SRL wyróżniono trzy grupy próbek związane z różną dawką azotu. Obydwa czynniki wyróżnione w analizie PCA łącznie wyjaśniły 57.8% wariancji badanych cech, przy czym czynnik 1 wyjaśnił 41,3%, a czynnik 2 – 16,5%. Czynnik 1 był związany z charakterystyką korzeni i ilością

wydzielanego C, natomiast czynnik 2 wiązał się głównie z wariantem eksperymentu (dawką azotu). W przypadku podstawowych właściwości fizykochemicznych substratu wykorzystanego w doświadczeniu nie wykazano istotnych różnic będących efektem zastosowanego wariantu nawożenia. Wartości pH w H_2O jak i w KCl nie różniły się między wariantami uwzględnionymi w doświadczeniu. Kwasowość hydrolityczna mieściła się w przedziale od 2,30 do 2,88 cmol(+)·kg^{-1}, odpowiednio dla dawki azotu 0,75 i 2,25 kg ha^{-1}. Kwasowość wymienna wahała się w przedziale od 2,18 do 2,74 cmol(+)·kg^{-1}, dla dawki azotu 0,75 kg ha^{-1} i wariantu kontrolnego. W przypadku kwasowości wymiennej nie wykazano statystycznie istotnej różnicy. Ilość zakumulowanego azotu i węgla wykazała niewielkie wahania pomiędzy poszczególnymi wariantami doświadczenia. Najwyższy stosunek C/N odnotowano dla wariantu doświadczenia z dawką 0,75 kg ha^{-1}, a najniższy dla wariantu doświadczenia z dawką 2,25 kg ha^{-1}. Najniższą aktywność czterech analizowanych enzymów stwierdzono przy zastosowaniu dawki azotu 4,5 kg ha^{-1}. W przypadku CB istotnie wyższą aktywność odnotowano w wariancie doświadczenia z dawką azotu 0,75 kg ha^{-1}, a najniższą w przypadku dawki azotu 4,5 kg ha^{-1}. W przypadku aktywności BG nie wykazano wpływu zastosowanych wariantów nawożenia. Dla NAG istotnie najwyższą aktywność odnotowano w wariancie z dawką azotu 2,25 kg ha^{-1}. Aktywność PH była istotnie zróżnicowana pomiędzy badanymi wariantami, z najwyższą aktywnością dla wariantu z dawką azotu 2,25 kg ha^{-1}, natomiast najniższą w wariancie z dawką 4,5 kg ha^{-1} (Tab. 6).

Tabela 6. Podstawowe właściwości i aktywność enzymatyczna (nmol MUB·g^{-1} dry soil·h^{-1}) gleb, w których rosły sadzonki buka po zakończeniu doświadczeniu

Dawka azotu	pH		Hh	Hex	N	C	C/N	CB	BG	NAG	PH
	H_2O	KCl									
0	4,87± 0,08	4,01± 0,05	2,92± 0,36	2,74± 0,35	0,79± 0,05	46,31± 1,23	59,1± 5,7	19,94± 30,68^{ab}	295,04± 155,02	339,49± 102,07^{a}	3317,38± 494,98^{ab}
0,75	4,91± 0,18	4,07± 0,19	2,30± 0,43	2,18± 0,41	0,76± 0,05	46,93± 1,16	62,0± 6,0	34,54± 41,94^{a}	204,95± 96,39	254,78± 45,45^{b}	2975,89± 1028,25^{b}
2,25	4,92± 0,06	4,03± 0,10	2,88± 0,46	2,73± 0,41	0,80± 0,03	46,82± 0,73	58,3± 1,6	24,36± 14,00^{ab}	287,05± 93,30	372,72± 83,82^{a}	3859,47± 619,73^{a}
4,50	4,89± 0,12	4,05± 0,12	2,69± 0,56	2,55± 0,54	0,79± 0,02	46,59± 1,19	59,0± 2,5	2,24± 5,01^{b}	187,56± 145,03	232,48± 87,40^{b}	2099,90± 807,20^{c}

średnia ± błąd stan.; Hh – kwasowość hydrolityczna (cmol(+)·kg^{-1}), Hex – kwasowość wymienna (cmol(+)·kg^{-1}); C, N, C/N (%); CB – celobiozydaza, BG – β-glukozydaza, NAG – N-acetylo-β-D-glukozaminidaza, PH – fosfataza; małe litery w górnym indeksie wartości średnich (a,b,c) oznaczają istotne różnice we właściwościach pomiędzy dawkami azotu; TukeyHSD<0.05

Zawartość pierwiastków w liściach i korzeniach sadzonek różniła się istotnie w zależności od zastosowanej dawki azotu. W przypadku liści najniższą koncentrację azotu odnotowano w wariancie kontrolnym, a najwyższe w wariancie z dawką azotu 2,25 kg ha^{-1}. Istotnie najniższą zawartość azotu w korzeniach odnotowano w wariancie z dawką azotu 0,75 kg ha^{-1}, a najwyższą w wariancie 4,5 kg ha^{-1}. Ilość węgla zakumulowanego w liściach oraz korzeniach wykazała niewielkie zróżnicowanie. Istotne statystycznie różnice odnotowano w przypadku stosunku C/N.

Dla liści i korzeni istotnie najwyższe wartości C/N odnotowano w wariancie kontrolnym i z najniższą dawką azotu 0,75 kg ha^{-1}. Zawartość Ca w liściach wahała się w granicach 6413,8 mg/kg dla dawki 4,5 kg ha^{-1}, do 8682,5 mg/kg dla sadzonek z wariantu kontrolnego. W przypadku liści najwyższą zawartością Ca charakteryzowały się sadzonki z wariantu kontrolnego oraz z wariantu z najniższą dawką azotu. W przypadku korzeni najniższą zawartość Ca odnotowano w wariancie kontrolnym. Wykorzystane w doświadczeniu dawki azotu skutkowały zmianami w zawartości Mg w liściach i korzeniach badanych sadzonek. Liście i korzenie sadzonek w wariantach z wyższymi dawkami azotu tj., 2,25 i 4,5 kg ha^{-1} charakteryzowały się istotnie wyższą zawartością Mg. Najwyższe zawartości K i P w liściach odnotowano w wariancie z dawką azotu 4,5 kg ha^{-1}, a najniższe w wariancie kontrolnym. W przypadku zawartości K i P w korzeniach nie odnotowano statystycznie istotnych różnic (Tab. 7).

Tabela 7. Zawartość chemiczna podstawowych pierwiastków w liściach i korzeniach w zależności od wariantu nawożenia azotem

	Dawka azotu	N	C	C/N	Ca	K	Mg	Na	P
Liście	0	0,82±0,1^{b}	46,60±0,3	57,2±5,2^{a}	8682,5±647,3^{a}	1804,3±361,8^{b}	2600,7±197,0^{a}	453,5±37,6	267,0±58,2^{b}
	0,75	0,86±0,2^{b}	46,97±0,7	58,1±15,7^{a}	8148,0±1100,1^{ab}	1886,1±958,2^{b}	2509,9±239,8^{a}	482,1±102,3	271,9±67,3^{b}
	2,25	1,31±0,2^{a}	46,34±0,7	36,1±5,5^{b}	7034,1±122,5^{ab}	3243,4±359,9^{a}	2238,8±88,2^{b}	479,2±63,9	387,2±63,9^{a}
	4,5	1,21±0,2^{a}	46,37±1,3	39,0±4,9^{b}	6413,8±766,5^{b}	3405,7±837,1^{a}	2159,5±159,5^{b}	416,0±51,4	414,0±42,9^{a}
Korzenie	0	0,59±0,2^{ab}	44,98±1,6	80,4±18,0^{ab}	1534,5±229,5^{b}	2958,4±322,4	1237,3±205,9^{b}	357,8±118,5	365,6±31,8
	0,75	0,58±0,1^{b}	46,12±0,6	82,4±17,8^{a}	1969,7±133,5^{a}	3064,8±382,5	1488,3±201,7^{ab}	468,8±49,7	407,2±45,0
	2,25	0,73±0,6^{ab}	45,80±1,2	63,8±10,8^{ab}	1926,6±383,1^{a}	3298,7±125,8	1531,6±177,9^{a}	467,3±47,2	366,0±47,8
	4,5	0,82±0,6^{a}	45,45±0,9	57,5±12,5^{b}	1967,4±277,2^{a}	3193,9±287,7	1423,5±204,4^{ab}	441,2±116,2	359,0±38,9

średnia ±błąd stan.; C, N (%); Ca, K, Mg, Na, P (mg/kg); małe litery w górnym indeksie wartości średnich (a,b,c) oznaczają istotne różnice we właściwościach pomiędzy dawkami azotu; TukeyHSD<0.05

W badaniach wykazano, że zastosowane dawki nawożenia azotem wpływają na charakterystykę systemów korzeniowych, a w następstwie na ilość węgla transferowanego do podłoża przez korzenie wraz z wydzielinami. Stwierdzono silną, dodatnią korelację pomiędzy zawartością C pochodzącego z wydzielin korzeniowych, a wskaźnikami SRL i SRA. Ponadto badania potwierdziły znaczenie nawożenia azotem w kształtowaniu gospodarki żywieniowej sadzonek buka. Efektem wyższych dawek azotu był istotny wzrost zawartości N w liściach i korzeniach badanych sadzonek. Wyniki wskazują, że buk może być stosowany jako gatunek plastyczny, dobrze przystosowujący się do warunków zwiększonej depozycji azotu.

7. Podsumowanie i wnioski

1. Przeprowadzone analizy potwierdziły znaczenie warunków klimatycznych w kształtowaniu cech morfologicznych korzeni bez względu na badany gatunek drzewa. Wraz ze wzrostem wysokości n.p.m. zmniejsza się biomasa korzeni oraz zostaje ograniczony ich przyrost zarówno w przypadku buka jak i jodły.
2. W ekosystemach górskich, poza warunkami klimatycznymi, w kształtowaniu cech systemu korzeniowego mają znaczenie właściwości gleby. Cechy systemu korzeniowego badanych gatunków, tj. buka i jodły w szczególności były skorelowane z pH, zawartością kationów zasadowych oraz zawartością wybranych mikroelementów.
3. Przeprowadzone badania wskazują na silną zależność pomiędzy składem frakcyjnym glebowej materii organicznej, a charakterystykami systemu korzeniowego buka i jodły w gradiencie wysokości.
4. Wyniki doświadczenia potwierdzają znaczenie warunków klimatycznych w kształtowaniu składu frakcyjnego gleb leśnych. W najwyższych położeniach gradientu wysokości z najniższą temperaturą, odnotowano najwyższą zawartość C i N lekkiej frakcji glebowej materii organicznej, co jest bezpośrednim następstwem wolniejszego procesu dekompozycji.
5. Oprócz warunków klimatycznych na stabilizację glebowej materii organicznej wpływa skład gatunkowy drzewostanu. Drzewostany bukowe powodują wyższą akumulację frakcji ciężkiej – związanej z substancją mineralną frakcji glebowej materii organicznej. Wyniki sugerują, że unikanie jednogatunkowych drzewostanów iglastych, ewentualnie wprowadzanie do nich domieszki gatunków liściastych, które stymulują tworzenie się ciężkiej frakcji glebowej materii organicznej – związanej z substancją mineralną, ma uzasadnienie w gospodarce leśnej.
6. Dalsze badania dotyczące wpływu warunków klimatycznych na kształtowanie morfologii korzeni drzew są potrzebne, szczególnie w regionach górskich, gdzie zachodzące zmiany mogą mieć charakter dynamiczny. Uzupełnienie wiedzy w zakresie kształtowania się systemu korzeniowego drzew rosnących w ekosystemach górskich, zapewni lepsze zrozumienie mechanizmu radzenia sobie przez drzewostany z różnymi typami stresu środowiskowego.
7. Aktywność biochemiczna gleby wyrażona aktywnością enzymatyczną jest silnie skorelowana z cechami morfologicznymi systemu korzeniowego oraz ilością C z wydzielin korzeniowych, na co wskazują wyniki badań dotyczące wpływu sześciu gatunków drzew wzrastających w identycznych warunkach glebowo-siedliskowych .

8. Spośród badanych gatunków drzew system korzeniowy jesionu wraz z wydzielinami, mają stymulujący wpływ na liczebność i różnorodność mikroorganizmów glebowych. Podczas gdy badane gatunki iglaste tj. sosna i modrzew oddziaływały mniej korzystnie na skład grzybów i bakterii.
9. Przeprowadzone badania sugerują, że należy zmniejszać udział jednogatunkowych drzewostanów iglastych, które prowadzą do pogorszenia właściwości gleby oraz zmniejszenia różnorodności mikroorganizmów, co w konsekwencji może mieć negatywny skutek dla ich stabilności. W celu poprawy właściwości gleby i zwiększenia różnorodności biologicznej organizmów glebowych należy wprowadzać gatunki liściaste takie jak jesion, grab i dąb.
10. Doświadczenie z symulowaną suszą wskazało na znaczenie uwilgotnienia podłoża w kształtowaniu cech morfologicznych systemu korzeniowego młodych sadzonek drzew oraz ich wydzielin korzeniowych. Cechy morfologiczne korzeni okazały się czynnikiem determinującym wysięk eksudatów w warunkach suszy. Sadzonki dębu bezszypułkowego rosnące w warunkach symulowanej suszy charakteryzowały się istotnie niższymi wartościami wskaźników SRA i SRL, co spowodowało mniejsze wydzielanie C wraz z wysiękiem korzeni.
11. W warunkach symulowanej suszy zmniejszała się ilość wydzielin korzeniowych, co miało bezpośredni wpływ na kształtowanie aktywności enzymatycznej, która przeciętnie zmniejszyła się o połowę, a w pojedynczych przypadkach nawet o 90%.
12. Wiedza na temat czynników kształtujących akumulację C w glebie oraz związek między tym procesem a wysiękami korzeniowymi w warunkach zmieniającego się klimatu jest niezbędny do zrozumienia obiegu C w ekosystemach leśnych. Lepsze zrozumienie mechanizmów i czynników wpływających na dynamikę C w glebach leśnych pozwoli na przewidywanie tych zjawisk w przyszłości, co przyczyni się do ukierunkowanego zapobiegania skutkom zmian klimatu.
13. Wyniki doświadczenia symulującego nadmierną depozycję azotu wykazały, że zastosowanie różnych dawek azotu miało wpływ na charakterystykę systemów korzeniowych, a co za tym idzie, na ilość węgla wydzielanego przez systemy korzeniowe wraz z ich wydzielinami. Ponadto badania potwierdziły znaczenie nawożenia azotem w kształtowaniu stanu odżywienia sadzonek buka.
14. Uzyskane wyniki wskazują, że buk może być wykorzystany jako gatunek o dużych zdolnościach adaptacyjnych, który przystosowuje się do warunków zwiększonej depozycji azotu.

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Fine root morphology and soil properties under influence of different tree stands along an altitudinal climosequence in the Carpathian mountains

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ABSTRACT

In an era of climate change, understanding the factors that impact root systems can improve our understanding of carbon cycling in forest ecosystems. The study objective was to determine the impact of climatic conditions on the biomass and morphology of roots of different tree species along an elevation gradient, and consequently on properties of montane forest soils. The study plots were established at three different elevations (600, 800 and 1000 m a.s.l.) along a slope with an inclination of 15°. The research plots were located in a beech stand (*Fagus sylvatica* L.) and fir stand (*Abies alba* Mill.). Soil samples were collected from each study plot, for which basic physical and chemical properties were determined. Additionally, we determined the morphology, production and decomposition rate of fine roots. Our analyses confirmed the significance of climatic conditions in the formation of soil properties, in particular the amount of accumulated carbon and nitrogen content. A decrease of root biomass and reduced root growth were recorded with increasing elevation. The characteristics of roots were linked with the properties of the studied mountain soils, in particular pH, alkaline cation content and content of selected micronutrients. Limitation of root growth in higher elevations affected both study species. Additional research into the formation of tree root morphology is needed, especially in mountainous regions where changes may occur more dynamically. This will provide a better understanding of how stands can cope with different types of environmental stress.

1. Introduction

Montane climate has a specific character, which is linked to the terrain and elevation above sea level (a.s.l.). With increasing elevation, the temperature and humidity change; the climate becomes more harsh, cool and humid. In addition, mountainous areas are characterised by long-lasting snow cover and low annual temperature amplitude. The vegetation affecting soil properties also changes along an elevation gradient (Staszel et al., 2021). Climatic conditions on mountains (temperature, humidity), and the quantities and diversity of microorganisms and organism inputs (plant litter, roots) have a major significance for the creation of soil properties (Bu et al., 2012; Wang et al., 2017; Zhang et al., 2021). These factors subsequently determine the efficiency and stability of ecosystems, which are controlled by precipitation, air temperature and light intensity (Morales et al., 2007). Plants and soil, despite being different components of the biogeochemical cycle, are strictly interlinked and interact (Liu et al., 2021). According to Zhang et al. (2021), vegetation type and soil pH might be the main factors controlling the spatial variability of soil organic carbon (SOC) and total nitrogen in mountain ecosystems. The composition of plant assemblages, in particular the number of plant species, changes as a result of climatic limitations. The number of trees, shrubs and undergrowth plants is reduced with increasing elevation, which translates to the activity of soil microorganisms (Klimek et al., 2020). In addition, the growth and biomass of fine roots decreases with the altitudinal gradient, which could be related to rainfall or nutrient availability (Sierra Cornejo et al., 2020). At lower elevations in montane areas which have favourable climatic conditions with a longer vegetative period plants produce greater amounts of biomass, which supplies the upper soil layer, whereas at higher elevations a decrease in biomass is observed, resulting in a lower influx of detritus to soil (Kotas et al., 2018).

Soil properties may determine a number of the features of root systems. They significantly affect the possibility of development of root systems, especially by mechanical resistance. The presence of highly rocky or dense soil horizons usually results in less root penetration into the soil. Apart from the graining characteristics, the water and air content in the soil significantly affect the ability to penetrate by the roots (Crow, 2005). Among the chemical characteristics of soil, acidification

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especially the presence of toxic aluminium and the availability of phosphorus exerts the strongest influence on the development of plant root systems (Persson et al., 1995; Lockwood et al., 2003; Robles-Aguilar et al., 2019). Fahey et al. (2005) estimated C input to soil in the Hubbard Brook northern hardwood forest from fine and woody roots to be about 90 and 40 g C·m^{-2}·yr^{-1}, respectively. Using these estimates, the ratio of aboveground to belowground detrital C input to soil is roughly 1.6. According to Joslin and Henderson (1987), the amount of residue reaching soil from root decay is five times greater than from plant litter fall. The roots are one of the more important components of plant biomass, thanks to which vegetation can control the intake of water and nutrients while also impacting biogeochemical cycles. Despite their minor size, fine roots constitute the main source of carbon in soil due to annual root dieback (Jackson et al., 1997; Jones et al., 2004). In addition, the amount of nutrients that are released by the death of fine roots is much greater than that released by the decomposition of plant litter decay (Yuan and Chen, 2010). Depending on the soil type, roots comprise approximately 30% of its biomass, but also 40%–85% of net primary production (Hoffmann and Usoltsev, 2001). In times of changing climatic conditions, trees have to adapt to the environment surrounding them. To this end, they use either extensive or adaptive strategies, as appropriate, which differ according to species, climate factors like temperature and precipitation, and also soil properties (Finér et al., 2011). In the case of an extensive strategy, biomass and the length of fine roots are increased, and this adaptation enables growth even under difficult conditions (Ostonen et al., 2007). This also impacts the distribution of roots in the soil profile, which consequently greatly influences the cycles of carbon and nitrogen in soil. Stress situations associated with excessive drought or damage can be reflected in the chemical and morphological characters of roots, which include specific root length (SRL), root tissue density (RTD) and root diameter (Weemstra et al., 2016). In response to stress factors, roots become more plastic, allowing them to seek more nutrients more easily and quickly, which will translate into faster growth and productivity of the tree (Hodge, 2004; Kembel and Cahill, 2005). Detailed knowledge of the factors influencing root biomass and morphology will allow the cultivation of stands that are more stable and more resistant to change. The detailed response of the root systems of various temperate forest species to climate change is currently unknown.

Knowledge on the factors affecting the root systems of different tree species is important for a better understanding of the carbon cycle in forest ecosystems. Our study utilised climatic sequence in a mountain ecosystem for the monitoring of the effect of climatic conditions on the creation of biomass and root morphology. The objective of our study was to determine the biomass and morphology of the roots of beech and fir tree stands growing in different climatic conditions. An additional study objective was to determine the growth of roots and the rate of root decomposition in diverse climatic conditions. The following research hypotheses were tested: i) along with the height along the elevation gradient, the climatic conditions and soil properties change and, as a consequence, the rate of decomposition processes of organic debris in the soil changes; ii) the location along the elevation gradient is important in shaping the morphological features of tree roots; iii) properties of montane forest soils, such as acidity and quality of organic matter, are related to the root characteristics of the studied tree species.

2. Materials and methods

2.1. Study area and soil sampling

The study was conducted in the Jałowiec Massif (49°38′ N, 19°20′ E) in the Western Beskidy Mountains, in the southern part of Poland. The study took place in Magurska Nappe on the sandstone and shales, with domination of Cambisols (WRB, 2014). The study plots were established at three different elevations (600, 800 and 1,000 m a.s.l.) along a slope with an inclination of 15°. The tested soils were characterised by a similar texture (average sand content was 54%, silt 42% and clay 3%). The average temperature of the growing season for the study plots was 12.4 °C at 600 m a.s.l., 11.3 °C at 800 m a.s.l. and 10.2 °C at an elevation of 1,000 m a.s.l. Average soil moisture at 600, 800 and 1,000 m a.s.l. differed, at 22.74%, 29.60% and 34.10%, respectively. The research plots were located in a beech stand (*Fagus sylvatica* L.) and fir stand (*Abies alba* Mill.), both at the age of 60. In each variant of elevation, 3 research plots (0.10 ha) were designated separately for each species. The samples were taken after removing the litter from a depth of 0–15 cm. Three soil samples from different locations were collected on each plot. In total, 54 soil samples were collected for the analysis. The field study was realised in 2021.

2.2. Root analysis

On each study plot, soil samples with a known volume of 15 cm × 15 cm × 15 cm were collected in three replications to determine root biomass, from which coarse roots with diameter >2 mm and fine roots with diameter <2 mm were separated. The extracted root system fragments were scanned at 400 dpi resolution and then analysed using a WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Ville de Québec, QC, Canada) to determine diameter, length and root area. Air-dried roots were further desiccated at 70 °C for 24 h to constant weight and then weighed. Root tissue density (RTD) (kg·m^{-3}), specific root area (SRA) (m^2·kg^{-1}), and specific root length (SRL) (m·g^{-1}) were calculated according to Ostonen et al. (1999). Following the analysis of the roots, they were used for the determination of the root decomposition rate in the later stage of the experiment. Bags (15 cm × 20 cm) containing 10 g of root matter were prepared for the determination of root decomposition. Bags with roots were buried to a depth of 10 cm in each study area for the period from May to October. After this period, the root litter mass loss in the bags was determined. The annual fine root (diameter <2.0 mm) biomass increase (FRBI) was determined with the core method (Böhm, 1985). The experiment sought to determine the root production between May and October 2021.

2.3. Laboratory analysis

Air-dried soil samples were sieved through a 2-mm mesh. Physical and chemical properties were determined in these prepared samples (Ostrowska et al., 1991). Soil pH was determined by the potentiometric method in water and 1 mol·L^{-1} KCl. Hydrolytic acidity (*Y*) was determined by the Kappen method. Total nitrogen and carbon content was determined using a LECO CNS True Mac Analyser (Leco, St. Joseph, MI, USA). The amount of alkaline cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in 1 mol·L^{-1} ammonium acetate was determined by an ICP-OES (iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK). The Cd, Cr, Cu, Ni, Pb and Zn contents were determined after mineralisation in a 2:1 mixture of concentrated nitric and perchloric acids by ICP-OES. The gravimetric method was used to determine soil moisture. The soil samples were dried at 105 °C for 48 h and weighed after drying. The humidity was determined according to Eq. (1):

$$Mw = \left(\frac{Mn - Md}{Md}\right) * 100\ (\%) \quad (1)$$

where Mw is the weight of moisture, Mn is the weight of the fresh sample, and Md is the weight of the dried sample.

2.4. Statistical analysis

Spearman correlation coefficients were computed to determine the mutual relationship between individual root parameters and soil properties. Principal component analysis (PCA) was used to evaluate relationships between soil and root characteristics. A general linear model (GLM) was used to investigate the effects of elevation and different stands and the interactive effect of these two factors on fine root biomass

and morphology. Tukey's test was used to evaluate differences between mean trait values. The results were considered to be statistically significant at $\alpha < 0.05$. All statistical analyses were performed using R statistical software (R Core Team, 2021), RStudio (RStudio Team, 2020), and Statistica 10 software (2010).

3. Results

3.1. Root analysis

In the case of beech, longer roots were observed compared to fir (Fig. 1a). The studied tree species differed statistically significantly in root system length. The length of the beech root systems ranged from 3,957.80 to 6,452.58 cm, and fir from 2,269.63 to 2,475.62 cm at an elevation of 1,000 and 600 m a.s.l., respectively. A tendency for reduced root length with increasing elevation could be seen for both species. Root diameter in the case of fir increased with elevation from 0.77 to 0.93 mm, but these differences were not statistically significant (Fig. 1b). Statistically significant differences in root diameter between species were recorded at elevations of 800 and 1,000 m a.s.l. Root biomass for beech and fir did not change statistically significantly with increasing elevation (Fig. 1c). Root biomass differed significantly between the tested tree species only at an elevation of 600 m a.s.l. In the case of SRA, significant differences between the study species were recorded at an elevation of 1,000 m a.s.l., with an average of 7.34 $m^2 \cdot kg^{-1}$ for fir and 11.70 $m^2 \cdot kg^{-1}$ for beech. Fir SRA did not change significantly along the elevation gradient. The highest beech SRA was recorded at an elevation of 1,000 m a.s.l. (Fig. 2a). RTD and SRL of both species did not change significantly along the elevation gradient (Fig. 2b and c). At an elevation of 600 m a.s.l., significant differences of RTD, and at 1,000 m a.s.l., significant differences of SRL, were recorded between beech and fir. The study species did not differ significantly in root biomass, irrespective of location along the elevation gradient (Fig. 3a). In the case of fir, no statistically significant differences in root biomass were determined along the elevation gradient. The lowest beech root biomass was recorded at the highest locations; the average varied from 3.27 to 5.66 $g \cdot dm^{-3}$. No significant differences resulting from the effect of species and elevation could be recorded for the decomposition rate (Fig. 3b). At 800 m a.s.l., the values oscillated from 24.38% to 29.24% for beech and from 19.48% to 31.35% for fir. In the case of root growth, statistically significant differences were recorded at 600 and 1,000 m a.s.l. (Fig. 3c). The root growth of beech did not change significantly along the elevation gradient; for fir, significantly greater growth was recorded at 800 m a.s.l. GLM analysis confirmed the significance of location along the elevation gradient on root biomass and root growth of the examined tree species (Table 1). Tree species was significant for formation of length and diameter of roots, SRL and root production. The simultaneous importance of tree species and location along the elevation gradient was recorded for root weight (Table 1).

3.2. Soil properties

The conducted analyses indicate differences in the physicochemical properties resulting from the effect of different tree species and location along the elevation gradient (Tables 2 and 3). At an elevation of 600 m a.s.l., statistically significant differences in nitrogen (N) content were recorded between the study species. At this elevation, soils under the effect of beech were characterised by higher N content compared to soils under fir influence (Table 2). At an elevation of 800 m a.s.l., the differences between the study species were recorded for pH in KCl, C and N content, C/N ratio, and Mg, K and Na content. At 1,000 m a.s.l., no differences between the tested species were recorded other than for Ca content (Table 2). Significant differences between the study species were also recorded for micronutrient content (Table 3). At an elevation of 600 m a.s.l., differences between species in the content of Mn were recorded; at 800 m a.s.l., in Co, Fe, Mn and Pb content, and at 1,000 m a.s.l., in Cd, Co, Cu, Mn, Ni and Pb content. Soils at the lowest elevation were characterised by different properties in comparison to soils at elevations of

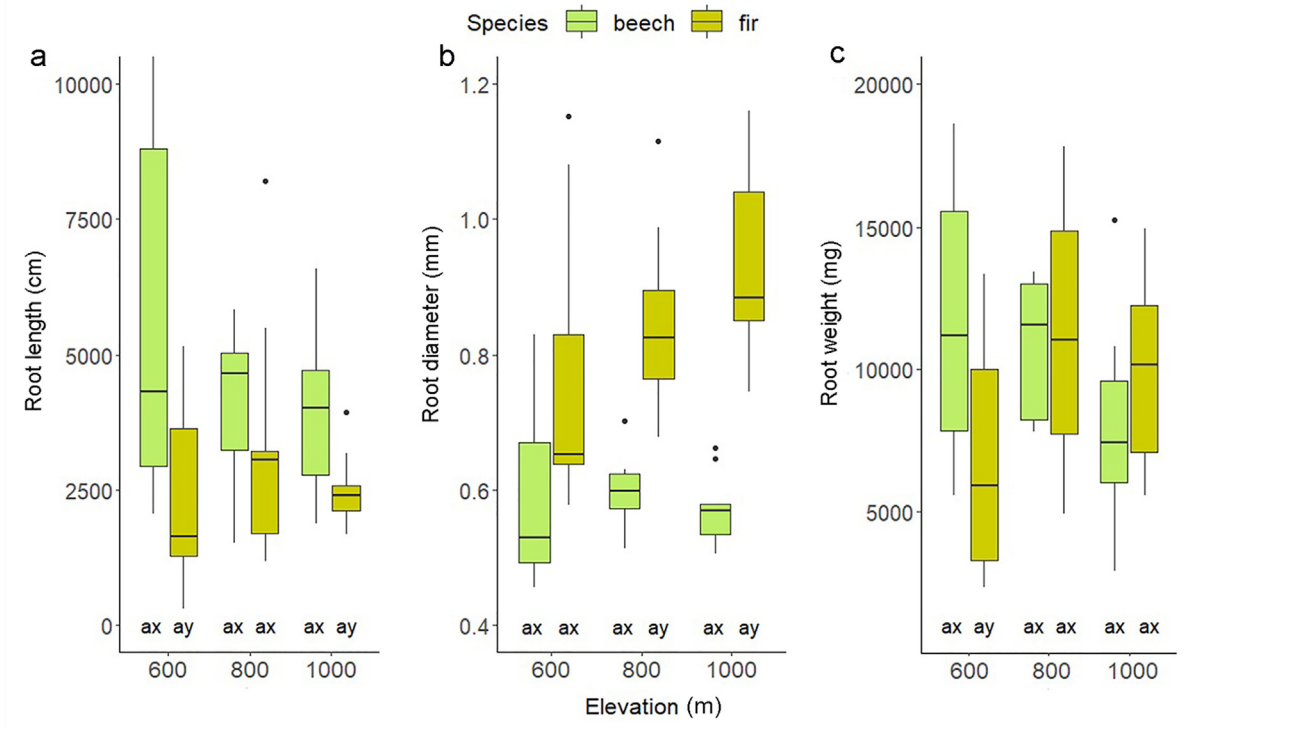

Fig. 1. Root length (cm), root diameter (mm) and root weight (mg) at different elevations. Different lowercase letters (a, b, c) indicate significant parameter differences between different heights, (x, y) indicate significant parameter differences between species; TukeyHSD $p < 0.05$ (box-whisker plots with median, 25- and 75-percentiles, and extremes).

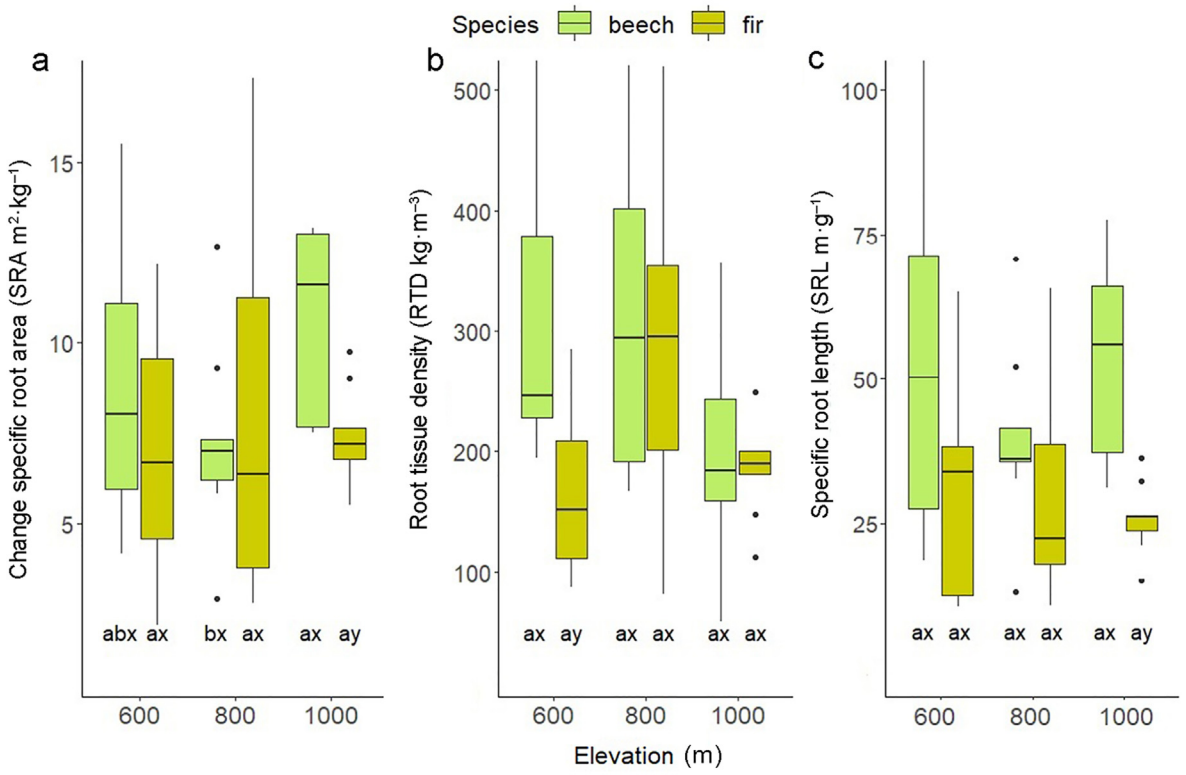

Fig. 2. Change specific root area (SRA $m^2 \cdot kg^{-1}$), root tissue density (RTD $kg \cdot m^{-3}$) and specific root length (SRL $m \cdot g^{-1}$) at different elevations. Different lowercase letters (x, y) indicate significant parameter differences between species, letters (a, b, c) indicate significant parameter differences between different heights; TukeyHSD $p < 0.05$.

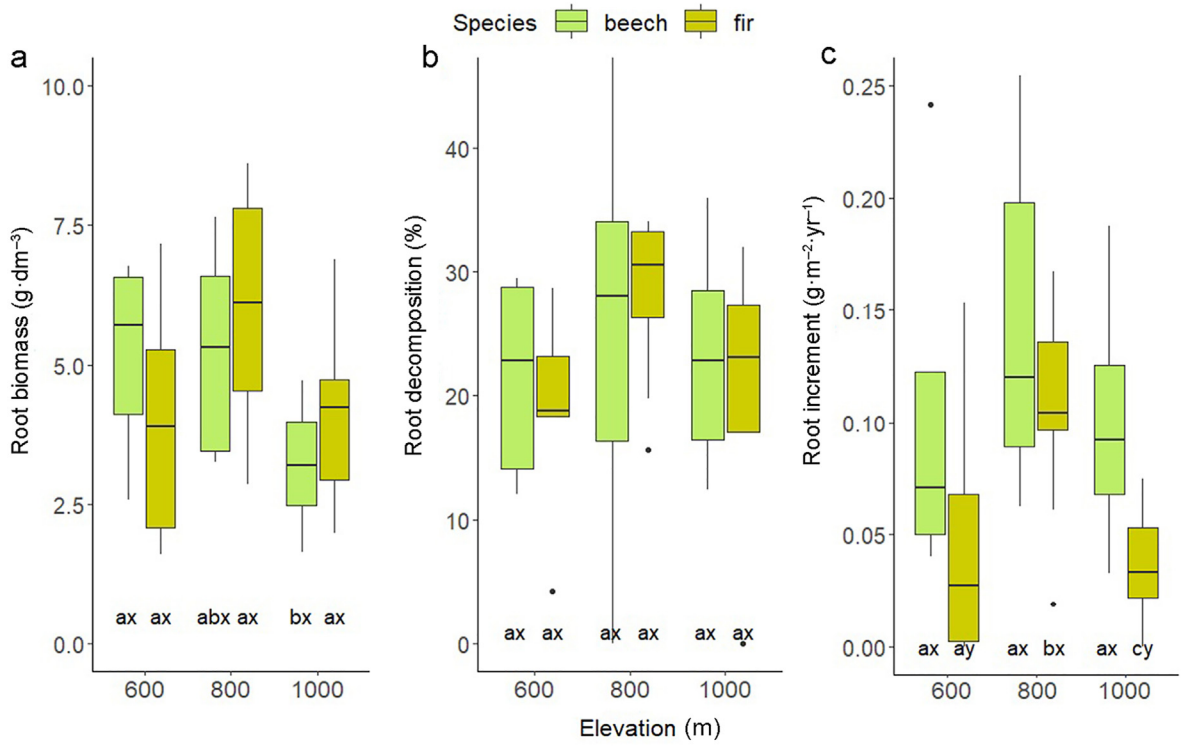

Fig. 3. Root biomass ($g \cdot dm^{-3}$), root decomposition (%) and root increment ($g \cdot m^{-2} \cdot yr^{-1}$) at different elevations. Different lowercase letters (x, y) indicate significant parameter differences between species, letters (a, b, c) indicate significant parameter differences between different heights; TukeyHSD $p < 0.05$.

Table 1
Summary of GLM analysis for root parameters influenced by different tree species in gradient (a.s.l.).

	Length		Diameter		Weight		SRA		SRL	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Elevation	0.8431	0.4366	0.0211	0.3065	1.6969	0.1941	1.2995	0.2821	0.6782	0.5123
Species	8.8972	**0.0045**	0.9214	**<0.001**	0.4913	0.4867	3.0327	0.0880	13.5058	**0.0006**
Elevation × Species	2.0804	0.1360	0.0345	0.1484	4.2219	**0.0205**	2.1182	0.1314	1.3166	0.2776
	RTD		**Biomass**		**Decomposition**		**Increment**			
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*		
Elevation	1.3633	0.2656	5.1241	**0.0096**	2.3459	0.1067	3.7390	**0.0310**		
Species	0.2692	0.6062	0.0559	0.8141	0.3806	0.5402	11.2197	**0.0016**		
Elevation × Species	1.9940	0.1473	3.1443	0.0521	0.3560	0.7023	0.6031	0.5512		

Significance effect ($p < 0.05$) are shown in bold.

Table 2
Chemical properties of study soils under influence of different species in gradient.

Elevation (m)	Species	pH (H_2O)	pH (KCl)	*Y*	C	N	C/N
600	Beech	4.84 ± 1.04^{ax}	4.18 ± 0.40^{ax}	2.42 ± 0.53^{ax}	5.23 ± 0.91^{bx}	0.37 ± 0.05^{bx}	14.12 ± 1.58^{ax}
	Fir	4.91 ± 0.20^{ax}	3.91 ± 0.18^{ax}	2.61 ± 0.37^{ax}	4.51 ± 0.62^{bx}	0.32 ± 0.04^{by}	14.01 ± 0.61^{ax}
800	Beech	4.09 ± 0.19^{bx}	3.45 ± 0.12^{bx}	4.90 ± 1.02^{bx}	6.72 ± 1.65^{abx}	0.43 ± 0.09^{abx}	15.65 ± 0.81^{abx}
	Fir	3.94 ± 0.15^{bx}	3.27 ± 0.14^{by}	5.83 ± 1.04^{bx}	10.44 ± 2.92^{by}	0.55 ± 0.10^{ay}	18.66 ± 1.81^{by}
1000	Beech	4.04 ± 0.25^{bx}	3.40 ± 0.19^{bx}	5.32 ± 0.98^{bx}	8.80 ± 2.02^{ax}	0.49 ± 0.09^{ax}	17.89 ± 1.76^{bx}
	Fir	3.65 ± 0.08^{by}	3.02 ± 0.11^{by}	8.69 ± 1.71^{by}	14.81 ± 5.60^{ay}	0.75 ± 0.23^{ay}	19.64 ± 1.31^{by}
Elevation (m)	**Species**	**Ca**	**Mg**	**K**	**Na**	**P**	
600	Beech	142.46 ± 70.39^{ax}	17.47 ± 8.41^{ax}	11.35 ± 2.49^{ax}	1.13 ± 0.71^{ax}	536.77 ± 109.84^{bx}	
	Fir	93.76 ± 28.64^{ax}	12.89 ± 4.19^{ax}	11.63 ± 3.25^{bx}	0.83 ± 0.07^{bx}	458.72 ± 56.84^{ax}	
800	Beech	19.49 ± 7.62^{bx}	4.17 ± 0.92^{bx}	6.56 ± 1.64^{bx}	0.65 ± 0.11^{bx}	640.32 ± 119.98^{bx}	
	Fir	14.57 ± 5.72^{bx}	4.18 ± 1.09^{by}	7.15 ± 3.68^{ay}	0.93 ± 0.15^{ay}	627.93 ± 89.45^{bx}	
1000	Beech	9.99 ± 4.14^{bx}	3.64 ± 0.64^{ax}	7.44 ± 2.45^{bx}	0.70 ± 0.09^{bx}	859.73 ± 68.14^{ax}	
	Fir	17.71 ± 16.92^{bx}	5.60 ± 2.09^{aby}	8.43 ± 4.33^{by}	1.24 ± 0.32^{aby}	689.51 ± 52.15^{by}	

Mean ± SD C, N (%); Ca, K, Mg, Na and P (cmol(+)·kg^{-1}); *Y* - hydrolytic acidity (cmol(+)·kg^{-1}); small letters in the upper index of the mean values mean significant differences between height (a, b, c) and species (x, y).

Table 3
Microelements content (mg·kg^{-1}) in study soils under influence of different species in gradient.

Elevation (m)	Species	Cd	Cr	Co	Cu	Fe
600	Beech	1.15 ± 0.54^{bx}	75.20 ± 14.81^{ax}	10.75 ± 2.59^{bx}	11.44 ± 3.73^{ax}	$20538.89 \pm 5446.69^{ax}$
	Fir	0.71 ± 0.17^{ax}	68.20 ± 5.26^{ax}	8.97 ± 0.93^{ax}	10.75 ± 2.06^{ax}	$16763.89 \pm 1355.30^{bx}$
800	Beech	0.34 ± 0.06^{ax}	69.83 ± 5.32^{ax}	6.00 ± 1.38^{bx}	10.55 ± 2.36^{ax}	$16138.06 \pm 1349.93^{ax}$
	Fir	0.37 ± 0.06^{bx}	65.68 ± 9.16^{ax}	4.23 ± 1.02^{by}	10.26 ± 1.10^{ax}	$14560.56 \pm 1555.96^{ay}$
1000	Beech	0.51 ± 0.21^{abx}	66.53 ± 7.04^{ax}	3.28 ± 0.21^{ax}	13.47 ± 2.44^{ax}	$15400.56 \pm 1824.26^{ax}$
	Fir	0.35 ± 0.06^{by}	71.27 ± 5.39^{ax}	4.85 ± 1.53^{by}	10.53 ± 0.89^{ay}	$16208.33 \pm 687.62^{abx}$
Elevation (m)	**Species**	**Mn**	**Ni**	**Pb**	**Zn**	
600	Beech	2076.07 ± 1941.08^{ax}	40.70 ± 16.26^{bx}	50.21 ± 8.20^{bx}	92.97 ± 28.20^{ax}	
	Fir	780.84 ± 367.54^{ay}	35.65 ± 6.76^{ax}	43.92 ± 7.65^{ax}	82.20 ± 21.38^{ax}	
800	Beech	396.30 ± 119.86^{bx}	23.90 ± 4.44^{bx}	65.32 ± 17.11^{bx}	56.20 ± 6.10^{abx}	
	Fir	266.27 ± 116.10^{by}	18.41 ± 5.32^{bx}	88.66 ± 17.87^{by}	53.32 ± 3.68^{bx}	
1000	Beech	182.17 ± 27.07^{cx}	15.39 ± 2.16^{ax}	133.25 ± 30.83^{ax}	53.11 ± 10.17^{bx}	
	Fir	326.69 ± 151.17^{by}	19.13 ± 3.12^{by}	75.85 ± 20.14^{by}	51.19 ± 5.95^{bx}	

Mean ± SD; small letters in the upper index of the mean values mean significant differences between height (a, b, c) and species (x, y).

800 and 1,000 m a.s.l. (Table 2). At 600 m a.s.l., soils were characterised by significantly higher pH, lower acidity, higher content of alkaline cations and lower C/N ratio in comparison with soils at 800 and 1,000 m a.s.l., irrespective of species. Significantly higher C and N content was recorded for soils at an elevation of 1,000 m a.s.l., irrespective of species. In the case of micronutrients, a less pronounced significance of location along the elevation gradient was recorded (Table 3).

3.3. The relationship between root traits and soil properties

The conducted analysis confirmed the relationships between root traits and soil properties along the elevation gradient (Fig. 4). Root length correlated positively with pH and content of Ca, Mg, Na, Cd, Fe, Mn, Ni and Zn. Root diameter correlated positively with hydrolytic acidity, content of C and N, and C/N ratio. Root diameter correlated negatively with pH and content of Ca, Cd, Cu, Fe, Mn, Ni and Zn. The content of Cu and Pb correlated positively with SRA and negatively with root biomass. SRL exhibited a positive correlation with pH and content of Ca, Mg, Cd, Cu, Fe, Ni and Zn. In addition, root growth correlated positively with Mn content (Fig. 4). The conducted PCA analysis confirmed relationships between the parameters describing roots and soil properties. In addition, the PCA confirmed the distinction between beech and fir tree stands (Fig. 5). Factors 1 and 2 explained 46.8% of variance of the tested characteristics. Factor 1 was related to the chemical properties of the tested soils and location along the gradient; Factor 2 was related to root parameters. In addition, PCA analysis confirmed the distinction between the properties of soils located at the elevations of 600 and 1,000 m a.s.l. Soils in these locations differed in acidification, C and N content and the content of alkaline cations.

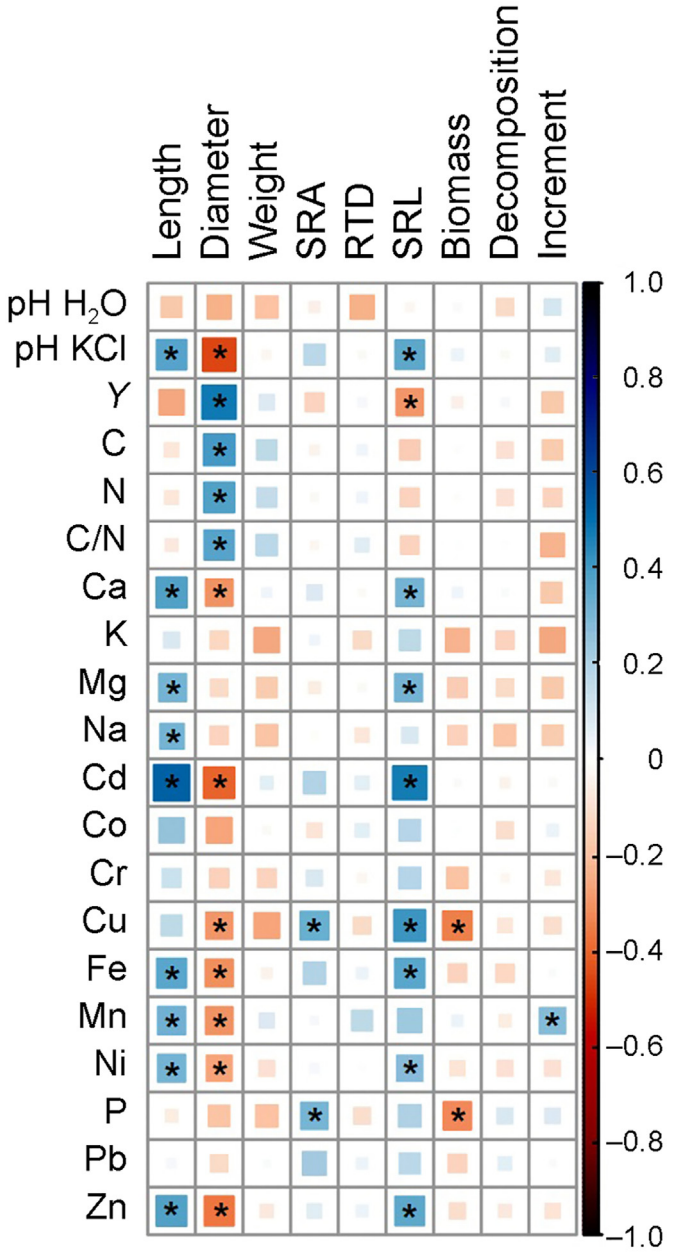

Fig. 4. Correlation between root characteristics and soil parameters. Significance effect * ≤0.05.

4. Discussion

4.1. Soil properties

The conducted research confirmed the validity of the research hypotheses. Our analyses confirmed the significance of climatic conditions in the formation of soil properties, in particular the amount of accumulated carbon and nitrogen content. Carbon and nitrogen resources increased with elevation. Soils at the lowest elevations were characterised by significantly different physicochemical properties as compared with soils from the higher locations. Independently of tree species, at an elevation of 600 m a.s.l. soils were characterised by significantly higher pH, lower hydrolytic acidity, higher content of alkaline cations and lower C/N ratio in comparison with soils at 800 and 1,000 m a.s.l. Our results are congruent with the results of earlier studies, which demonstrated that in granite-based soils, total organic carbon stock increases with elevation a.s.l. and soil acidification increases along an elevation gradient (Bojko and Kabała, 2016; Lasota et al., 2016; Staszel et al., 2021). The results obtained in the present study are likely the effect of the increasing precipitation and reduced temperature at higher locations along the elevation gradient. Greater accumulation of acidic organic matter at higher montane locations is the outcome of retardation of its decomposition, which is directly linked to low temperatures inhibiting the biological decomposition of organic residues (Prescott, 2010; Klimek et al., 2020). Lower C/N at lower elevations confirmed the higher efficiency of decomposition of soil organic matter, which is directly linked with the biological activity of soils. At lower elevations the climate is milder, which creates better conditions for the growth and activity of microorganisms. In a study by Zhao et al. (2021), climate factors including MAT, MAP and their combined effects controlled the main elevation patterns of soil microorganisms. Acidification of soils located at higher elevations is also linked with the quality of soil organic matter. Soils from lower elevations are accompanied by mor-type humus, which is dominated by poorly decomposed organic residues. Mor humus is formed under the conditions of low biological activity in the soil, where organic matter mineralisation occurs at a slow rate (Lasota et al., 2020). On the other hand, Mull is a type of well-humified organic matter associated with high biological activity. Mull humus is characteristic of soils at lower elevations. More favourable thermal and humidity conditions prevail at lower locations, which affects the decomposition rate of soil organic matter. In our study, we have obtained confirmation of the results of Bojko and Kabała (2017), stating that humus forms reflect the accumulation of carbon in soils of the elevation gradient. The resources of C and N and other soil properties are not affected solely by the climate, but also by vegetation. Along the elevation gradient, differences in the amount of organic matter provided may result from the intensity and differentiation of plant layer development. It is known that the elevation gradient is one of the decisive factors forming the spatial patterns of species diversity (Lomolino, 2001). In addition, the availability of light, humidity and soil depth change along the elevation gradient, leading to local differences in the species composition of plants (Cirimwami et al., 2019). An additional cause for soil acidification and lower levels of alkaline cations at higher elevations is the increase of precipitation, which affects the leaching processes (Kocowicz, 1998). Our research showed the role of root systems in shaping the accumulation of soil organic carbon in the studied soils. In particular, underground biomass in the form of fine roots not only takes up nutrients but can also, through root exudates or decaying dead roots, contribute to the deposition of about 58%–96% of the total C in the soil (Shahzad et al., 2015). The content of organic acids extracted by the roots not only influences organic carbon, but also contributes to the formation of microbial processes within the rhizosphere (Badri and Vivanco, 2009; Erktan et al., 2018).

4.2. Root analysis

The conducted study also confirmed the validity of the second hypothesis, concerning the importance of location along the elevation gradient and species on the traits of root systems of trees. In this study, we were able to observe statistically significant differences in the length and diameter of roots, SRL and root growth. Independently of location, beech was characterised by the highest root length compared to fir. Our study confirmed the typical species traits of the analysed trees. The silver fir develops a strong taproot, which can develop independently of the habitat. In addition, fir develops a regular crown comprising of lateral roots, creating anchor-like branching (Köstler et al., 1968). The European beech initially develops a taproot, but this alters into a very dense heart-shaped root system, particularly in the direct vicinity of the trunk (Köstler et al., 1968). In addition, the present study demonstrated the role of species in SRL determination; higher SRL was recorded for beech. Analysis of morphological characters can provide information on the strategies of plants, their adaptation to climatic conditions and to the environment, elevation above sea level or availability of nutrients (Ostonen et al., 2007; Valverde-Barrantes et al., 2015; Freschet et al.,

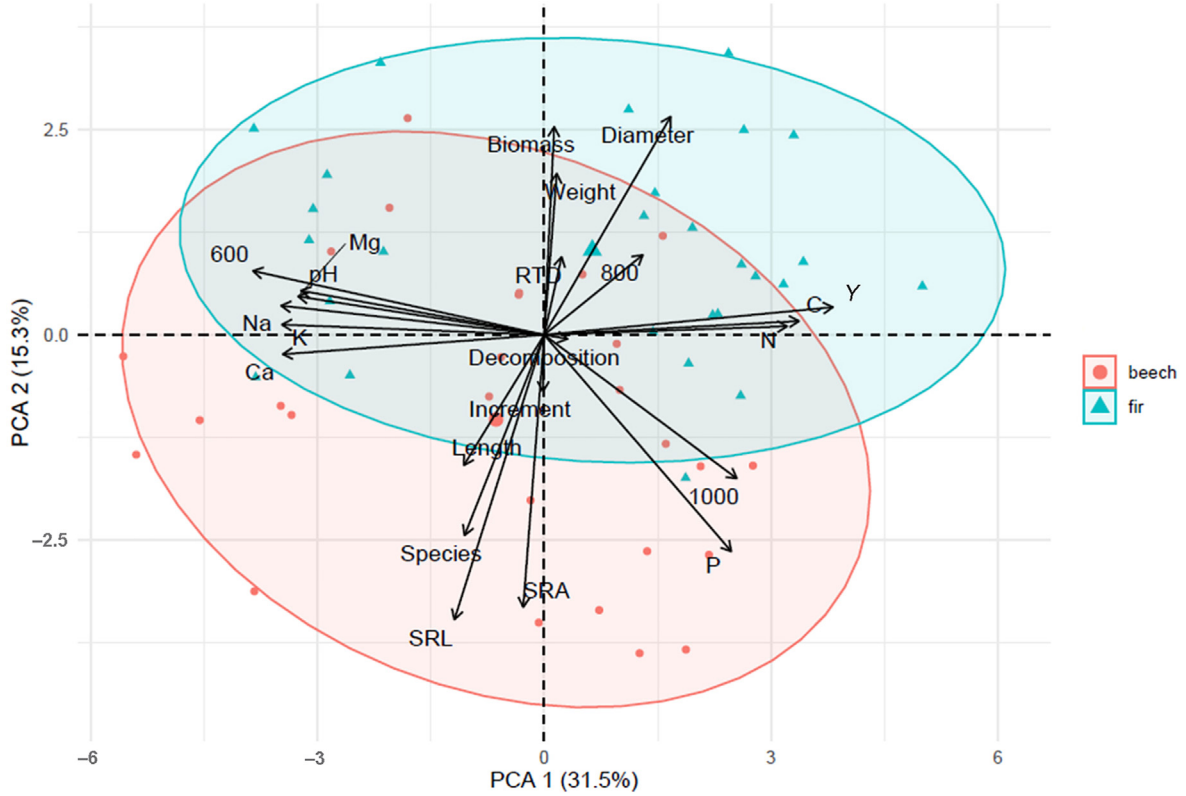

Fig. 5. Projection of variables onto the plane of the first and second PCA factors.

2017). Better characteristics of root systems in the case of beech predispose it for creating tree stands at higher elevations in mountains. Ryser (1996) noted that the response of a plant to environmental conditions results in changes to root tissue density (RTD) and diameter, which may have a contrasting effect on SRL. Changes in the morphology of roots at different elevations affect SRL and SRA. In addition, the elevation and species have a marked impact on fine root growth during the vegetation season. This relationship was also confirmed by the GLM analysis. The greatest root biomass and growth was recorded at an elevation of 800 m a.s.l., which may be associated with more favourable humidity conditions at a sufficiently long vegetative season. Greater root growth was recorded for beech than for fir. This may have stemmed from the seasonal fine root production which, depending on the species, occurs in spring—at the beginning of the vegetative season, or at the end of it (Persson, 1983; Burton et al., 2000). Research conducted in a beech forest in Italy demonstrated increased biomass and root length in mid-summer and at the beginning of autumn (Montagnoli et al., 2014). Other research on a coniferous species provided evidence that the peak of production occurred between spring and summer (Tingey et al., 2005). On the other hand, another study on the Douglas fir conducted over a period of several years also demonstrated growth in autumn and winter (Santantonio and Hermann, 1985). Differences in the growth of roots between the species investigated in the present study can be explained by the study period. Our experiment on root growth was conducted from spring through autumn, with omission of the winter months, which may be of key significance for the growth of coniferous roots.

4.3. The relationship between root traits and soil properties

The conducted study confirmed the relationship between root characteristics and properties of montane forest soils. Root length correlated positively with pH, alkaline cation content and selected micronutrient content in the studied soils. Soil acidification, and its resultingly increased Al content, may directly alter root characteristics, disturb root growth and limit root functions (Vanguelova et al., 2007). Świątek and Pietrzykowski (2021) identified chemical properties of soils as factors affecting the biomass of fine roots. The pH values between 3.5 and 4.1 increased the growth of fine roots, whereas availability of exchangeable Mg inhibited such growth. In the present study, a reversed relationship between fine root growth and Mg content was observed. Soil richness in alkaline cations (Ca, Mg and Na) stimulated the root growth of the tested tree species (Świątek and Pietrzykowski, 2021). According to Meier et al. (2020), on fertile soils rich in alkaline cations, the growth of fine roots of beech is intensive. Root growth can also be associated with the microbial activity of soils. Acidic soils with low pH are characterised by lower microorganism activity. Earlier research demonstrated that the combination of arbuscular mycorrhizal fungi (AMF) and green algae resulted in carbon decomposition and formation of soil aggregates through pH reduction and increased C bacteria biomass, and consequently root density (Al-Maliki and Breesam, 2020). A clear correlation between upper soil pH values and ectomycorrhizal fungi investigated in five different forest tree species was not observed by Shirkonyer et al. (2013).

5. Conclusions

The conducted study confirmed the significance of climatic conditions for the properties of montane forest soils and the biomass and morphology of beech and fir roots. With decreasing temperature shortening the vegetative period at higher positions along the elevation gradient, the organic residue decomposition process is limited, soil pH is reduced and the conditions for the development of root system deteriorate. At the highest elevations, characterised by a harsher climate, lower root biomass and poorer root production were observed. Limitation of root growth occurred in both study species; however, in the case of beech, greater adaptive capacity to the changing environmental conditions was observed. It can be expected that, in the event of climate warming, beech will be predisposed for expanding its distribution range.

Authors' contributions

K.S., E.B. and J.L.: conceived and designed the investigation; analysed and visualized the data; E.B. and J.L.: concepts research methodology; K.S., E.B. and J.L.: preparation of manuscript.

Availability of data and material

The data that support the findings of this study are available from the corresponding author on reasonable request.

Declaration of competing interests

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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Soil Organic Matter Fractions in Relation to Root Characteristics of Different Tree Species in Altitude Gradient of Temperate Forest in Carpathian Mountains

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Abstract: The roots are a key functional component of belowground systems and one of the main factors influencing the quality and quantity of soil organic matter. Our research aimed to determine the fractional composition of the soil organic matter (SOM) in soils under various tree species on an altitude gradient. In our research, we related the SOM fractions with the root characteristics. There is a lack of information on the relationship between the SOM fractions and the root properties. We assessed labile and heavy fractions of SOM content in forest mountain soils with a climosequence approach. The study plots were established at 600, 800, and 1000 m above sea level in a beech stand (*Fagus sylvatica* L.) and a fir stand (*Abies alba* Mill.). In this case, three research plots with beech and fir were designated in each altitude variant. Forest stands growing in the same soil conditions were selected for the study. The research used stands of similar age with the same tree canopy density. The basic physicochemical properties (pH, hydrolytic acidity, carbon and nitrogen content, base cations content) and the fractional composition of the SOM were determined from soil samples. In addition, we determined the basic characteristics of the roots (diameter, length, biomass, decomposition, production). The correlation between soil organic matter fractions and root characteristics was recorded. This study confirmed the importance of climatic conditions in shaping the fractional composition of forest soils. In the highest locations, characterized by lower temperatures, the light fraction of the SOM exhibited the highest C and N content, which is the effect of slower decomposition processes. Apart from climatic conditions, the stabilization of SOM is influenced by the tree species composition of a forest stand. Beech forest stands lead to a larger accumulation of a heavy fraction of SOM. This study indicates a positive correlation between the light fraction of SOM, root biomass, and decomposition rate of roots. Our research shows that avoiding single-species coniferous stands and introducing admixtures of deciduous species, which increase the heavy SOM fraction, is justified in forest management.

Keywords: beech; fir; soil; fine root; carbon accumulation; organic matter fractions

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1. Introduction

Organic matter is a basic component of soil that determines its physical, chemical, and biological properties [1–3]. Properties influenced by organic matter include soil structure, moisture capacity, diversity, and activity of soil organisms and nutrient availability. Conserving and increasing the amount of soil organic matter (SOM) offers many benefits, including climate change mitigation [4,5]. Climate change leads to increased temperatures, which drives the decomposition process rate in soils [6]. Temperature changes alter the activity of microorganism communities involved in decomposition processes, in turn reducing the soil carbon pool [7]. Physical fractionation of SOM allows extracting free light fraction (fLF), the occluded light fraction (oLF), which may become stabilized by occlusion inside aggregates, and heavy fraction, which is the mineral-associated fraction

(MAF) [8,9]. The light fraction of SOM is unstable and remains in the soil for weeks to years, in contrast with the heavy fraction, which can persist for decades. The light fraction of SOM is less resistant to changes resulting from land management [10]. According to De Feudis et al. [11], an increase of 1 °C in mean annual air temperature reduces the amount of organic carbon associated with the light fractions in the rhizosphere.

As a result of the supply of organic remains from above-ground biomass and root systems, forest soils are characterized by a high content of organic carbon. Roots are among the key elements affecting the soil environment through the supplied biomass and root exudates [12,13]. Plant roots react to changes in thermal conditions in the soil environment, which results in changes in the physiology of the roots, their growth rate, and the possibility of obtaining resources [14]. According to Cesarz et al. [15], the impact of root systems on the decomposition rate of SOM and energy channels depends on the tree species.

Our study considered soils in beech and fir stands. Previous studies have confirmed these species' different influences on the physical, chemical and biological properties of soil [3,16]. Trees affect soil properties through several pathways, the most important of these being litter and roots [17–19]. Coniferous stands, especially spruce and pine, acidify the soil environment, influencing the activity and diversity of microorganisms, which directly affect the soil's organic matter decomposition rate. Błońska et al. [16] confirmed the stronger acidifying effects of fir stands compared to deciduous species. According to Likulunga et al. [19], tree species influence soil composition and properties, affecting soil fungal composition and diversity. Root-derived resources from trees are important for soil microbial communities' structure and functioning, so soil microbial responses to tree species will be equally diverse and reflected in the quantity and quality of SOM [20]. A strong correlation of thermal conditions with the growth characteristics of roots in the latitudinal gradient has been proven [21]. It has also been shown that different species have a thermal minimum at which root growth occurs [22]. Considering the above, we decided to conduct our experiment in a climosequence.

This study aimed to determine the amounts of SOM fractions concerning the species composition of stands in an altitude gradient. The selected approach will explain the impact of thermal conditions on shaping soil properties, especially the organic matter fractions. Tree roots' contributions to the accumulation of SOM must be better evaluated to improve forest stand management and carbon stabilization in forest soils. Knowledge of long-term C storage determinants in soil remains limited, especially in mountain forest soils. We suppose that the climate conditions in the altitude gradient determine the biomass and growth of the root systems of the studied tree species, which in turn is reflected in the fractional composition of the SOM. We tested the following hypotheses: (1) reduced temperature and increased humidity in the altitude gradient affect decomposition rate and the share of light and heavy fractions of SOM; (2) the soils of beech stands are characterized by a lower share of the light fraction and higher share of the heavy fraction of SOM compared with the soils of fir stands; and (3) beech and fir stand in a different way influence the fractional composition of SOM in mountain forests through their root systems.

2. Materials and Methods

2.1. Study Site and Experimental Design

The study was conducted in the Jałowiec Massif in the Żywiec Beskids of southern Poland (49°39′64″ N; 19°28′67″ E). The study was conducted in Magurska Nappe on sandstone and shale; Cambisols [23] dominated the chosen study plots, which were established at altitudes of 600, 800, and 1000 m above sea level (a.s.l.). Selected heights constitute the boundaries of the climatic and plant zones in the Western Carpathians. The study plots were located at the same heights. The study plots were located along a slope with a 15° incline and northern exposure. The tested soils were sandy loams (average sand content was 54%, silt 42% and clay 3%). The average growing season temperature for the study plots at 600 m a.s.l. was 12.4 °C, for the study plots at 800 m a.s.l. was 11.3 °C, and for those at an altitude of 1000 m a.s.l., it was 10.2 °C. The average soil moistures at 600, 800,

and 1000 m a.s.l were 22.74%, 29.60%, and 34.10%, respectively. The research plots were located in a beech stand (*Fagus sylvatica* L.) and a fir stand (*Abies alba* Mill.). The research used stands of similar age with the same tree canopy density. The age of the stands was 60 years. Study plots were located in the managed forest coming from natural regeneration. The history of all the stands is similar. At each altitude, three research plots (10 acres) were designated in beech and fir stands. In total, 18 study plots were included in the research. Three soil samples from different locations were collected from each plot. Soil samples were collected in the same way on each study plot. The samples were taken after removing the litter to a depth of 15 cm. Organic material not related to the soil profile was treated as litter. Composite soil samples consist of three subsamples from different points. In total, 54 soil samples were taken for analysis.

2.2. Laboratory Analysis

The potentiometric method was used to determine soil pH in H_2O and 1M KCl. The Kappen method was used to determine hydrolytic acidity (Y). The content of nitrogen and carbon was analyzed by the LECO CNS True Mac Analyser (Leco, St. Joseph, MI, USA). The alkaline cations (Ca^{2+}, Mg^{2+}, K^{+}, Na^{+}) in 1M ammonium acetate were determined by the ICP-OES (iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK). The physical fractionation of SOM was performed according to the method described by Sohi et al. [24]. A 15-g sample of soil was placed in a 200-mL centrifuge tube and 90 mL of NaI (1.7 g cm^{-3}) was added. Each tube was gently shaken for 1 min and centrifuged for 30 min. The free light fraction (fLF) was removed using a pipette and collected on a glass fiber filter. The soil remaining at the bottom of the centrifuge tubes was mixed with another portion of 90 mL of NaI and subjected to sonication (60 watts for 200 s) to destroy aggregates. After centrifugation, the matter released from the aggregate-occluded light fraction (oLF) was collected on the glass fiber filter. The remaining fraction was assumed to consist of the mineral associated fraction (MAF) of SOM [8,16]. The fractions were analyzed for carbon and nitrogen (CfLF, CoLF, CMAF, NfLF, NoLF, and NMAF, respectively) by the LECO CNS True Mac Analyzer (Leco, St. Joseph, MI, USA).

Three soil samples with known volumes (block with dimensions of 15 × 15 × 15 cm) were collected from each study plot to determine root biomass. The roots were divided into two groups: coarse roots with diameters >2 mm and fine roots with diameters <2 mm. The roots were scanned and analyzed (diameter, length, projected area) using the WinRhizoTM Pro 2003 system (Regent Instruments INC., Ville de Québec, QC, Canada). After drying (70 °C, 24 h), the roots were weighted. After analyzing the roots, they were used to determine the root decomposition rate in the later stage of the experiment. Bags (15 × 20 cm) containing 10 g of root matter were prepared to determine roots decomposition. Bags with roots were buried to a depth of 10 cm in each study area from May to October. After this period, the loss of roots in the bags was determined. The annual fine root (diameter < 2.0 mm) biomass increase was determined with the core method [25]. The experiment sought to determine the root production expressed in grams between May and October 2021.

2.3. Statistical Analysis

The Shapiro-Wilk test was used to check the normal distribution. The differences between soil properties were determined using ANOVA. The Pearson correlation coefficient was used to determine the relationship between the tested properties of soils in the altitude gradient. The Pearson correlation was used to determine the strength of the relationship between the studied variables. A general linear model (GLM) allowed us to assess the importance of species and the height of a.s.l.in shaping the fractional composition of soil organic matter. Principal component analysis (PCA) was used to interpret factors in certain datasets. Statistica 12 software (StatSoft 2012) was used for the analysis.

3. Results

This study confirmed the differentiation of the properties of the examined soils due to the influence of beech and fir stands in different climate conditions in the altitude gradient. With increased altitude a.s.l., the studied soils exhibit increased acidity, regardless of tree species (Table 1). With increased altitude, the pH of the studied soils decreases and the hydrolytic acidity increases. Soils at 600 m a.s.l. had a significantly higher pH than soils at the other altitudes (4.84 in the soil of beech stand and 4.91 in the soil of fir stand). At an altitude of 1000 m a.s.l., the pH in the soils of the beech stands was 4.04, and in the soils of the fir stands, it was 3.65. Significantly, the lowest hydrolytic acidity was recorded in soils at 600 m a.s.l. 2.42 cmol(+)·kg^{-1} in the beech stand soils and 2.61 cmol(+)·kg^{-1} in the fir stand soils. The C and N concentration also increased with increasing altitude a.s.l. Significantly higher C and N concentration was measured in soils at 1000 m a.s.l. In the soils of beech stands, the C concentration ranged from 5.23% to 8.80% and in the soil of fir stands, it ranged from 4.51% to 14.81%. In the soils of beech stands, the N concentration ranged from 0.37% to 0.49% and in the soil of fir stands, it ranged from 0.32% to 0.75%. The content of basic cations decreased with increasing altitude. Significantly higher cation content was recorded in the lowest-lying soils. At an altitude of 600 m a.s.l. the Ca content in the soils of the beech stands was 142.46 cmol(+)·kg^{-1}, while at an altitude of 1000 m a.s.l. the Ca content was 9.99 cmol(+)·kg^{-1} (Table 1).

Table 1. Chemical properties of study soils under influence of different species in altitude gradient.

Species	Altitude [m]	pH H_2O	pH KCl	Y	C	N	C/N	Ca	Mg	K	Na
Beech	600	4.84 ± 1.04 a	4.18 ± 0.40 a	2.4 ± 0.5 a	5.23 ± 0.91 b	0.37 ± 0.05 b	14.1 ± 1.6 a	142.46 ± 70.4 a	17.47 ± 8.4 a	11.35 ± 2.5 a	1.13 ± 0.7 a
	800	4.09 ± 0.19 b	3.45 ± 0.12 b	4.9 ± 1.0 b	6.72 ± 1.65 ab	0.43 ± 0.09 ab	15.7 ± 0.8 ab	19.49 ± 7.6 b	4.17 ± 0.9 b	6.56 ± 1.6 b	0.65 ± 0.1 b
	1000	4.04 ± 0.25 b	3.4 ± 0.19 b	5.3 ± 0.9 b	8.80 ± 2.02 a	0.49 ± 0.09 a	17.9 ± 1.8 b	9.99 ± 4.1 b	3.64 ± 0.6 a	7.44 ± 2.5 b	0.70 ± 0.1 b
Fir	600	4.91 ± 0.20 a	3.91 ± 0.18 a	2.6 ± 0.4 a	4.51 ± 0.62 b	0.32 ± 0.04 b	14.0 ± 0.6 a	93.76 ± 28.6 a	12.89 ± 4.2 a	11.63 ± 3.3 b	0.83 ± 0.1 b
	800	3.94 ± 0.15 b	3.27 ± 0.14 b	5.8 ± 1.0 b	10.44 ± 2.92 b	0.55 ± 0.10 a	18.7 ± 1.8 b	14.57 ± 5.7 b	4.18 ± 1.1 b	7.15 ± 3.7 a	0.93 ± 0.2 a
	1000	3.65 ± 0.08 b	3.02 ± 0.11 b	8.6 ± 1.7 b	14.81 ± 5.60 a	0.75 ± 0.23 a	19.6 ± 1.3 b	17.71 ± 16.9 b	5.60 ± 2.1 ab	8.43 ± 4.3 b	1.24 ± 0.3 ab

Mean ± SD; C, N (%); Ca, K, Mg, Na (cmol(+)·kg^{-1}); Y—hydrolytic acidity (cmol(+)·kg^{-1}); small letters in the upper index of the mean values mean significant differences of soil properties in altitude gradient (a, b).

No statistically significant differences in root length were determined along the altitude gradient for beech and fir trees (Table 2). Fir root diameter increased with altitude a.s.l., but these differences were not statistically significant (Table 2). No statistically significant differences in root biomass were determined in beech along the altitude gradient. The significantly highest fir root biomass was recorded in the highest locations. No significant differences originating from tree species and altitude a.s.l. were recorded for the decomposition rate (Table 2). Statistically significant differences in root growth were recorded at 600 and 1000 m a.s.l. Beech root growth did not change significantly along the altitude gradient. Significantly greater fir growth was recorded at 800 m a.s.l.

Table 2. Characteristics of the fine roots of different tree species in the altitude gradient.

Species	Altitude [m]	Diameter [mm]	Length [cm]	Biomass [g·dm^{-3}]	Decomposition [%]	Production [g]
Beech	600	0.59 ± 0.13 a	6452.57 ± 5339.64 a	2.14 ± 0.54 a	24.37 ± 13.56 a	0.11 ± 0.08 a
	800	0.59 ± 0.05 a	4153.94 ± 1428.64 a	2.17 ± 0.38 a	29.24 ± 22.05 a	0.14 ± 0.07 a
	1000	0.57 ± 0.05 a	3957.79 ± 1702.15 a	2.34 ± 0.59 a	23.67 ± 7.99 a	0.10 ± 0.05 a
Fir	600	0.77 ± 0.21 a	2269.63 ± 1760.69 a	1.76 ± 0.33 b	19.48 ± 6.89 a	0.04 ± 0.04 b
	800	0.84 ± 0.14 a	3378.09 ± 2218.55 a	1.93 ± 0.44 b	31.35 ± 13.69 a	0.11 ± 0.05 a
	1000	0.93 ± 0.13 a	2475.61 ± 708.62 a	2.56 ± 0.49 a	19.60 ± 12.00 a	0.04 ± 0.02 b

Mean ± SD; small letters in the upper index of the mean values mean significant differences in altitude gradient (a, b).

Differences in the fractional composition of SOM resulting from the impact of tree species and location in the altitude gradient were noted (Figures 1 and 2). Regardless of tree species, the CfLF content increased with the altitude a.s.l. Significantly lower CfLF content was found in the lowest-lying soils (Figure 1). There were no significant differences in CoLF content along the altitude gradient, although CoLF trended downward. In soils

with beech, a significantly lower CMAF content was recorded in the highest soils. In soils with fir, there is a downward trend of CMAF content along the height gradient. The lowest CMAF content was recorded in soils at 1000 m a.s.l. In soils with beech, a higher CMAF content was noted compared with soils with firs (Figure 1). The NfLF content increased along the altitude gradient regardless of tree species (Figure 2). Significantly lower NfLF content was measured in the lowest soils (600 m a.s.l.). In soils with beeches, significantly lower NoLF content was found in the highest soils (1000 m a.s.l.). No significant differences in NoLF content were found in soils with firs along the altitude gradient. A decrease in CMAF content was measured along the altitude gradient. Significantly lower NMAF content was recorded in soils with firs in the highest locations (Figure 2). The CfLF and NfLF content were negatively correlated with soil pH and the basic cation content (Table 3). The CfLF and NfLF content were significantly positively correlated with hydrolytic acidity ($r = 0.821$ and $r = 0.834$, respectively) and C ($r = 0.821$ and $r = 0.834$, respectively) and N content ($r = 0.913$ and $r = 0.893$, respectively). The NoLF content was negatively correlated with hydrolytic acidity and C content and positively correlated with the pH and Ca and Mg content. The CMAF and NMAF content were correlated with soil acidification and C and N content. The fractional composition of SOM was correlated with the properties of the studied trees' root systems, such as diameter, biomass and decomposition rate. The CfLF and NfLF content were positively correlated with the root diameter ($r = 0.489$ and $r = 0.510$, respectively), biomass ($r = 0.337$ and $r = 0.320$, respectively), and decomposition rate ($r = 0.381$ and $r = 0.393$, respectively) (Table 4). The CoLF and NoLF content were significantly positively correlated with root length. The CMAF and NMAF content were negatively correlated with root diameter ($r = -0.421$ and $r = -0.326$, respectively), and CMAF content was positively correlated with root production ($r = 0.310$) (Table 4).

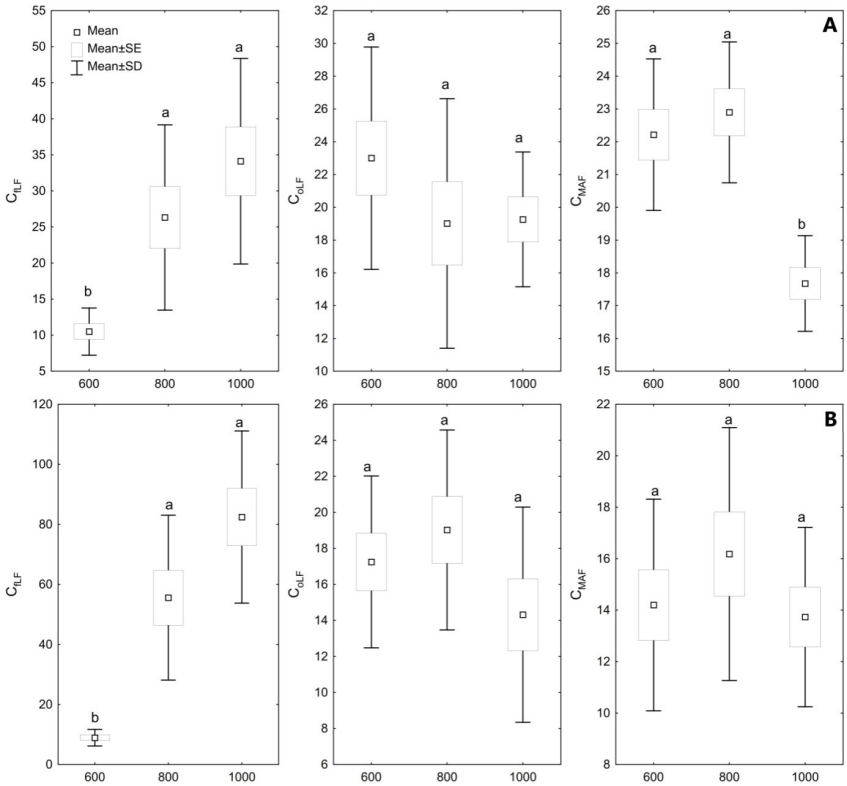

Figure 1. Carbon of different fraction of soil organic matter in soils under influence of different forest stands in altitude gradient (CfLF—carbon of free light fraction (g kg^{-1}), CoLF—carbon of occluded light fraction (g kg^{-1}), CMAF—carbon of mineral associated fraction (g kg^{-1}); (**A**)—soils under influence of beech, (**B**)—soils under influence of fir; small letters mean significant differences in altitude gradient (a, b)).

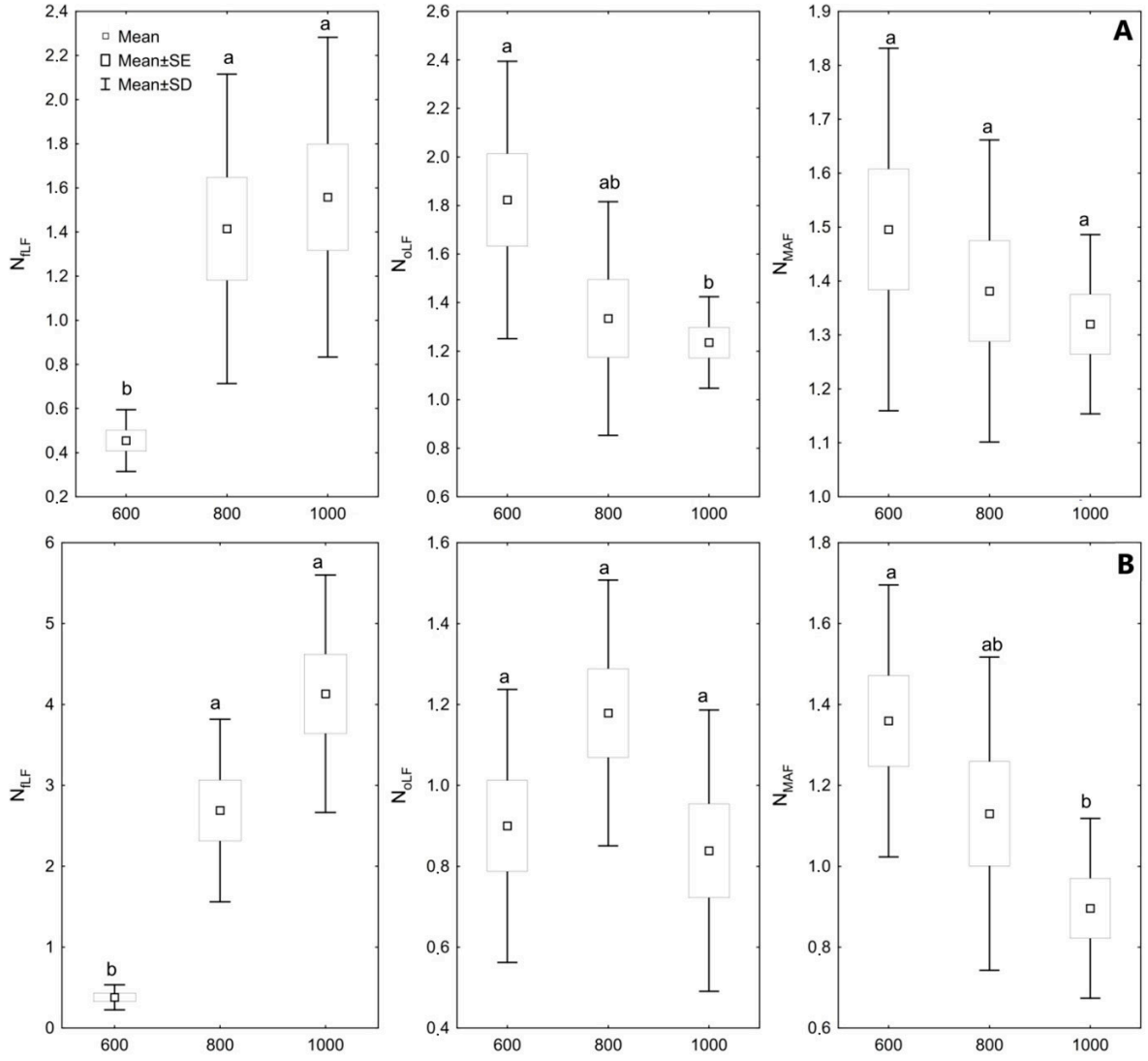

Figure 2. Nitrogen of different fraction of soil organic matter in soils under influence of different forest stands in altitude gradient (NfLF—nitrogen of free light fraction (g kg^{-1}), NoLF—nitrogen of occluded light fraction (g kg^{-1}), NMAF—nitrogen of mineral associated fraction (g kg^{-1}); (**A**)—soils under influence of beech, (**B**)—soils under influence of fir; small letters mean significant differences in altitude gradient (a, b)).

Table 3. Correlation between soil organic matter fractions and soil properties.

	pH H_2O	pH KCl	Y	N	C	Ca	K	Mg	Na
CfLF	−0.597 *	−0.746 *	0.821 *	0.913 *	0.927 *	−0.456 *	−0.012	−0.368 *	0.342 *
NfLF	−0.607 *	−0.756 *	0.834 *	0.893 *	0.895 *	−0.481 *	−0.066	−0.394 *	0.312 *
CoLF	−0.065	0.238	−0.251	−0.241	−0.245	0.231	−0.057	0.231	−0.004
NoLF	0.061	0.401 *	−0.334 *	−0.239	−0.289	0.349 *	−0.011	0.309 *	0.042
CMAF	0.376 *	0.325 *	−0.309 *	−0.381 *	−0.417 *	0.142	−0.116	0.089	−0.253
NMAF	0.444 *	0.432 *	−0.491 *	−0.559 *	−0.581 *	0.196	0.043	0.141	−0.458 *

Significance effect * $\leq$ 0.05; CfLF—carbon of free light fraction, NfLF—nitrogen of free light fraction, CoLF—carbon of occluded light fraction, NoLF—nitrogen of occluded light fraction, CMAF—carbon of mineral associated fraction, NMAF—nitrogen of mineral associated fraction.

Table 4. Correlation between soil organic matter fractions and root characteristics.

	Diameter	Length	Biomass	Decomposition	Production
CfLF	0.489 *	−0.083	0.337 *	0.381 *	−0.124
NfLF	0.510 *	−0.106	0.320 *	0.393 *	−0.123
CoLF	−0.240	0.369 *	0.265	−0.071	−0.066
NoLF	−0.434 *	0.515 *	0.234	−0.079	0.218
CMAF	−0.421 *	0.136	−0.202	0.030	0.310 *
NMAF	−0.326 *	0.161	−0.471 *	−0.165	0.121

Significance effect * $\leq$ 0.05; CfLF—carbon of free light fraction, NfLF—nitrogen of free light fraction, CoLF—carbon of occluded light fraction, NoLF—nitrogen of occluded light fraction, CMAF—carbon of mineral associated fraction, NMAF—nitrogen of mineral associated fraction.

GLM analysis confirmed that the location a.s.l. and tree species affect the content of light and heavy fractions of SOM (Table 5). Tree species was a significant factor for all SOM factions' N and C content. Altitude a.s.l. affected the CfLF, NfLF, CMAF, and NMAF content. The simultaneous importance of tree species and altitude was noted for CfLF, NfLF, and NoLF content (Table 5). The conducted PCA analysis confirmed relationships between the SOM fractions and tree species and location in the altitude gradient. In addition, the PCA confirmed the distinction between the organic matter fractions of soils under beech and fir tree stands (Figure 3). Factors 1 and 2 explained 52.0% of the variance of the tested characteristics. Factor 1 explained 34.7% of the variance, while factor 2 explained 17.4% of the variance. Factor 1 was related to the SOM fractions and altitude gradient. Factor 2 was related to tree species and root characteristics. PCA analysis confirmed the distinction between the fractional composition of soils located at altitudes of 600 and 1000 m a.s.l. In addition, the conducted analyses show the correlation between root properties and SOM fractions content. The PCA analysis confirmed the importance of the location conditions and tree species in shaping root characteristics.

Table 5. Summary of GLM analysis for soil organic matter fractions.

	CfLF		NfLF		CoLF		NoLF		CMAF		NMAF	
	F	p	F	p	F	p	F	p	F	p	F	p
Species	26.5109	0.0000	28.6168	0.0000	4.8850	0.0318	20.9384	0.0000	48.067	0.0000	11.1602	0.0016
Altitude	33.3297	0.0000	36.9183	0.0000	1.4836	0.2370	3.1637	0.0512	6.273	0.0037	5.1995	0.0090
Species * Altitude	8.7124	0.0005	10.5703	0.0000	1.2513	0.2952	4.4425	0.0169	1.790	0.1778	1.0656	0.3525

CfLF—carbon of free light fraction, NfLF—nitrogen of free light fraction, CoLF—carbon of occluded light fraction, NoLF—nitrogen of occluded light fraction, CMAF—carbon of mineral associated fraction, NMAF—nitrogen of mineral associated fraction.

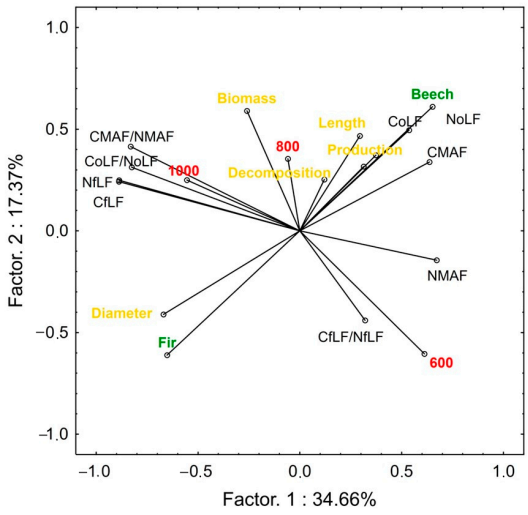

Figure 3. Projection of the variables on the factor plane (CfLF—carbon of free light fraction, NfLF—nitrogen of free light fraction, CoLF—carbon of occluded light fraction, NoLF—nitrogen of occluded light fraction, CMAF—carbon of mineral associated fraction, NMAF—nitrogen of mineral associated fraction).

4. Discussion

The decreasing the temperature and increasing the humidity along the altitude gradient with a simultaneous increase in carbon accumulation is reflected in the shares of light fractions of SOM. Previously studies have confirmed that climatic factors strongly influence carbon accumulation in forest soils [26–28] and grassland [29], but no one identified the change in the fractional composition of SOM depending on the location condition. In mountainous areas, there is a gradual gradient of thermal and pluvial factors with increasing elevation above sea level. The analyzed climate sequence included three positions (at altitudes of 600, 800, and 1000 m a.s.l.) and the extreme positions differed by 2.2 °C in average annual temperature. Differences in thermal conditions translate directly to the length of the growing season. Between the lowest (600 m) and the highest (1000 m) positions, the difference in the length of the vegetation period may reach up to 1 month. Changes in thermal conditions and humidity in the altitude gradient can lead to changes in the efficiency of ecosystems and changes in the rate of decomposition of organic matter.

The mechanism of climatic factors' influence on the accumulation of organic matter in soil and its fractional composition is complex. On the one hand, reducing the temperature at high elevations reduces the rate of biochemical changes and slows the decomposition of detritus reaching the soil [7,30]. Large amounts of C in the soil is effect of rhizosphere processes. Fast turnover of exudates and microbial biomass C in the rhizosphere may lead to local changes in the rate of microbial decomposition of various C pools: dead plant residues and/or soil organic matter (SOM) [12]. Lauchner et al. [31] proved in their research the importance of temperature in shaping the root exudation rate of beech trees. In our study, a higher C content of the stable fraction of SOM was noted in soils at a lower position (600 m a.s.l.). According to Sun et al. [32], the preferential preservation of higher stability C compounds in warmer climates might be explained by the increased decomposition of labile SOC and accumulation of recalcitrant SOC components at increasing temperatures. At the same time, the amount of rainfall increases with increasing altitude, favoring the acidification of the humus forming on the surface and displacing easily dissolved, mobile fractions of humic compounds. This phenomenon has been confirmed by the results obtained in our experiment. The soils of the highest positions observed a significant increase in acidification in surface horizons. This phenomenon can be explained by the higher leaching intensity of rainwater, which occurs in greater quantities in higher mountainous locations [33]. The slower decomposition of organic matter in higher positions causes greater accumulation of the light fraction of organic matter and a simultaneous decrease in the accumulation of the heavy fraction associated with soil mineral particles. This was also observed in other studies conducted in mountain areas [11,34]. De Feudis et al. [11] also found a significant increase in the content of the light fraction in the rhizosphere zone of soil in higher locations, which was explained by the influence of root systems in more restrictive locations with harsh climates.

The soils of beech stands are characterized by a lower share of light fractions and a higher share of heavy fractions of SOM than those of fir stands. The beech species produces a strongly developed and dense root system, creates mountain stands at much higher altitudes and should contribute to stronger intra-soil stabilization of organic matter than fir. The obtained results prove that the soil under the beech stands is characterized by a higher C content associated with the mineral fraction in the occlusions (CoLF) and mineral particles (CMAF). The content of these fractions is 25–50% higher than in the fir stands growing under similar conditions. Several reports have noted the particular importance of the organic matter roots supply to the soil for the composition and activity of microorganisms in the soil [19,20]. The supply of easily available C by roots stimulated the growth and maintenance of microorganisms [34]. Root-derived organic matter and detritus reaching the soil surface are important to soil microorganisms [35–37]. Research by Meier et al. [38] conducted in mature beech stands growing in various habitat conditions suggests that the beech root system secretes large amounts of exudates, especially under unfavorable soil conditions (acidic and N-deficient), and a significant portion of the assimilates produced

by trees feeds the external ecosystem C cycle. We found a lower amount of C associated with the mineral part of the soil under fir stands. In our study, the soils under firs at 800 and 1000 m a.s.l. are characterized by a significantly higher organic matter content on the surface horizon; this is mainly poorly decomposed organic matter (CfLF) that is not bound to mineral substances.

Our findings indicate a relationship between the fractional composition of SOM and root biomass. The root biomass and the increase in the percentage of roots positively affected the carbon reserve of the light fraction of SOM (CfLF), while the growth of fine roots positively affected the carbon reserve of the heavy SOM fraction (CMAF). The differences in the root system characteristics of both studied species are not insignificant here. Beech should be treated as a species with greater root biomass, total length, and growth of fine roots. Fir is characterized by thicker roots, as a rule, with lower root biomass and growth of fine roots [39]. Fine roots, and especially their necromass, significantly affect mineralization processes, shaping the kinetics of the root decomposition process in the soil profile [40]. In mountainous areas, the density and biomass of fine roots not only depend on the species composition of the vegetation but also correlate with environmental factors [41]. Our research confirmed the importance of the location above sea level in changing the characteristics of the root. Higher root densities in colder environments might serve two purposes, to increase the absorbing root surface area under conditions of reduced nutrient supply and to enhance the stimulation of microbial activity under low temperatures [31]. Sierra-Cornejo et al. [42] suggest that biomass and other features of fine roots in mountain ecosystems may be affected by water and nitrogen availability. Previous studies [43] showed that with increasing altitude, the C: N ratio increased significantly, which suggests that N may limit the decomposition of litter and soil organic matter at high altitudes.

5. Conclusions

The decreasing the temperature and increasing the humidity along the altitude gradient with a simultaneous increase in carbon accumulation is reflected in the shares of light fractions of SOM. Soils at higher locations are dominated by the light fraction of SOM, which results from slower decomposition processes. Regardless of their location along the altitude gradient, the soils of beech stands are characterized by a lower share of the light fraction and a higher share of the heavy fraction of SOM relative to the soils of fir stands. Coniferous species such as firs, by supplying above ground and underground biomass can reduce the soil pH, which may slow down the decomposition processes. We confirmed the importance of roots in shaping the fractional composition of SOM. Our research shows that avoiding single-species coniferous stands and introducing admixtures of deciduous species, which increase the heavy SOM fraction, is justified in forest management. The light fraction of SOM tends to degrade faster and is, therefore, less stable than the heavy fraction. The species composition of stands should be selected to foster an increase in the stable, heavy fraction of SOM.

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The impact of root systems and their exudates in different tree species on soil properties and microorganisms in a temperate forest ecosystem

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Abstract

Background The species composition of tree stands plays an important role in shaping the properties of forest soils. The aim of our research was to determine the influence on soil properties of the root systems of six species of trees which form forest stands in the temperate climatic zone. The research covered areas including six tree species – Scots pine (*Pinus sylvestris L.*), European larch (*Larix deciduas Mill.*), English oak (*Quercus robur L.),* English ash (*Fraxinus excelsior L.),* European beech (*Fagus sylvatica L.*) and European hornbeam (*Carpinus betulus L.).* In our study, we determined the characteristics of the roots and the amount of carbon excreted alongside their exudates. Enzymatic activity, and the composition and diversity of the fungi and bacteria, were also determined in addition to the basic physico-chemical properties of the soil samples.

Results A strong relationship between the root characteristics and soil properties, including the pH, basic cation content and phosphorus content, was confirmed. In addition, the enzymatic activity of phosphatase, β-glucosidase, N-acetyl-β-D-glucosaminidase and β-D-cellobiosidase were positively correlated with the root characteristics. The study on soil bacteria across different tree species revealed *Proteobacteria* and *Actinobacteriota* to be the most abundant phylum. Fungal analysis showed *Basidiomycota* and *Ascomycota* as the dominant phyla. *Ascomycota* dominated in hornbeam and oak soils. *Mortierellomycota* was remarkably more present in pine soil.

Conclusions This analysis of root systems and soil properties confirmed the distinctness of ash stands, which were also more abundant in various microorganisms. It was also found that soils affected by different tree species were characterised by varied fungal and bacterial composition. The ash had particularly beneficial impact on soil microbiota.

Keywords Enzyme activity, Fungal and bacterial diversity, Next-generation sequencing (NGS), Root characteristics

Background

Forests provide numerous benefits for the environment, including climate regulation, water supply, habitats for biodiversity, and erosion control [1–3]. Protecting and restoring forest ecosystems is the key to mitigating climate change and slowing global biodiversity loss [4]. Global change is exposing forest ecosystems to an increased frequency of climate extremes and both the emergence and spread of pests and pathogens [5]. These

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phenomena affect the stability of forest tree stands. Forest ecosystems can be optimally restored and maintained using climate-based tree species distribution models to predict which tree species will tolerate the climate change [6]. Thus, it is important to understand the influence of different species on soil properties, which, in the future, will facilitate the management of forest ecosystems [7, 8].

Woody vegetation plays an important role in the forest ecosystems and it may affect long-term productivity and sustainability by influencing biochemical processes in the soil environment, such as the microbial activity and diversity, carbon sequestration and nutrient turnover rates [9–11]. Soil properties, such as soil organic carbon, nitrogen and nutrient contents and microbes, not only affect forest growth, but they also modulate ecological soil functionality and biochemical cycles [12, 13]. Changes in aboveground communities can impact the biodiversity of soil microorganisms which, in turn, can be important for regulating the balance between the decomposition and stabilisation of soil organic matter [14]. Soil microorganisms are an important component of biodiversity as they are involved in several ecological processes, with the species composition of the stand being the main factor affecting biodiversity [15]. According to Qiao et al. [16], biodiversity contributes to the stabilisation of forest ecosystems from local to larger spatial scales. Tree species impact soil microbial communities through their litter, roots, mycorrhizal fungi and exudates [17]. Bacteria and fungi living in the soil react differently to the changes in soil characteristics caused by the alterations in the species composition of the stand. According to Guo et al. [18], bacteria are more sensitive to the soil carbon:nitrogen ratio than fungi. The species or compositions of plants in the forest can also play a significant role in promoting the abundance of microbes by shaping their access to nutrients and modifying local environmental conditions [19].

Root systems are vital in shaping soil properties, including microbiological activity [20]. The roots shape the physical, chemical and biological properties of soils through the biomass supplied by dead roots, and their exudates [8, 21]. Root exudates include non-volatile rhizodeposits and soluble organic compounds, such as sugars, amino acids and organic acids [22]. Low-molecular-weight (LMW) root exudates and mucilages can both be used by microbes as a carbon source [23]. Root exudates are considered to be a key determinant of rhizosphere microbial community structure [24, 25].

Due to the unpredictable future threats and the need to improve the stability of forest ecosystems, an alteration in the species composition of stands should be considered. However, the proper reconstruction of forests requires knowledge about the role of individual tree species in shaping the biodiversity of forest ecosystems. So far, several studies have been devoted to the impact of trees on the forest ecosystem and soil environment in particular through the addition of aboveground biomass. There is, however, insufficient knowledge on the impact of the characteristics of root systems on the properties of forest soils and their biodiversity. Our research was focused on bridging this gap.

We examined in detail the root systems of six tree species found in forest stands in the temperate climatic zone. The main objective of our research was to determine the role of root systems in shaping the composition and diversity of soil microorganisms in connection with the basic soil properties. We tested the following research hypotheses: 1) physicochemical properties and enzymatic activity are strongly correlated with the morphological features of the roots of the examined trees, 2) root-exuded carbon positively affects the formation of the physicochemical properties of soils and, consequently, enzymatic activity, 3) ash root systems, together with their exudates, have the most beneficial influence on the properties of the tested soils, 4) the examined tree species, through their root systems and their exudates, have different effects on the soil microorganisms, and 5) coniferous species (pine and larch) have a similar effect on the amount and diversity of fungi and bacteria in the soil.

Materials and methods

Study sites

The study was carried out on experimental plots owned by the Department of Ecology and Silviculture at the University of Agriculture in Kraków, Poland. The spaces were located 25 km north of Kraków, in southern Poland (50°11.46.35N, 20°3.54.28E). The study covered the area containing six species of trees–Scots pine (*Pinus sylvestris* L.), European larch (*Larix deciduas* Mill.), English oak (*Quercus robur* L.), English ash (*Fraxinus excelsior* L.), European beech (*Fagus sylvatica* L.) and European hornbeam (*Carpinus betulus* L.). The stands contained single species without admixtures of other species, and were of similar age (70–80 years) and density. Each of the six plot variants (0.1 ha study plots) was tested in five repetitions. In total, 30 study plots were designated for the investigation (six tree species × five repetitions = 30 study plots). On each study plot, five points were designated for the detailed analysis of the root systems and soil properties. Soil and root samples were collected for the analysis from all points. The whole area was characterised by the presence of Luvisols developed from homogeneous loess. The soil samples were collected after the organic horizon was removed from the A horizons, which were 15-cm-thick humus–mineral horizons. Soil samples for

laboratory analyses were collected 100 cm from the trunk of trees of the studied species, within the range of their root systems. The collection and analysis was carried out in 2022.

Chemical analysis

In the collected soil samples, pH in H_2O and KCl was determined using the potentiometric method. The carbon (C) and nitrogen (N) contents were measured with an elemental analyser (LECO CNS TrueMac Analyzer, Leco, St. Joseph, MI, USA). The P content was measured using a ICP-OES ThermoiCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, U.K.) after mineralisation of the mixture with concentrated nitric and perchloric acids at ratio of 3:1. The cation concentrations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) were extracted with ammonium acetate and determined through the inductively-coupled plasma analysis (ICP-OES Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK).

Analysis of enzymatic activity

Enzymatic activity was determined in the soil samples with natural moisture. These samples were sieved through a 2-mm mesh and stored at 4°C. The activity of six extracellular enzymes––β-glucosidase (BG), β-D-cellobiosidase (CB), β-xylosidase (XYL), N-acetyl-β-D-glucosaminidase (NAG), phosphatase (PH) and arylsulphatase (SP)––was determined using the fluorescence method [26–28].

Collection of root exudates and analysis of root morphology

The exudates were collected using a culture-based cuvette system [29]. The exudates were collected twice, in June and September 2022. Root exudates were collected from one branched fine root segments of similar length and branching. Each root system was carefully removed, using deionised water and fine forceps, in order to maintain the integrity of the root system. The root systems were placed in sterile glass syringes containing sterile glass beads and moistened with a carbon-free nutrient solution (0.5 mM ammonium nitrate/NH_4NO_3, 0.1 mM potassium dihydrogen phosphate/KH_2PO_4, 0.2 mM potassium sulphate/K_2SO_4, 0.15 mM magnesium sulphate/$MgSO_4$ and 0.3 mM calcium chloride/$CaCl_2$). After 24 h of stabilisation in the syringe, the roots were flushed three times with fresh carbon-free solution to remove the organic carbon exuded during the stabilisation period. The exudate-containing samples were then collected in 50-mL glass vials that were sealed with silicon caps and stored at 4°C until we were ready to determine the total organic carbon (TOC).

Trap solutions containing the exudates were collected from each cuvette and filtered through sterile syringe filters. The trap solutions were then analysed using a Shimadzu TOC analyser (Shimadzu, Japan). On each study plot, soil samples with a known volume of 15 × 15 × 15 cm were collected in three replications in order to determine the root biomass. The coarse roots (diameter > 2 mm) were separated from the fine roots (diameter < 2 mm) in these samples. The extracted root system fragments were scanned at 400 dpi resolution and then analysed using a WinRhizo Pro 2003b image analysis system (Regent Instruments Inc., Ville de Québec, QC, Canada) in order to determine their diameter, length and root area. The air-dried roots were further desiccated at 70°C for 24 h to a constant weight and then weighed. The root tissue density (RTD) (kg m^{-3}), specific root area (SRA) (m^2 kg^{-1}) and specific root length (SRL) (m g^{-1}) were calculated according to Ostonen et al. [30]. The annual fine root biomass increase was determined using the core method [31]. We sought to determine the root production between April and October 2022.

Preparation of the soil fungal and bacterial DNA library

The DNA was isolated from the soil picked from the organic (O) horizon ($n = 3$) and from one additional sample from the humus mineral soil (A) horizon. The DNA was isolated from 1 g of soil in accordance with the protocol of the Genomic Mini AX Bacteria + (A&A Biotechnology, Poland). Mechanical lysis was carried out using zirconia balls in FastPrep-24 homogeniser. Lyticase (A&A Biotechnology, Poland) was also used in the enzymatic lysis. Fungal DNA libraries were prepared for the ITS1 rDNA region amplified using ITS1F [32] and ITS2 primers according to the Illumina 16S Metagenomic Library preparation protocol. Bacterial DNA libraries were prepared for the V3–V4 16S rDNA region amplified using 341F and 785R primers [33]. A polymerase chain reaction (PCR) was carried out in a reaction mixture containing 15 ng of genomic DNA using a Q5 Hot Start High-Fidelity 2X Master Mix (New England Biolabs, USA). An indexing PCR was prepared using the Nextera XT index kit (Illumina). After indexing, the samples were purified using AMPure XP beads, verified in a bioanalyser (Agilent Technologies, US) and with qPCR. The DNA libraries were sequenced on an Illumina MiSeq platform (2 × 300 bp paired end) by Genomed (Poland). The sequencing depth was 50 000 reads per sample.

Next-generation sequencing (NGS) data for the fungi were processed using QIIME software [34]. The samples were demultiplexed, and fastq files were generated using MiSeq Reporter v.2.6 software (Illumina). Adapter and low-quality (below Q20) sequences were removed using the cutadapt tool [35]. Paired sequences were joined

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Table 1 Basic properties of soils under the influence of different tree species

	pH H_2O	pH KCl	Ca (cmol kg^{-1})	K (cmol kg^{-1})	Mg (cmol kg^{-1})	Na (cmol kg^{-1})	P (mg kg^{-1})	N (%)	C (%)	C/N
Ash	4.86 ± 0.51^{a}	4.23 ± 0.55^{a}	5.65 ± 3.30^{a}	0.15 ± 0.09^{a}	0.99 ± 0.67^{a}	0.05 ± 0.02^{a}	388.96 ± 75.71^{a}	0.28 ± 0.06^{a}	3.63 ± 0.87^{b}	12.7 ± 0.4^{b}
Beech	4.25 ± 0.12^{ab}	3.67 ± 0.02^{ab}	1.11 ± 0.43^{b}	0.11 ± 0.43^{a}	0.22 ± 0.06^{a}	0.02 ± 0.01^{b}	276.17 ± 16.93^{a}	0.31 ± 0.09^{a}	5.64 ± 1.48^{a}	18.3 ± 0.9^{a}
Hornbeam	4.14 ± 0.13^{abc}	3.55 ± 0.05^{abc}	0.88 ± 0.33^{b}	0.89 ± 0.33^{a}	0.23 ± 0.05^{a}	0.01 ± 0.01^{b}	238.46 ± 44.67^{a}	0.19 ± 0.03^{a}	2.54 ± 0.64^{b}	13.7 ± 1.4^{b}
Larch	3.71 ± 0.03^{bc}	3.14 ± 0.08^{bc}	1.21 ± 0.58^{b}	1.21 ± 0.58^{a}	0.27 ± 0.12^{a}	0.04 ± 0.01^{a}	367.23 ± 182.71^{a}	0.27 ± 0.13^{a}	5.19 ± 2.70^{a}	18.6 ± 0.8^{a}
Oak	3.86 ± 0.13^{bc}	3.40 ± 0.09^{bc}	1.03 ± 0.44^{b}	1.03 ± 0.44^{a}	0.33 ± 0.06^{a}	0.02 ± 0.01^{b}	264.01 ± 65.47^{a}	0.26 ± 0.04^{a}	3.26 ± 0.87^{b}	12.4 ± 1.6^{b}
Pine	3.93 ± 0.16^{abc}	3.33 ± 0.16^{abc}	2.10 ± 0.98^{b}	2.10 ± 0.98^{a}	0.44 ± 0.13^{a}	0.02 ± 0.01^{b}	275.51 ± 59.73^{a}	0.30 ± 0.07^{a}	4.75 ± 1.56^{a}	15.4 ± 1.7^{a}

Mean ± SD; small letters in the upper index (a, b, c) mean significant differences between different species

using the seqprep algorithm. The usearch61 tool was used for chimera removal [36]. The fungal reads were clustered using the uclust algorithm [36] and checked against ITS sequences using the UNITE v.8.2 database (Unite Community 2018) and the BLAST algorithm [37]. Operational taxonomic units (OTUs) were filtered for very low abundance, and only OTUs with a relative abundance of at least 0.01% were used in further analysis. Data on the occurrence of fungal species were used to calculate the relative abundance of fungal types and to generate an abundance heatmap (including Euclidean cluster analysis) using log10 + 1 transformed abundance data for fungal species that totalled more than 500 readings for at least five plots with a given tree species in the Illumina metabarcode. R packages (https://cran.r-project.org) were applied to analyse the microbial diversity and visualise the results. Heatmaps were drawn using the R superheat package.

The NGS bacteria data were processed using QIIME [34]. The samples were demultiplexed, and fastq files were generated using MiSeq Reporter v.2.6. Adapter and low-quality sequences were removed using cutadapt [35]. The paired sequences were joined and clustered using the DADA2 algorithm [38], which removed the chimera. Clustered reads were checked against 16S sequences from the Silva 138 database [39]. Bacterial amplicon sequence variants were processed and analysed as described above for the fungal OTUs. The libraries with the analysed sequences of fungi have been deposited with the Gene Bank https://www.ncbi.nlm.nih.gov/ in project number PRJNA951397 and for bacteria on the number PRJNA956107.

Statistical analysis

Statistical analyses were performed using the statistical software R (R Core Team 2020) and R Studio (RStudio Team 2020). Spearman correlation coefficients for the soil and root characteristics were calculated. Principal component analysis (PCA) was used to evaluate the relationships between the soil properties and root characteristics. The Shapiro–Wilk test was used to assess normality, and Levene's test was used to check the homogeneity of the variances. The Kruskal–Wallis test was used to assess the differences between the average values of the soil and root properties.

Results

Soil and root characteristics

The different species investigated in this study had different effects on the physicochemical properties of the studied soils (Table 1). Significantly higher pH values (average pH in $H_2O = 4.86$) were found in the soils affected by ash, the lowest in the soils of the larch stand (average pH in $H_2O = 3.71$) (Table 1). A significantly higher content of alkaline cations, especially calcium, was recorded in the soils of ash stand. No significant differences in phosphorus and nitrogen content were noted in the soils under the influence of the different species. Significantly higher carbon content (average carbon content = 5.64, 5.19 and 4.75%, respectively) was recorded in the beech, larch and pine stands. The soils of the ash stand were characterised by the lowest (average = 3.63%) carbon content (Table 1). Significantly higher carbon:nitrogen ratios were recorded in the soils with stands of larch (18.6), beech (18.3) and pine (15.4) (Table 1). The soils affected by the different tree species were characterised by having different enzymatic activity (Fig. 1). Significantly higher activity of CB, BG, NAG and PH was recorded in the soils of the ash stands. In terms of XYL and SP activity, there were no significant differences between the studied tree species (Fig. 1).

Significantly higher amounts of carbon released together with the exudates were in the ash stands (Fig. 2). The root systems of the ash stands differed significantly from the other species in terms of length, diameter and surface area. There were no significant differences between the species in terms of SRL and SRA. Significantly lower RTD was recorded in the ash stands. In addition, a significantly higher root increment was noted in the ash stands (Fig. 2). The ash-root biomass was significantly higher than in the other studied species (beech, oak and pine) (Fig. 3).

Statistical analysis confirmed the relationship between the characteristics of the roots and the properties of the tested soils (Fig. 4). Root area and length were strongly positively correlated with the basic cation content. The carbon released with the exudate was positively correlated with pH and the cation content, especially calcium. The SRA and SRL were positively correlated with the phosphorus and, to a lesser extent, the carbon content. A negative correlation was observed between RTD and the content of sodium and phosphorus. Additionally, a strong relationship was observed between the root characteristics and the enzymatic activity in the soils (Fig. 4). The CB, BG, NAG and PH activity strongly positively correlated with surface area and root length, and, to a lesser extent, with diameter. In addition, root growth was strongly positively correlated with the root exudate. A weak positive relationship was noted between the carbon of the exudates and the CB and BG activity. The RTD negatively correlated with BG activity and, to a lesser extent, with NAG activity (Fig. 4). The PCA performed analysis confirmed the relationship between the root characteristics and soil properties (Fig. 5). The PCA analysis explained 45.5% of the variability of the studied features. Factor 1 was especially related to the features of

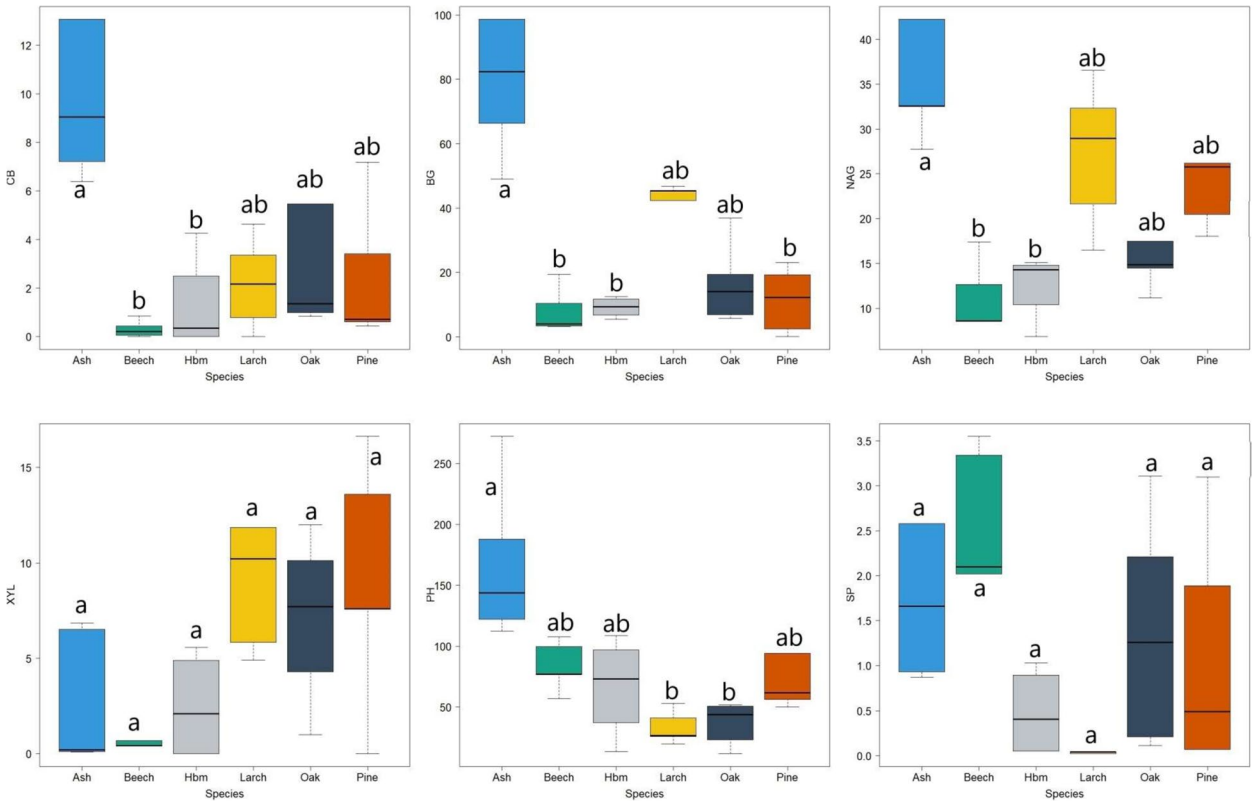

Fig. 1 Enzymatic activity (nmol MUB g^{-1} ·C ·$h.^{-1}$) of soils under the influence of various tree species (Hbm – hornbeam; PH—phosphatase, BG—β-glucosidase, NAG—N-acetyl-β-D-glucosaminidase, XYL—β-xylosidase, CB—β-D-cellobiosidase, SP – arylsulphatase; letters (**a**, **b**) mean significant differences between tree species)

the roots, whereas Factor 2 was related to those features that expressed the quality of soil organic matter. In addition, the PCA confirmed the separateness of root characteristics and soil properties of the ash stands (Fig. 5).

Fungal and bacterial diversity

The analysis of the share of bacteria phyla in different stand soils revealed that the most numerous phylum was *Proteobacteria*, with the relative share of this phylum being quite stable (amounting to 35.3–37.0%, on average) in the soils inhabited by all the tree species. The second largest group of bacteria was the *Actinobacteriota*, which was more numerous (36.8, 36.4 and 34.4%, respectively) in the soils of oak, hornbeam and larch stands and slightly less numerous (30.8, 29.4, 28.8%, respectively) in the soils of ash, beech and pine stands (Fig. 6). In the case of the *Acidobacteriota*, very similar abundance (10.4–13.3%) was found in the soils of five tree species with the exclusion of pine. In the soils of pine stands, the share of *Acidobacteriota* was slightly higher, amounting to 15.5%. The remaining phylum of bacteria––the *Verrucomicrobiota*––had a share of less than 5%, showing a slightly higher proportion (4.0%) in the soils of the beech stands, whereas it amounted to 2.7–3.6% for the remaining species. The bacteria from the phylum *Planctomycetota* were characterised by a very similar share (2.5–3.6%, on average) in the stand soils. In the case of the phylum *Chloroflexi*, a higher amount (3.9%) was found in the soil of the beech stands, whereas it was 1.4–2.4% for the remaining species. The phylum *Bacteroidota* prevailed (2.5%) in the pine stands, with their share being 1.9–2.1% in the other species. Candidate phylum WPS-2 and the Firmicutes were more numerous (1.7 and 2.3%, respectively) in the soil of the ash stands, while the phylum *Patescibacteria* showed a higher abundance (1.7%) under hornbeam. The phylum *Myxococcota* had a very equal share (0.8–1.1%). The remaining phyla had a share of less than 1% (Fig. 6).

The analysis of fungal taxonomic units proved that the most numerous phyla in the soils were those of the phyla *Basidiomycota* and *Ascomycota*. The *Basidiomycota* were most numerous (average relative abundance = 52.2%) in the beech soil, followed by the ash (44.2%), larch (40.3%) and pine (36.3%) soils. In the oak and hornbeam stands, the *Basidiomycota* were low in number (19.4 and 22.6%, respectively). The opposite situation was found for the *Ascomycota*, which

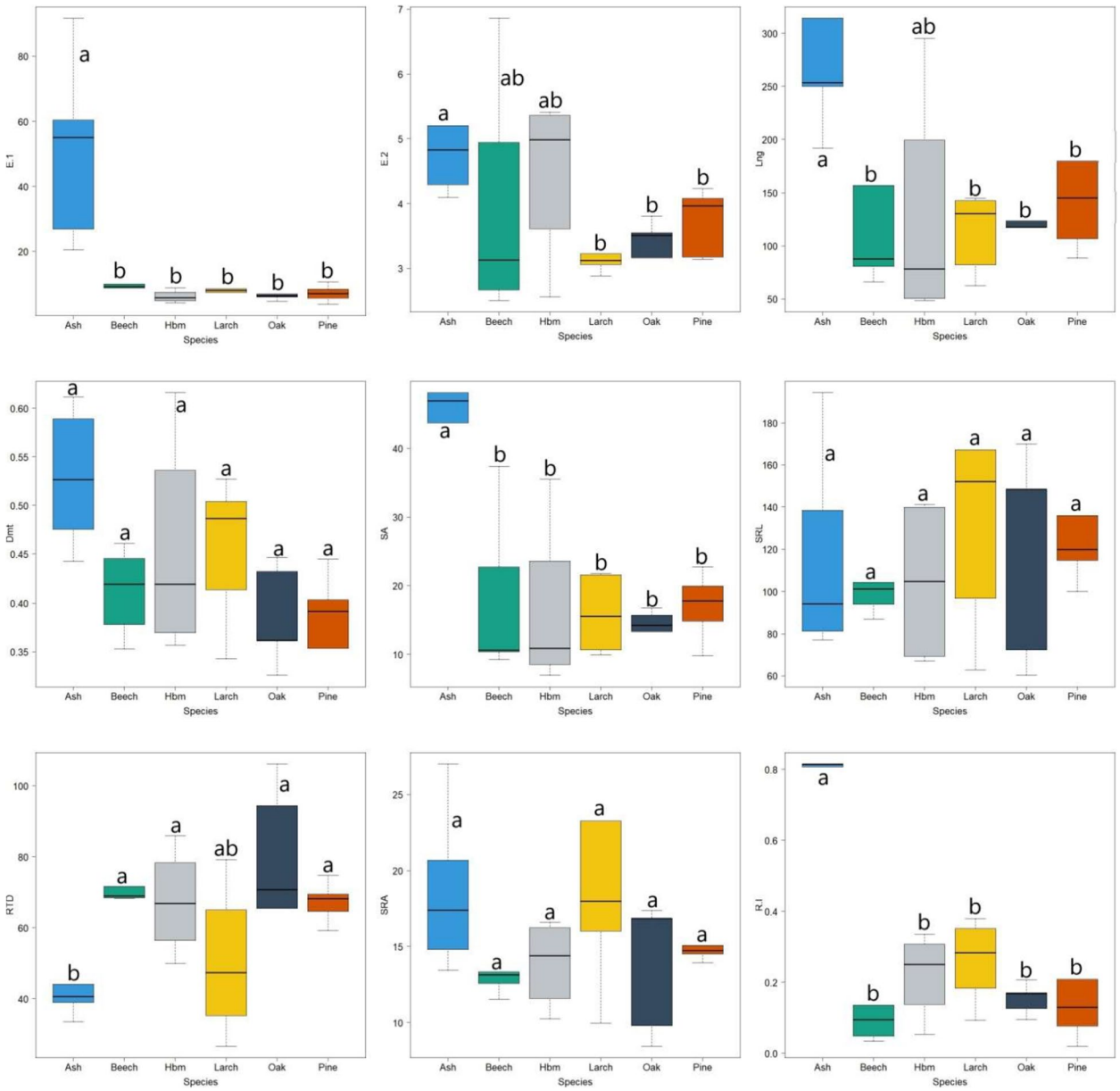

Fig. 2 Root characteristics of various tree species covered by the research (Hbm – hornbeam; E.1—root-exuded carbon at the beginning of the growing season (mg C g^{-1} day^{-1}), E.2—root-exuded carbon at the end of the growing season (mg C g^{-1} day^{-1}), Lng – length roots (cm), Dmt – diameter roots (mm), SA – surface area of roots, SRL—specific root area (m^2 kg^{-1}), RTD—root tissue density (kg m^{-3}), SRA—specific root length (m $kg.^{-1}$), R.I – root increase (g); letters (**a**, **b**) mean significant differences between tree species)

were most numerous (52.5 and 43.0%, respectively) in the soils under hornbeam and oak, but averaged from 24.5% (beech) to 32.7% (larch) in the other species (Fig. 7). The fungal phylum *Mortierellomycota* was most numerous (35.0%) in the soil of the pine stands, being much lower (19.0% under beech and ash to 22.4% under larch) in the soils of the other species. Relatively high abundances (12.4%, on average) of fungi were identified in the soil of the oak stands whereas, for the other species, the share was much smaller (1.5–3.1%). The remaining fungal phyla were not exceeding 0.1%.

The analysis of fungal taxonomic units using heatmaps showed clear similarities in the structure of the fungal populations between the plots with oak and hornbeam, and the ash and larch stands. The beech stands were similar to the pine stands, the latter being the most diverse in terms of fungal population structure (Fig. 8). Fungi of the genus *Mortierella* were most numerous (34.9%) in the

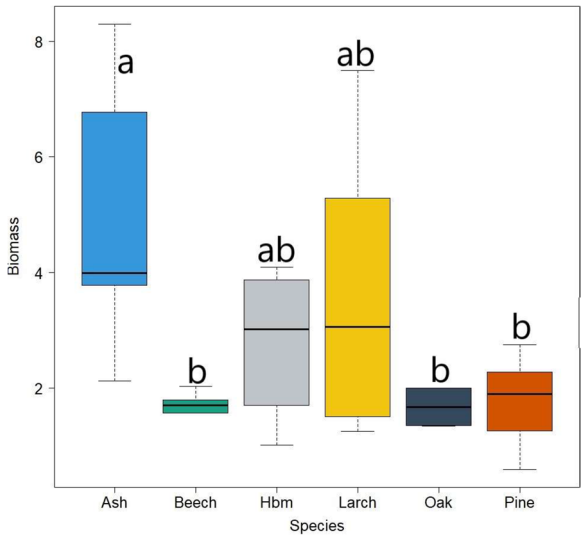

Fig. 3 Root biomass (g) of various tree species covered by the research (Hbm – hornbeam; letters (**a**, **b**) mean significant differences between tree species)

soils of pine stands, and less abundant (17.9–22.4%) in the other cases. The genus *Russula* was most pronounced (35.0 and 33.0%, respectively) under beech and ash, less abundant (23.1% larch, 23.7% pine) in the soils under conifers, and least numerous (3.7 and 4.9%, respectively) under hornbeam and oak. Fungi of the genus *Lactarius* had the highest shares (8.6 and 7.1%, respectively) under larch and beech, whereas its presence was much more insignificant (0.5–2.0%) in the soils under the other species. The remaining types were usually characterised by presence below 5%. The genus *Penicillium* was found to be present in similar amounts (2–4.1%) in the soils of all the studied tree species. Under oak, there was a slightly higher share of fungi from the genera *Oidiodendron*, *Geomyces*, *Aspergillus* and *Trichoderma*, as well as unidentified genera from the orders *Tremellales* and *Helotiales.* Under the hornbeam stands, there was a higher share of fungal genus *Cladophialophara*, as well as unidentified genera from the orders *Agaricales* and *Hypocreales* (Fig. 8).

The analysis of the bacterial population structure showed that the soils contained numerous types of bacteria, each with a relatively low share (Fig. 9). The bacterial genus with the highest relative representation (10.7–11.9%) under all the tree species was *Acidothermus*, except for under beech, where its representation was slightly lower (7.5%). The genus *Paraburkholderia* had a higher abundance (10.3 and 8.2%, respectively) in the soils of larch and ash stands, while its occurrence was lower (2.9–5.9%) for the other species. Also, in the soils under larch, there was a higher percentage (7.6%) of the bacterial genus *Cellulosimicrobium*. Under the beech and oak stands, its share was lower (4.6 and 4.2%, respectively), lower still (2.8 and 2.7%, respectively)

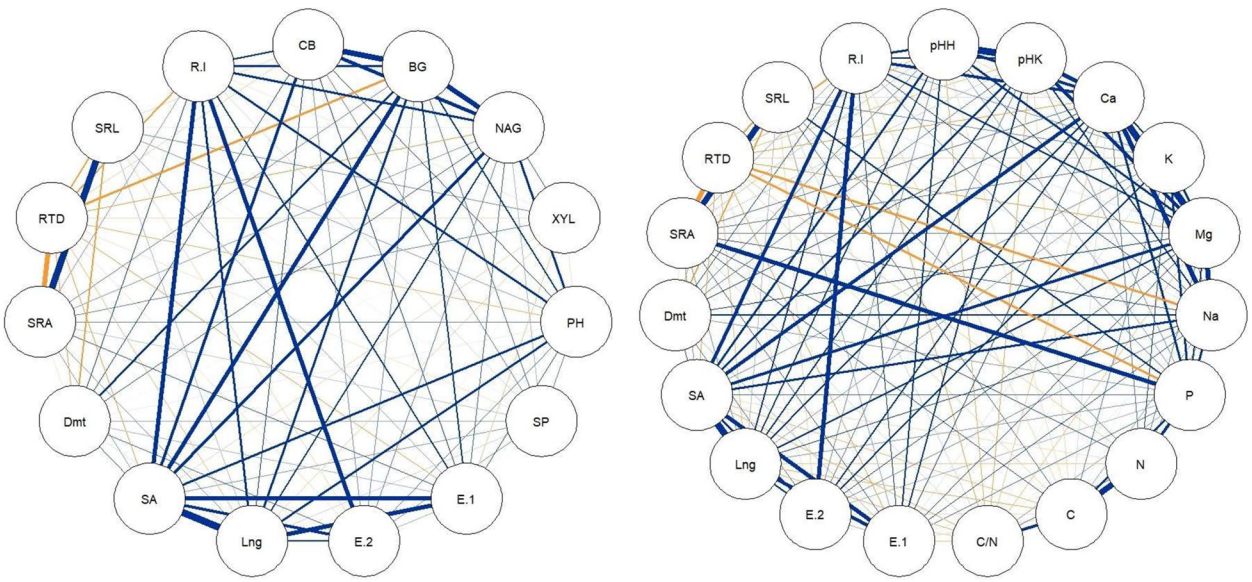

Fig. 4 Correlations between root features and soil properties influenced by different tree species (SRL—specific root length, RTD—root tissue density, SRA—specific root area, Dmt – diameter roots, SA – surface area of roots, Lng – length roots, E.1—root-exuded carbon at the beginning of the growing season, E.2—root-exuded carbon at the end of the growing season, R.I – root increase, PH—phosphatase, BG—β-glucosidase, NAG—N-acetyl-β-D-glucosaminidase, XYL—β-xylosidase, CB—β-D-cellobiosidase, SP – arylsulphatase; pHH – pH in H_2O, pHK – pH in KCl, navy blue—positive correlation, orange—negative correlation)

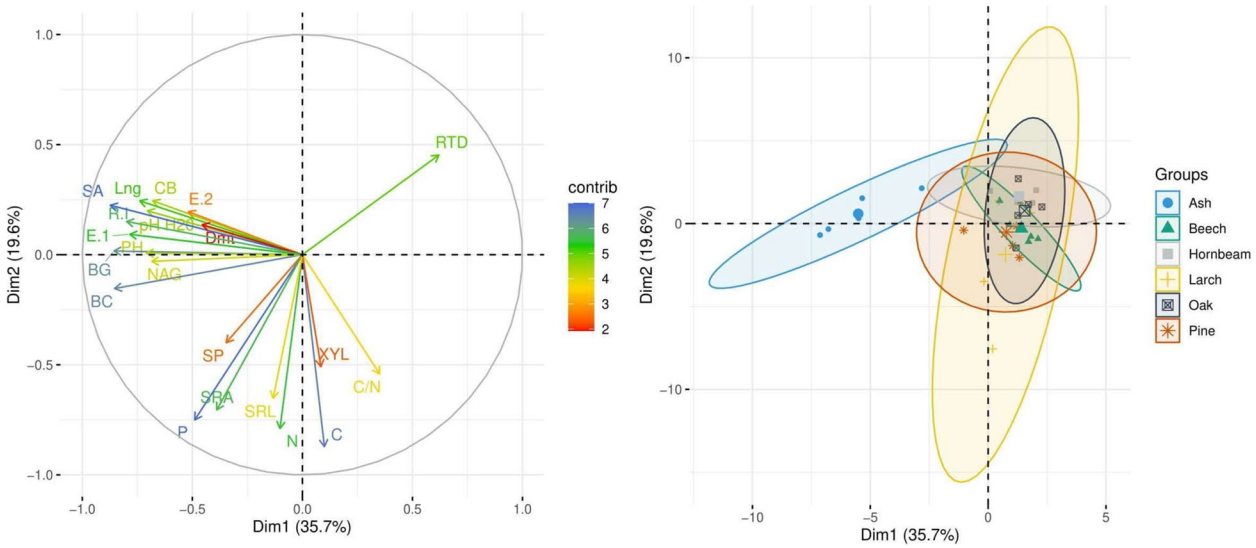

Fig. 5 Projection of variables on the plane of the first and second PCA factors (SRL—specific root area, RTD—root tissue density, SRA—specific root length, Dmt – diameter roots, SA – surface area of roots, Lng – length roots, E.1—root-exuded carbon at the beginning of the growing season, E.2—root-exuded carbon at the end of the growing season, R.I – root increase, PH—phosphatase, BG—β-glucosidase, NAG—N-acetyl-β-D-glucosaminidase, XYL—β-xylosidase, CB—β-D-cellobiosidase, SP – arylsulphatase, BC – base cations content)

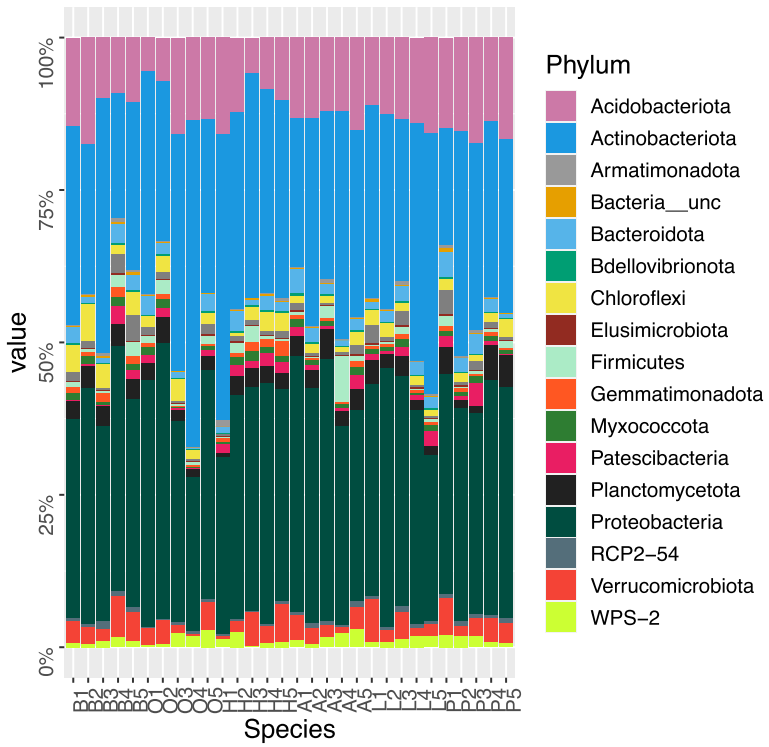

Fig. 6 Relative abundance of bacterial phyla based on the Illumina metabarcoding data

under hornbeam and ash, and the lowest (0.9%) under the pine stands. Under the hornbeam stands, there was a slightly higher share (4.3%) of the bacterial genus *Bradyrhizobium*, this share being 1.9–3.0% for the other soils. In the soil under the ash stands, the bacterial genus *Roseiarcus* was most numerous (3.2%), reaching only

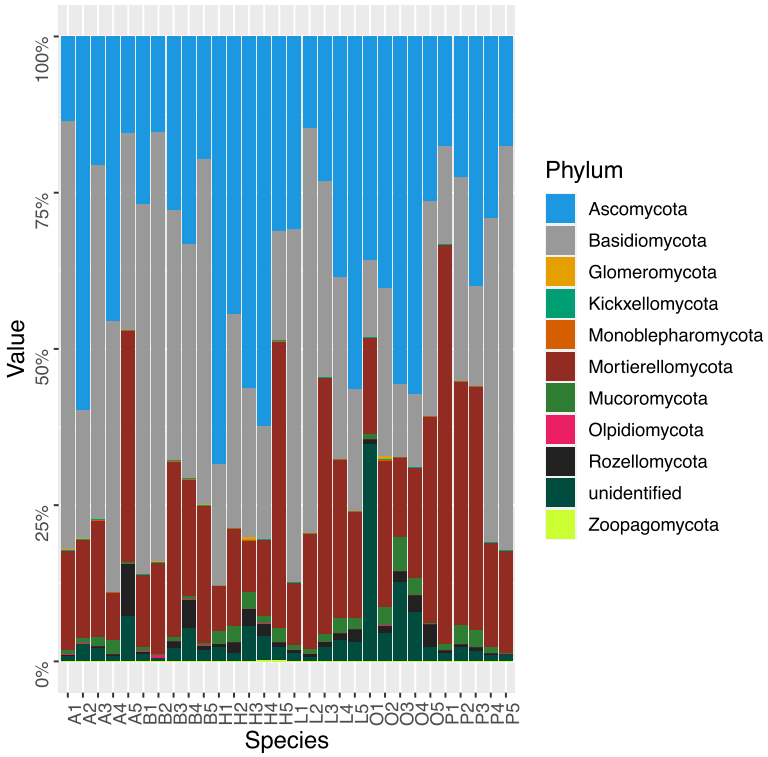

Fig. 7 Relative abundance of fungal phyla based on the Illumina metabarcoding data

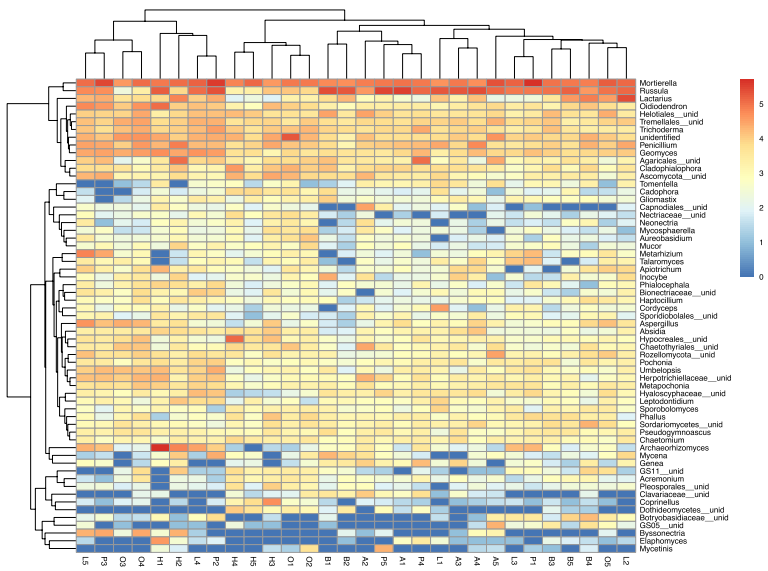

Fig. 8 Abundance heatmap based on the Illumina metabarcoding data constructed using log10 + 1 transformed abundance data for fungal species whose total number of reads excided 400

0.9–2.6% in the other stands. The remaining types of bacteria were characterised by very low (< 2%) relative abundances. Clusters isolated based on the dominant bacteria (Fig. 9) suggest the presence of three groups of bacterial microbiome surfaces. The microbiomes under the hornbeam stands (H3–H5) shared the greatest similarity to those of the oak stands (O1 and O2). The second large microbiome cluster covered most of the plots containing

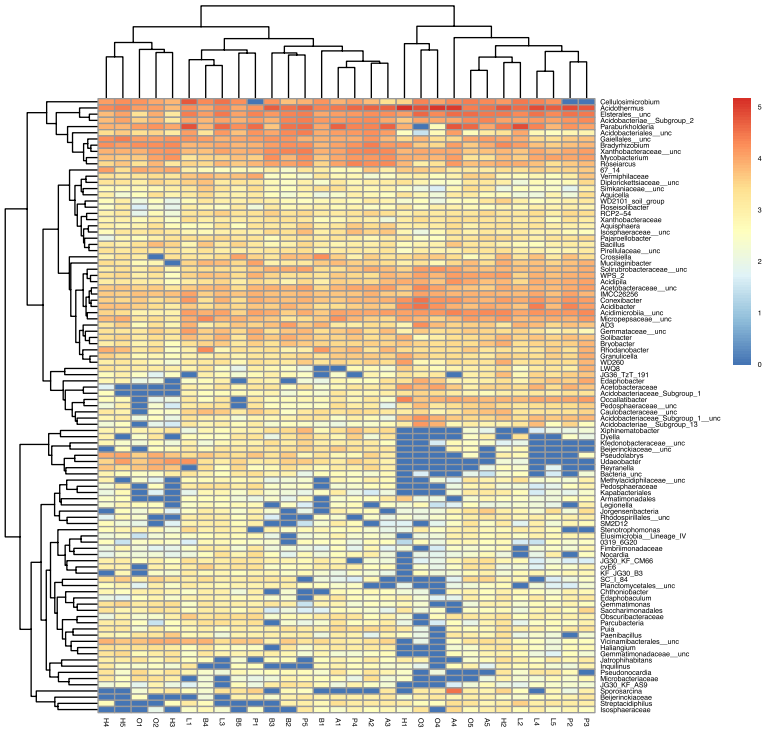

Fig. 9 Abundance heatmap based on the Illumina metabarcoding data constructed using log10 + 1 transformed abundance data for bacterial species whose total number of reads excided 400

ash stands (A1–A3), which shared the strongest similarities to all the plots with beech and three plots with pine (P1, P4 and P5). The microbiomes of the larch stands (L2, L4 and L5) were most similar under oak (O3–O5) and pine (P2 and P3) (Fig. 9).

Discussion

Our results indicate a strong relationship between the morphological features of the roots of the studied tree species and the physicochemical properties of, and enzymatic activity in, the corresponding soils. Root systems are the basic components of the surface horizons of soils and, as such, they have a significant impact on soil physical, chemical and biological properties, mainly via changes in the content of organic matter [40, 41]. Ash root systems, together with their exudates, had the most beneficial effect on the soil properties, differing significantly in terms of root morphology compared with the other species. The ash roots were significantly longer, and had higher surface areas and diameters. Webb et al. [42], in examining how the diversity of root morphology between tree species affected the hydrological properties of soils, found that ash, as a species, could be distinguished by the morphological features of its roots. According to these authors, ash has a great potential for improving the hydrological properties of forest soils because of its roots. In our study, we established a strong positive correlation between the morphological characteristics of the roots and the pH of the soil, as well as the base cation, carbon, nitrogen and phosphorus contents. Ash had the highest annual fine root biomass increase, which undoubtedly had a positive effect on the properties of the soils. According to Shi et al. [43], root biomass increase can change the structure of the soil and its physical properties, especially its hydraulic properties. Numerous studies have shown that longer, faster-growing roots have a greater effect on soil properties such as micropores presence and aggregates formation compared to less developed roots [44]. In the case of ash, we recorded statistically significantly lower RTD compared to the other species, and it is already known that tree-root characteristics are coordinated with, and their suitability is affected by, soil fertility gradients [45], with RTD increasing with decreasing nutrient availability [46] and high RTD being associated with infertile soils [47].

We determined that root-exuded carbon positively affected the physicochemical properties of the soils and, consequently, their enzymatic activity. We also found positive correlations between the root-secreted carbon and the pH of the soils and their enzymatic activity. Root exudates significantly affect the physical and chemical properties of the rhizosphere soil through their complex

and diverse composition consisting of three fractions–– diffuses, secretions and excretions [48, 49]. Root exudates have been divided into LMW compounds, which comprise mainly sugars, amino acids and phenol, high-molecular-weight compounds, mainly derived from mucus and extracellular enzymes, and ions [50]. Ash was characterised by the highest amount of root-exudate carbon and the highest enzymatic activity. The development of soil microorganisms is stimulated by providing easily assimilable carbon substrates together with exudates. Our results indicate that exudate composition is related to the tree species, and therefore, through appropriate selection of species composition, it would be possible to influence the soil properties. It is already known from previous studies that root exudates modulate the composition of soil microbial communities by accelerating biochemical reaction, which may improve the decomposition rate processes in soil organic matter [49]. According to Gianfreda [51], all processes and functions occurring in the rhizosphere are predominantly influenced by the activities of plant roots, rhizosphere microorganisms and root–microorganism interactions. Enzymes are recognized as the key players in all activities taking place within this environment. Our results confirm the hypothesis regarding the influence of tree species on the composition of the soil microbiota through their root systems and secretions. In the fungal population structures in the soils hosting the studied tree species, we found differences in the number of fungi specialized in the formation of ectomycorrhizal symbiosis. The taxa that most differentiated the tree species were the fungi *Russula* and *Lactarius*. These genera are known to tend to form ectomycorrhizal associations with a number of tree species in different climatic zones, including the temperate zone [52]. The richness and diversity of soil fungi are strongly related to the tree species composition of the stand [53], whereas the composition and diversity of saprotrophic fungal communities are strongly influenced by external factors, such as pH, the carbon:nitrogen ratio, or soil type and its moisture content [54, 55]. In our plots of different tree species, the soil subtype and its moisture conditions were the same. However, among the tree species, we found differences in the its degree of organic matter decomposition expressed as a C/N ratio, and also some differences in the degree of soil acidity. This might be associated with the impact of the detritus reaching the soil from the individual species, and especially its impact on the root systems. This seems to have been the most important factor influencing the population structure of the soil microorganisms.

The soil fungal and bacterial structures, as well as their functions, may be strongly interconnected [56, 57]. The structure of the fungal population forming mycorrhizal associations could be linked with certain groups of bacteria that are present in root zones, for example, the mycorrhizal zone created by selected *Russula* fungi [58]. The bacterial genera found to be strongly associated with *Russula* mycorrhizae include *Burkholderia–Paraburkholderia*, *Mycobacterium*, *Roseiarcus*, *Sorangium*, *Acidobacterium* and *Singulisphaera*. We observed that *Russula* was most prevalent in the beech and ash stands, but in the ash stands, *Burkholderia–Paraburkholderia* and *Roseiarcus* were even more abundant. *Paraburkholderia* bacteria, possessing suitable enzymatic abilities, exhibit high phenolic compound degradation activity, thus significantly accelerating the decomposition processes of soil organic matter [59]. *Roseiarcus* bacteria, like ectomycorrhizal fungi, are symbiont that positively impact plant growth and are an indicator of natural, undisturbed microbial environments [60].

At the beginning of our study, we hypothesised that coniferous species such as pine and larch would similarly affect the number and diversity of fungi and bacteria inhabiting the soil. However, this hypothesis was not fully confirmed by our results. The plots with larch, in terms of bacterial structure, had soils more similar to those of ash, while the plots with pine, in terms of bacterial structure, were more similar to the soils of the beech stands. Regarding fungal structure, the plots with pine were quite diverse and did not form a distinct cluster. The plots with pine showed greater similarity in terms of fungal organisms to the larch plots, while forming smaller clusters with the plots of beech, oak and ash stands. The distinctiveness of the soil bacterial community in the larch stands was reflected in the relatively high share of bacteria from the genera *Burkholderia–Paraburkholderia* and *Cellulosimicrobium*, which were less prevalent in the soils of the pine stands. *Cellulosimicrobium* is known for its strong cellulolytic properties [61, 62]. In our study, the soils of the larch stands exhibited a tendency towards higher cellulolytic enzyme activity and selected root parameters (i.e., SRA, RI) compared to the soils of the pine stands. At the same time, the fungal populations in the pine stands were characterised by a large share of the genus *Mortierella*, occurring in lower abundances in the larch stands. *Mortierella* is considered a saprotrophic microorganism, also found in plant root zones, possessing various enzymatic abilities, including the decomposition of polysaccharides (chitin, hemicellulose), enhancing phosphate-ion absorption, and synthesizing phytohormones, all beneficial to plant growth [63]. It should be noted that the conditions of the studied pine stands (on luvisols formed from loess) are considered unnatural (they were artificially introduced by humans), suggesting that differences in the fungal and

bacterial structure in this environment might be more reflective of adaptation to new, somewhat unfavourable habitat conditions for the pine stands. The studied pine stands, characterised by good health and no disease symptoms, might confirm their positive adaptation due to a specific set of symbiotic soil microorganisms.

Our findings indicate a positive impact of ash stands on shaping soil properties and enhancing their biodiversity. We observed the beneficial effect of root systems and their exudates on the composition and diversity of microorganisms, as well as on the activity of their enzymes. Currently, in Europe, there is an issue with dying ash stands. The common ash is found in almost all of Europe, and in Poland, it grows throughout the country, except in upper mountain forests [64]. The presence of the fungus *Chalara fraxinea*, the causative agent of ash dieback, has been detected in tissues of dying ash trees [65, 66]. The loss of ash stands could lead to a deterioration of soil properties and, consequently, a decline in the stability of forest ecosystems.

Conclusions

Our study has confirmed the importance of tree species in shaping soil properties through their root systems. We identified a strong relationship between the morphological features of the roots and the basic physicochemical properties of the soils and their enzymatic activity. Root-exudate carbon was found to be positively correlated with pH, calcium content and the activity of enzymes involved in the carbon and nitrogen cycles. Analysis of the morphological features of the roots and their exudates in connection with soil properties, confirmed the distinctive influence of ash tree stands. We observed differences in the composition of bacterial and fungal associations in relation to coniferous species such as pine and larch. The ash stands were also distinguished by their particular differences in microorganism diversity compared to the other species. The research examining bacteria in the soil of various tree species found that *Proteobacteria* and *Actinobacteriota* were the most prevalent phyla. In the analysis of fungi, *Basidiomycota* and *Ascomycota* emerged as the dominant phyla. The soils under hornbeam and oak trees were particularly rich in *Ascomycota*, while soil of pine trees showed a significant presence of the *Mortierellomycota* phylum. Our findings suggest that the formation of single-species coniferous stands should be avoided, as this leads to a deterioration of soil properties, a reduction in microorganisms diversity, and consequently, a decrease in the stability of the stand. To improve the soil properties and biodiversity, deciduous species such as ash, hornbeam and oak should be introduced into tree stands.

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Authors' contributions
K.S.S., E.B. and J.L.: conceived and designed the investigation; analysed and visualised the data; E.B., J.L.: concepts research methodology; K.S.S. A.S: field work, sample collection; K.S.S., E.B., J.L., A.S.: preparation of manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author on reasonable request. NGS sequence data of bacteria and fungi isolated from soil samples were deposited in the Gene Bank https://www.ncbi.nlm.nih.gov/ in project number PRJNA956107 and PRJNA951397.

Declarations

Ethics approval and consent to participate
We declare that the plant material in the form of tree roots was collected in accordance with the guidelines while maintaining all the ethical rules of our country. Fragments of root systems were taken in a way that was safe for individuals. They were small in quantity and did not cause any harm. The tree species that were selected for the experiment are common species in Poland. Due to the fact that the experimental area was not located in a protected area, the consent to collect tree roots was obtained from the Forest Inspector.

Consent for publication
Not applicable.

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Effect of drought on root exudates from *Quercus petraea* and enzymatic activity of soil

Karolina Staszel✉, Jarosław Lasota & Ewa Błońska

Root exudation is a key process that determines rhizosphere functions and plant-soil relationships. The present study was conducted with the objectives to (1) determine the root morphology of sessile oak seedlings in relation to drought, (2) assess root exudation and its response to drought, and (3) detect possible changes in the activity of soil enzymes in response to drought enhancement. In the experiment, sessile oak seedlings (*Quercus petraea* Matt.) were used, and two variants of substrate moisture (25% humidity—dry variant and 55% humidity—fresh variant) on which oaks grew were considered. Exudates were collected using a culture-based cuvette system. Results confirmed the importance of drought in shaping the morphology of roots and root carbon exudation of sessile oak. The oak roots in the dry variant responded with a higher increment in length. In the case of roots growing in higher humidity, a higher specific root area and specific root length were determined. Experimental evidence has demonstrated decreased root exudation under dry conditions, which can lead to a change in enzyme activity. In the study, enzyme activity decreased by 90% for β-D-cellobiosidase (CB), 50% for β-glucosidase (BG) and N-acetyl-β-D-glucosaminidase (NAG), 20% for β-xylosidase (XYL) decreased by, and the activity of arylsulphatase (SP) and phosphatase (PH) decreased by 10%.

The conducting observations and measurements of climate elements confirm that the climate changes on a global scale, with the tendency of air temperature increase. The global average temperature is projected to increase between 1.5 °C and 6.4 °C by 2100[1]. An undoubted effect of changes in thermal conditions, wind speed, and amount of precipitation will be the occurrence of extreme meteorological phenomena, including rapid and strong periods of drought[2]. Such an increase air temperatures and simultaneous changes in rainfall distribution during the year affect plant growth and, among others, root systems. Früchtenich et al.[3] indicate that mesic, deciduous European oaks (*Q. robur* and *Q. petraea*) tend to use an avoidance strategy to cope with potential drought stress on sandy soil by establishing deep root systems, thereby increasing access to water supply during the initial growth phase. Root development may be affected directly by elevated soil temperatures or indirectly by changes in the physiology, development, and resource acquisition of the shoot in response to warmer air temperatures or by a combination of both factors[4]. An earlier study had also demonstrated that root exudates are active players in whole-ecosystem responses to environmental perturbation[5]. Furthermore, by collecting root exudates from intact root systems in solution, many studies have shown that drought affects the composition of root exudates[6,7]. Root exudation is a crucial process that determines rhizosphere functions and plant-soil relationships[8]. Root exudation is a key plant function with a large influence on soil organic matter dynamics and plant-soil feedbacks in forest ecosystems[9].

Plant exudates also play an essential role in the shaping of soil extracellular enzyme activity. They may have a significant effect on the expression and repression of extracellular enzyme activity in the rhizosphere. According to Gianfreda[10], the enzyme activity profile of the rhizosphere is a footprint of plant-microorganism interactions. Soil enzymes, as indicators of microbial nutrient demand and metabolic processes, play an important role in soil organic carbon (C) mineralization, the nitrogen (N) cycle, the phosphorus (P) cycle, and the sulfur (S) cycle[11]. Soil enzymes are central in the response of terrestrial ecosystems to climate change, and studying these enzymes can be crucial for the models' implementation[12]. Under drought conditions, enzyme production and activity are reduced as the nutrient requirement for enzyme production exceeds the net increase in nutrient availability for microbes[13]. For example, Sardans and Penuelas[14] found that the enzymes involved in the N cycle, protease and urease, were affected the most by drought, and the reduction of soil moisture decreased enzyme activity.

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pH H_2O	pH KCl	N	C	C/N	Hh	Hex	P	Ca	K	Mg	Na
3.99	2.81	0.70	48.82	70.0	62.21	4.00	89.60	3.53	1.08	1.45	0.01

Table 1. The physico-chemical characteristics of substrate used for experiment. C, N (%);Hh– hydrolytic acidity (cmol(+)·kg^{-1}), Hex—exchangeable acidity (cmol(+)·kg^{-1}); P, Ca, K, Mg and Na (cmol(+)·kg^{-1}).

The study covered sessile oak, which is the basic forest-forming species in a temperate climate in Europe, spcifically. Despite the fact that it is a species with a relatively high tolerance to lower soil moisture, simulations of changes in climatic conditions together with an assessment of the species' reactions suggest the possibility of a significant reduction in its survival and deterioration of growth conditions in the areas of its south-eastern range[15,16]. This research is an attempt to improve the understanding of how sessile oak trees respond to drought in temperate climate. The present study was conducted with the aims to (1) determine root morphology of sessile oak seedlings (*Quercus petraea* L.) in relation to drought, (2) assess root exudation and its response to drought, and (3) detect the possible changes in the activity of soil enzymes in response to drought enhancement.

Materials and methods

Description of experiment. The experiment was carried out in the Laboratory of Forest Environment Geochemistry and Land Intended for Reclamation at the Faculty of Forestry at the University of Agriculture in Krakow. In the experiment, 3-month-old sessile oak seedlings were used. Oaks grew in a typical substrate used in forest nurseries, the properties of which are presented in Table 1. The growth condition of sessile oak seedlings was assessed in line with Regulation of the Minister of the Environment[17]. Seedlings with similar growth characteristics were selected for experiment. The single seedlings were grown in uniform plastic pots 15 cm in diameter and height. The research considered two variants of substrate moisture (25% humidity – dry variant and 55% humidity – fresh variant) on which oaks grew. The humidity sensors in an hourly interval were used to monitor the soil moisture in the post. In the event of change in soil humidity it was brought back to the initial state. The experiment was conducted from March 20 to April 20, 2021. Samples for analysis were taken four times every week. In each variant of moisture and each campaigns of the experiment four seedlings were samples from one pots. The completed experiment complies with local and national regulations.

Root exudate collection. Exudates were collected in four sampling campaigns during the experiment using a culture-based cuvette system[18]. In each variant of humidity, four oaks were sampled. Root exudates were collected from one branched fine root segments of similar length and branching. Each root system was carefully removed with deionized water and fine forceps to maintain the integrity of the root. The root systems were placed into a sterile glass syringe with sterile glass beads moistened with a C-free nutrient solution (0.5 mM NH_4NO_3, 0.1 mM KH_2PO_4, 0.2 mM K_2SO_4, 0.15 mM $MgSO_4$, 0.3 mM $CaCl_2$). After 24 h of stabilization in the syringe, the roots were flushed three times with a clean C-free solution to remove the organic C exuded during the stabilization period. Exudate-containing samples were collected in 50 mL glass vials with silicon caps and stored at 4 °C until the determination of total organic carbon (TOC). Trap solutions containing exudates were collected from each cuvette and filtered through sterile syringe filters. A total of 32 exudate samples were analyzed (4 seedlings in two variants of moisture x four times extractions = 32). Trap solutions were then analyzed using the Shimadzu TOC—Total Organic Carbon analyzer (Shimadzu, Japan).

Root morphology analysis. After root exudate collection, the sampled all roots were clipped from the tree, scanned at 400 dpi, and analyzed with a WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Ville de Québec, QC, Canada) to establish diameter, length, and projected area. Air-dried roots were further desiccated at 70 °C for 24 h to constant weight and then weighed. Root tissue density (RTD; kg m^{-3}), SRA (m^2 kg^{-1}), and SRL (m g^{-1}) were subsequently calculated as described by Ostonen et al.[19]. Root branching intensity was expressed as the number of root tips per 1 mg of dry mass.

Enzyme activity analysis. The soil samples for the determination of enzymatic activity were taken from the rhizosphere zone of oak seedlings. Soil samples of natural moisture were stored at 4 °C prior to analysis. The enzymatic activity was determined at the beginning and the end of the experiment (in the 1st and 4th series of experiment). The enzyme activities were determined using fluorogenically labeled substrates[20,21]. Six fluorogenic enzyme substrates based on 4-methylumbelliferone (MUB) were used: MUB-β-D-cellobioside for β-D-cellobiosidase (CB), MUB-β-D-xylopyranoside for β-xylosidase (XYL), MUB-N-acetyl-β-D-glucosaminide for N-acetyl-β-D-glucosaminidase (NAG), MUB-β-D glucopyranoside for β-glucosidase (BG), MUB-phosphate for phosphatase (PH), and MUB-sulfate potassium salt for arylsulphatase (SP). A soil sample of 2.75 g was mixed with 92 mL buffer. After that, the soil suspension was pipetted into wells on a microwell plate containing substrate and a modified universal buffer. Fluorescence was determined by first incubating the soil suspensions for 1.5 h at 35 °C in 96-well microplates (Puregrade, Germany) and then measuring with fluorogenic substrates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm.

Statistical analysis. The Spearman correlation coefficients between different root parameters and exudation rates were calculated. Principal component analysis (PCA) was used to evaluate the relationships between root characteristics. Additionally, PCA analysis was used to evaluate the effect of moisture variant on root char-

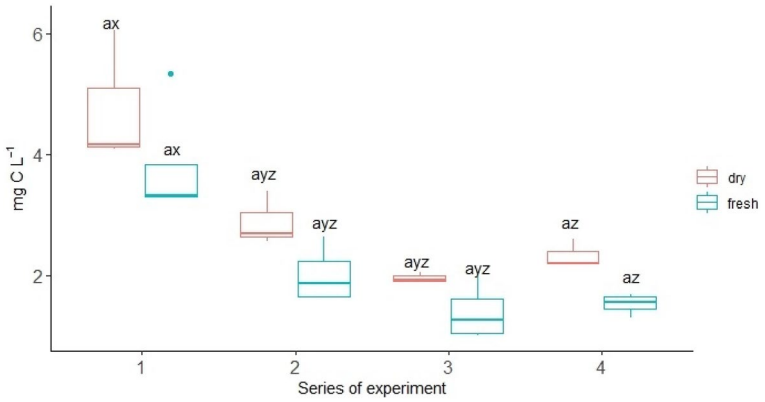

Figure 1. The change of total root-exuded carbon (mg C L^{-1}) over time depending on the moisture variant; different lowercase letters (a,b) indicate significant differences in parameters between the different moistures; different lowercase letters (x,y,z) indicate significant differences in parameters between the series; Tukey HSD $p < 0.05$.

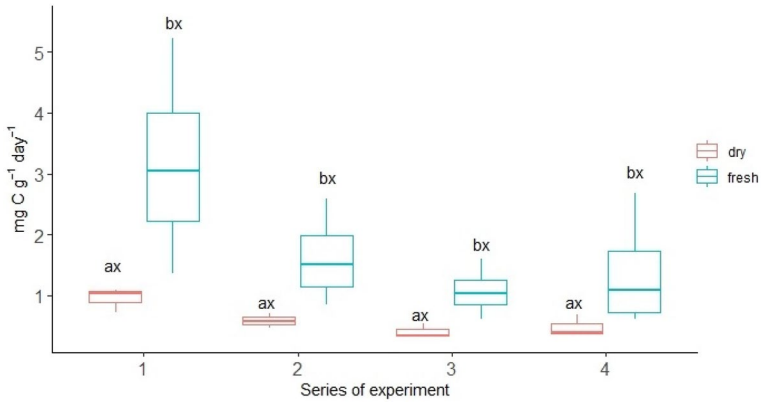

Figure 2. The change of root exuded C per unit root biomass over time depending on the moisture variant (mg C g^{-1} day^{-1}); different lowercase letters (a,b) indicate significant differences in parameters between the different moistures; different lowercase letters (x,y,z) indicate significant differences in parameters between the series; Tukey HSD $p < 0.05$.

acteristics. The ANOVA with a Tukey test was used to assess the differences between the average values of the properties. Different lowercase letters (a, b) in tables and figures indicate significant differences in parameters between the variant of moisture. Different lowercase letters (x, y, z) indicate significant differences between the series of experiment. All the statistical analyses were performed using the statistical programs R Studio[22] and Statistica 10.0 software (StatSoft Inc. USA 2010). The ANOVA with a Tukey test and PCA analysis were done in R while Spearman correlation was done in Statistica.

Results

Root carbon exudation and root morphology. On average, oak roots exuded 12.64 mg C g^{-1} day^{-1} in the fresh variant of the experiment (Table 1). The absolute amount of C root exudates collected were higher from the oak growing in the dry variant (Fig. 1). In all experimental series, there was a trend, not confirmed by statistical analysis, of a higher absolute amount of root exudate C in the dry variant compared to the fresh variant. However, when expressed per unit root biomass, this pattern disappeared. The higher root exudation rates per unit of root biomass were in the fresh variant (Fig. 2). In the first series of experiments, the root exudation rates in the fresh variant were three times higher than that of the dry variant. In subsequent series, the differences in root exudation rates in the fresh variant were smaller. In all experiment series, the root exudation rates were significantly higher in the fresh variant than in the dry variant (Fig. 2).

Moreover, the moisture variant had a significant effect on root morphology of oak (Table 2). In the case of roots in the dry variant, a higher diameter was noted. The dry variant was characterized by statistically significantly longer roots. SRA it was higher and amounted to 38.90 m^2 kg^{-1} in the case of the fresh variant while in the case of the dry variant, it was 26.90 m^2 kg^{-1}. In the fresh variant, a higher SRL of statistical significance was noted, compared to the dry variant (8.47 m kg^{-1} and 3.92 m kg^{-1}, respectively). Root tissue density did not differ between the examined variants. The exudation rate significantly correlated with the morphological features of

Variants	Length [cm]	Diameter [mm]	Weight [mg]	SRA [$m^2 kg^{-1}$]	RTD [$kg m^{-3}$]	SRL [$m kg^{-1}$]	Exudation rate
Fresh	65.57 ± 25.99^b	0.20 ± 0.10^a	10.95 ± 6.36^b	38.90 ± 15.70^a	64.49 ± 21.41^a	8.47 ± 6.56^a	12.64 ± 8.75^a
Dry	148.13 ± 65.67^a	0.23 ± 0.06^a	36.43 ± 8.14^a	26.90 ± 1.46^b	68.11 ± 16.32^a	3.92 ± 1.03^b	4.23 ± 1.80^b

Table 2. Mean morphological characteristics of roots and exudation rate (mg C g^{-1} day^{-1}) in different moisture variant. Mean ± standard deviation; root tissue density (RTD), specific root area (SRA) and specific root length (SRL); a, b—statistically significant parameters; $p < 0.05$).

	Exudation rate	Length	Diameter	Weight	RTD	SRA	SRL
Exudation rate	1.00						
Length	− 0.35	1.00					
Diameter	− 0.52*	− 0.25	1.00				
Weight	− 0.66*	0.82*	0.29	1.00			
RTD	0.09	0.29	− 0.61*	0.09	1.00		
SRA	0.69*	− 0.15	− 0.69*	− 0.63*	− 0.10	1.00	
SRL	0.72*	− 0.13	− 0.79*	− 0.61*	0.17	0.95*	1.00

Table 3. Correlation between different root parameters and exudation rate (mg C g^{-1} day^{-1}). *Correlation significant with $p < 0.05$.

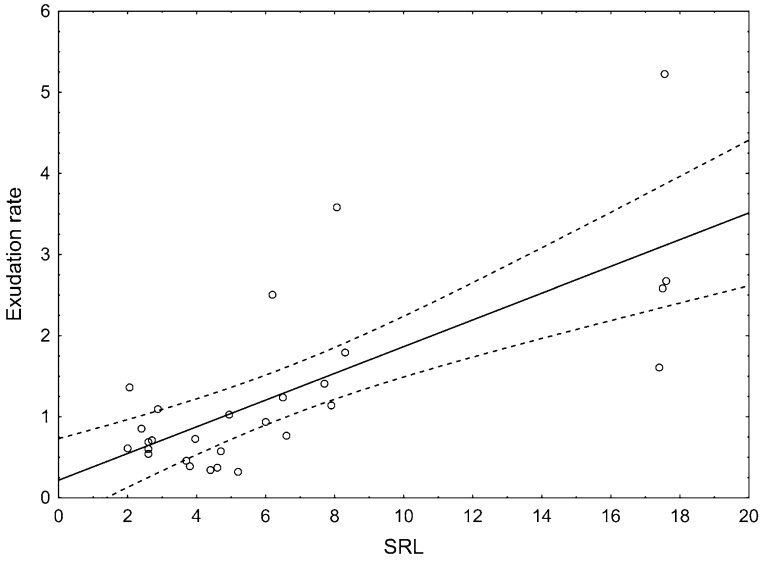

Figure 3. The positive relationship between root exudation rate (mg C g^{-1} day^{-1}) and specific root length (SRL).

the roots (Table 3). There was a statistically significant negative correlation between the exudation rate, diameter, and length of roots (r = -0.52 and r = -0.66, respectively). The strongest positive correlation was noted between the exudation rate and SRA (r = 0.69) and SRL (r = 0.72) (Table 3; Fig. 3). Factors 1 and 2, distinguished in the PCA analysis, explain a total of 81.9% of the variance of the tested characteristics (Fig. 4). The PCA analysis confirmed a positive correlation between the exudation rate, SRA, and SRL and a higher exudation rate in the fresh variant of the experiment.

Enzymes activity. Of the tested enzymes, significantly lower activity was noted in the dry variant compared to the fresh variant (Fig. 5). In the first series of experiments, the differences in the activity of the tested enzymes were smaller. In the fourth series of experiments, when comparing the dry variant to the fresh one, enzyme activity decreased by 90% in the case of CB, 50% for BG and NAG, 20% for XYL, and 10% for both SP and PH. In the fresh variant of the experiment, significant differences between series I and IV were noted only in the case of NAG activity. However, in the dry variant, a significant decrease in the activity of all tested enzymes (except SP) was noted between series I and IV of the experiment (Fig. 5).

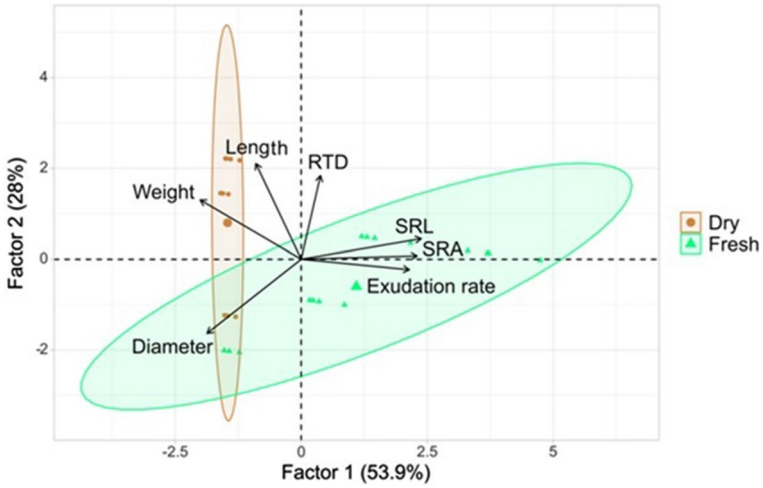

Figure 4. Principal component analysis (PCA) biplot of variables (root tissue density (RTD), specific root area (SRA) and specific root length (SRL), exudation rate – mg C g^{-1} day^{-1}).

Discussion

Root morphology. This research confirmed the importance of drought in shaping the morphology of roots and root C exudation of sessile oak. The oak roots in the dry variant responded with a higher increment in length. In the case of roots growing in higher humidity, a higher SRA and SRL were determined. It is well documented that tree species adapted to dry climatic regimes generally have higher root-to-shoot ratios and deeper root systems than species from mesic climatic conditions[23]. According to Brunner et al.[24], trees adapted to dry climates also invested more biomass into longer-lasting root organs, thus optimizing water uptake while minimizing water loss from transpiration. Such longer roots allow deeper soil penetration in search of water, typical of drought-prone plants[25]. In addition, tree species can delay drought stress by maximizing their access to water and minimizing transpirational water loss through biomass investments to the root, smaller leaf area, and strong stomatal control[26]. In this study, a decrease in soil humidity caused changes in the SRA and SRL. According to Ostonen et al.[27]. SRL is the most commonly measured morphological parameter of fine roots because it is believed to characterize the economic aspects of root systems and indicate environmental changes. Lozano et al.[28] showed that several plant species (most forbs and some grasses) had reduced SRA and SRL as a response to drought and can be interpreted as a drought coping mechanism.

Root exudates. Drought simultaneously affects root systems and soil nutrient availability, and roots can directly affect soil properties via the uptake of water and nutrients. Still, roots can also indirectly affect soil nutrient and C availability via root exudation[29]. This research confirmed significantly lower C exudation rates in the dry variant. The increase of the mean SRA and SRL coincided with increased C exudation. In the fresh variant, the C exudation rates were three times higher than in the dry variant. Results further show that root exudation is closely related to the morphology of the roots in drought conditions. In research by Meier et al.[9], they show that the quantity of C released with root exudation closely and positively relates to SRL. Results in this study further confirm the role of small diameter young root segments in shaping root exudation. Sell et al.[30] also indicated that greater mass-specific exudation flux was related to a higher proportion of pioneer roots, suggesting that an essential amount of C may be released by actively growing pioneer roots. According to Meier et al.[9], root systems with a high SRL have, on average, thinner roots, and the C costs of root construction and mycorrhizal symbiosis are reduced. Therefore, more C may be available for root exudation.

Enzymatic activities. The influence of drought on root growth and the intensity of root exudation induce changes in microbiological processes occurring in the rhizosphere around the roots. Karlovsky et al.[31] analyzed the effect of simulated drought on the flux of C compounds released with exudates into the soil and their assimilation by microorganisms. They found a slowdown in microbiological processes and lower absorption of exudates by microorganisms inhabiting the rhizosphere at the culmination point of drought. In our study, the effect of reducing microbial activity was confirmed by a substantial reduction in the activity of enzymes involved in C, N, and P transformations. The most significant changes were found in the activity of CB, BG, and NAG. This decrease in enzymes activity is probably related to the availability of SOC, which is commonly the most limiting factor for microbial growth in soils. The low C supply restricted the synthesis of enzymes in the soil of the dry variant. The enhanced labile C supply from root exudation has the potential to trigger increases in decomposition rates and thus N availability[8]. The change in enzymatic activity may also be a consequence of changes in the structure and composition of microorganism species induced by the drought phenomenon. Evidence for such changes as a result of drought stress was provided by the studies of Fuchslueger et al.[32]. In the course of drought, potentially slow-growing gram-positive bacteria adapted to drought develop, and the overall ratio of fungi to bacteria changes, leading to changes in enzyme activity. Furthermore, drought induces changes in the chemical

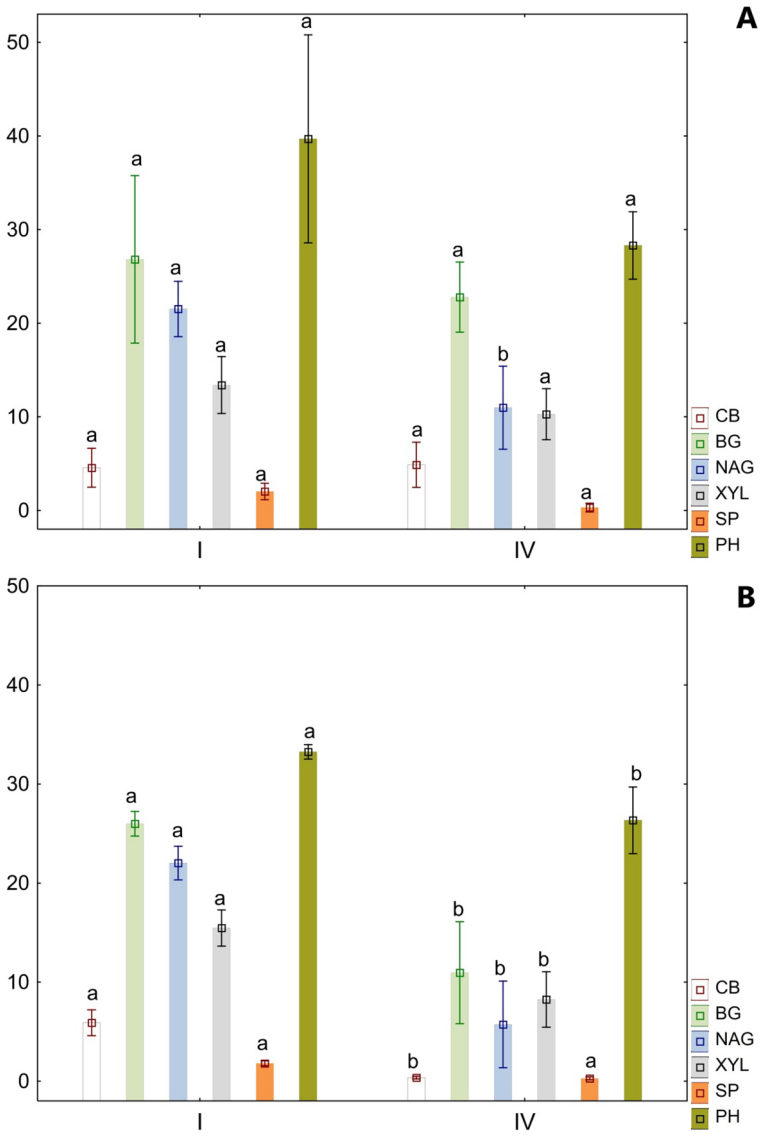

Figure 5. Change in enzymatic activity over time; A – fresh variant; B—dry variant; I, IV – series number; phosphatase (PH), β-glucosidase (BG), N-acetyl-β-D-glucosaminidase (NAG), β-xylosidase (XYL), β-D-cellobiosidase (CB),), arylsulphatase (SP) [nmol MUB·g^{-1} dry soil ·h^{-1}]; different letters (a,b) indicate significant differences in enzyme activites between the series of experiment.

composition of exudates, as shown in the studies by Gargallo-Garrig et al.[33], where the focus was on holm oak (*Quercus ilex*). Also, the significance of the influence of the quantity and the quality of root exudates on enzymatic activity in the rhizosphere has been confirmed in previous studies[34]. According to Zhang et al.[34] among the tested exudate components, alanine showed the strongest stimulating effect on BG, PH, and SP activity. Hommel et al.[35] studied the influence of drought on the rate of photosynthesis and the allocation of assimilates to the root in beech and common maple seedlings. In the case of beech, a higher assimilate allocation to the root was found under moderate drought conditions compared to an unlimited water supply. Similar experiments with sessile oak have not been carried out, but a similar phenomenon may explain the increased development of long roots found in the presented research. When considering the significant changes in the growth and structure of the root system induced by drought, namely a reduction of the degree of fine root density and a smaller number of elongation zones and root tips through which the greatest amount of exudates is subject to outflow, then the obtained results indicate a reduction in exudate excretion per unit mass of the root under the influence of simulated drought, seem to be justified.

Conclusions

This research shows that the amount of root exudates C have a positive and close relationship with the morphology of roots, especially with SRA and SRL. Root morphology is a driver of root exudation in drought conditions. The sessile oak seedlings growing in the dry variant were characterized by lower SRA and SRL, which resulted

in lower C released with root exudation. Experimental evidence has demonstrated decreased root exudation under dry conditions, which led to a change in enzyme activity. Compared to the fresh variant, enzyme activity results in the dry variant showed a decrease of 90% in CB, 50% in BG and NAG, a decrease of 20% in XYL, and SP and PH activity decreased by 10%. Knowledge about the factors shaping the accumulation of organic C in the soil and the relationship between these processes and root exudates is essential for understanding the C cycle in forest ecosystems. It is hoped that this better understanding of the mechanisms and factors influencing the dynamics of organic C in forest soils will allow for intentional prediction of these phenomena in the future, which will contribute to the prevention of the adverse effects of climate change.

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

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Author contributions

I declare following participation of co-authors in the publication: 1. concepts/ideas and objectives K.S., J.L., E.B. 2. research methodology E.B., J.L. 3. results K.S., E.B., J.L. 4. preparation of manuscript K.S., E.B., J.L.

Competing interests

The authors declare no competing interests.

Additional information

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ORIGINAL PAPER

Effect of nitrogen deposition on root systems and exudates of seedlings of beech *Fagus sylvatica* L. in a temperate climate

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ABSTRACT

The purpose of this experiment was to determine the effect of deposition of different doses of nitrogen on the root systems of common beech *Fagus sylvatica* L. seedlings and their exudates. We tried to explain how different doses of nitrogen can affect the assimilation of nutrients necessary for seedling growth, as well as the morphology of fine roots. The experiment was conducted on a nursery at the Forest Experimental Station of the Agricultural University of Kraków. Three different nitrogen doses were used in the experiment: 0.75 kg·ha^{-1}, 2.25 kg·ha^{-1}, 4.5 kg·ha^{-1} and a control variant without nitrogen. The experiment was conducted from May to September 2021. Seedlings with their root systems and their secretions were taken twice, at the beginning and end of the experiment. The content of micro- and macro-nutrients was determined in the above- and below-ground parts of the seedlings. In addition, the basic chemical properties and enzymatic activity of the substrate in which the seedlings grew were determined. In the study, we showed that a higher nitrogen dose influenced a higher amount of carbon released with exudates from fine roots, which was related to the overall root morphology. Higher specific root length (SRL) and specific root area (SRA) parameters showed a positive correlation with root exudates. In addition, a higher nitrogen dose had a positive effect on the nutritional status of the seedlings.

KEY WORDS

beech, enzyme activity, nitrogen deposition, seedlings, soil properties

Introduction

Mineral nutrients in the soil are needed to ensure adequate growth and metabolic processes occurring in the plant (Soares *et al*., 2019). Nitrogen is one of the macronutrients necessary for plant life. Nitrogen is the building material of proteins and is part of vitamins, nucleotides, nucleic acids, alkaloids and chlorophyll. This element stimulates the growth of aboveground parts, and additionally regulates the consumption of potassium, phosphorus and other nutrients. Many studies have focused on the effect of fertilization on seedling yield. Previous research indicates that intensive fertilization can lead to excessive vegetative growth (Shen *et al*., 2010) which can result in greater water and energy consumption, which is reflected in greater leaching of N beyond the reach of root systems (Rudnick *et al*., 2017; Wang *et al*., 2017).

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Root systems play an extremely important role for plants, primarily enabling them to anchor in the soil. During the growth stage, root development is very plastic and is mainly dependent on nutrient distribution and water availability (Hodge *et al*., 2009). Among others, the morphology of the roots and the amount of exudates secreted into the soil change in response to the amount of nutrients (Richardson *et al*., 2009; Staszel *et al*., 2022c). Above all, fine roots show high physiological activity in response to environmental changes such as drought or excessive acidity of soils (Guo *et al*., 2004; Hirano *et al*., 2007). Research by Trachsel *et al*. (2013) confirm that the production of fine and long roots, as well as their distribution in the soil, contributes to transporting more nutrients due to drought or low nitrogen deposition. Root development is additionally influenced by the form of nitrogen, which, with the participation of microorganisms, is converted into an oxidized and reduced readily available nitrate form (Philippot *et al*., 2007).

Depending on the availability of certain soil nutrients, the activity of microorganisms can vary significantly (Razaq *et al*., 2017). In particular, excessive nitrogen fertilization can affect the respiration processes, competitiveness, diversity and biomass of microorganisms as well as change their life strategies (Zhou *et al*., 2015). It is known from research that soil microbiota is mainly shaped by soil properties such as nutrient availability, pH, salinity and soil moisture, as a result of which they can quickly respond to a change in environmental conditions (Li *et al*., 2014; Zhao *et al*., 2014). The abundance and activity of microorganisms, expressed by enzymatic activity, can be used to assess the quality and fertility of soils, the correctness of nutrient cycling and the changes that occur in the soil environment (Piaszczyk *et al*., 2019; Błońska *et al*., 2021).

The common beech *Fagus sylvatica* L. is one of the most widespread and socio-economically valuable species for European forest ecosystems (Kolář *et al*., 2017). The high reproductive and productive potential of beech, as well as its relatively broad ecological value, is the reason for beech planting in Central European areas affected by the decline of spruce in recent years (Ammer *et al*., 2008). The purpose of this study was to determine the effects of deposition of different nitrogen doses on the root systems of beech *F. sylvatica* seedlings and their exudates. We tried to explain how different doses of nitrogen could affect the assimilation of nutrients necessary for seedling growth, as well as the morphology of fine roots. We assumed that the nitrogen doses applied would affect the morphology of the roots and their exudates. We suppose that as a result of the applied nitrogen doses, the degree of nutrition of beech seedlings will change.

Materials and methods

STUDY AREA AND SOIL SAMPLING. The research was carried out at the Forest Experimental Station Krynica of the Agricultural University of Kraków on the nursery in Kopciowa. The vegetation period was 164 days. The research was conducted on beech *Fagus sylvatica* seedlings fertilized with three different doses of nitrogen 0.75 kg·ha^{-1}, 2.25 kg·ha^{-1}, 4.5 kg·ha^{-1} and a control variant. Each variant contained 5 replicates (20 plots). The experiment was conducted from May to September 2021. In May, beech *F. sylvatica* seeds were sown into the substrates in plastic boxes. A standard mixture of fir-spruce sawdust and high peat at a ratio of 1:1 was used as a substrate. We used the same substrate in all experimental variants. In July, the first series of exudates secreted by the root systems over 24 h was taken, in which dissolved organic carbon was determined. The exudates were collected using a culture-based cuvette system (Philips *et al*., 2008). A second series was taken at the end of the experiment in September. Root exudates were collected from a single branched thin section of root of similar length and branching from 5 seedlings in each variants. The extracted solutions were analysed using a Shimadzu TOC-Total Organic Carbon analyzer (Shimadzu, Japan). In parallel with the exudates, root systems were sampled, 5 from each variant for the determination of basic root parameters. In addition, substrates were

sampled to determine enzymatic activity, enzymes involved in the cycling of C, N and P. At the end of the experiment in September, the morphology of seedling roots was analyzed in all variants and C, N, macro and micronutrient content were determined. The detailed analysis included 30 seedlings per plot (about 600 seedlings in total). In addition, the properties of the substrates were analyzed.

Root analysis. Extracted root systems collected were scanned at 400 dpi resolution and then analyzed using a WinRhizo™ Pro 2003b image analysis system (Regent Instruments Inc., Ville de Québec, QC, Canada) to determine diameter, length, and projected area. Air-dried roots were further desiccated at 65°C for 24 hours to constant weight and then weighed. Root tissue density (RTD [$kg \cdot m^{-3}$]), specific root area (SRA [$m^2 \cdot kg^{-1}$]) and specific root length (SRL [$m \cdot g^{-1}$]) were then calculated as described by Ostonen *et al.* (1999).

Chemical analysis. After drying to an air-dried state, all substrate samples were sieved through a 2-mm mesh. Physicochemical properties were determined in these prepared samples (Ostrowska *et al.*, 1991). pH was determined by the potentiometric method in water and 1M KCl. Hydrolytic acidity (Y) was determined by the Kappen method. Total nitrogen and carbon content was determined using a LECO CNS True Mac Analyser (Leco, St. Joseph, MI, USA). The cation concentrations (Ca^{2+}, Mg^{2+}, K^+, and Na^+) were extracted with ammonium acetate and determined through inductively-coupled plasma analysis (ICP-OES Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, UK). Soil samples for the determination of enzymatic activity were stored at 4°C. The activity of four extracellular enzyme [β-glucosidase (BG), β-D-cellobiosidase (CB), N-acetyl-β-D-glucosaminidase (NAG), phosphatise (PH)] were determined using fluorogenically labelled substrates (Pritsch *et al.*, 2004; Turner, 2010; Sannaullah *et al.*, 2016). Fuorescence was measured on a multi-detection plate reader (Biotek Synergy) with excitation at 355 nm and emission at 460 nm wavelengths.

In the leaves and roots samples, the concentration of macro and microelements was determined by an ICP (ICP-OES Thermo iCAP 6500 DUO, Thermo Fisher Scientific, Cambridge, U.K.). Dried samples of leaves and roots were mineralized in a mixture of HNO_3 and $HClO_4$ (3:1). Carbon (C) and nitrogen (N) in the leaves and roots samples were measured with an elemental analyzer (LECO CNS TrueMac Analyzer (Leco, St. Joseph, MI, USA)).

Statistical analysis. Spearman correlation coefficients between individual root parameters and root exudates were calculated. Principal component analysis (PCA) was used to evaluate the relationship between root parameters and nitrogen doses. In addition, a cluster analysis was performed. Regression lines were used to test the relationship between root exudates and the SRL parameter. A *post-hoc* test was used to evaluate differences between the mean values of the traits. Results were considered statistically significant at $\alpha<0.05$. All statistical analyses were carried out using R statistical software (R Core Team, 2022), R Studio (RStudio Team, 2022) and Statistica 13 software (Tibco Software Inc., 2017).

Results

Root analyses. Between the first and second series of sampling, there were no statistically significant differences between exudation rate and nitrogen dose. The amount of carbon exuded from the roots differed in the second series of sampling. This series showed statistically significant differences between the rate of 2.25 $kg \cdot ha^{-1}$ and 4.5 $kg \cdot ha^{-1}$ (Fig. 1). Root length differed significantly in the second series. The longest root systems were recorded at the 0.75 $kg \cdot ha^{-1}$ rate and the shortest at the 4.5 $kg \cdot ha^{-1}$ rate. Root length varied between 823.03 and 602.38 cm.

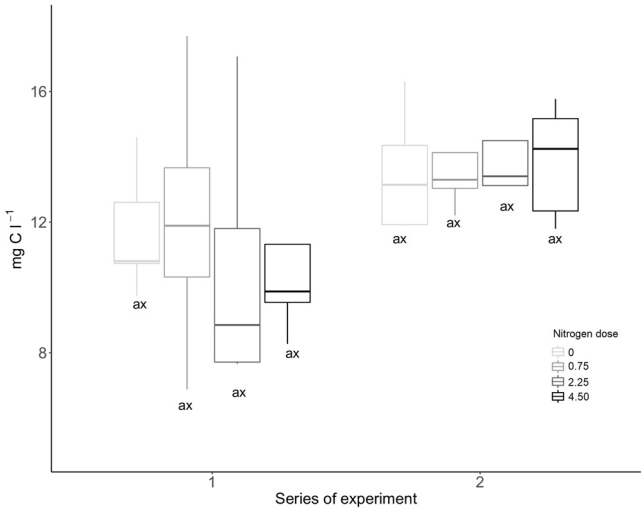

Fig. 1.

The change of total root-exuded carbon (mg C l^{-1}) over time depending on the nitrogen fertilization variant
different lowercase letters (a, b, c) indicate significant differences in parameters between the different nitrogen fertilization; different lowercase letters (x, y) indicate significant differences in parameters between the series; *post-hoc* $p<0.05$

The diameter and weight of roots in the first series of sampling shows no statistically significant differences between the applied doses, while in the second series of sampling the weight of roots differs according to the applied nitrogen dose especially between the dose of 4.5 kg·ha^{-1} and 2.25 kg·ha^{-1}. In the second series of the experiment, SRA differences were recorded between the dose of 2.25 kg·ha^{-1} and 4.5 kg·ha^{-1}. For SRL in the second series of the experiment, differences were recorded at the dose of 0.75 kg·ha^{-1} and 2.25 kg·ha^{-1}. RTD at a dose of 4.5 is significantly different from the other doses (Table 1). Statistical analyses confirmed a significant relationship between the amount of exudates and root parameters. A strong positive correlation was noted between the amount of exudates and SRL and SRA (Figs. 2, 3). A negative statistically significant correlation was found for length, diameter, dry weight, RTD and exudation rate. A strong positive correlation was noted between SRA and SRL (Fig. 2). Cluster analysis carried out taking into account exudation rate, root tissue density (RTD) and specific root length (SRL) distinguished three groups associated with different nitrogen dose (Fig. 4). Factors 1 and 2 distinguished in the PCA analysis together explained 57.8% of the variance in the traits studied (Fig. 5). Factor 1 explained 41.3 % of the variance, factor 2 explained 16.5 % of the variance of the studied parameters. Factor 1 was related to root characteristics and exudation rate, while factor 2 was mainly related to the variant of the experiment.

Chemical analyses. As for the basic physicochemical properties of the substrates used in the experiment, no significant differences were shown as an effect of the applied fertilization (Table 2). The pH values in H_2O as well as in KCl showed no significant differences between the variants included in the experiment. Hydrolytic acidity ranged from 2.30 cmol(+)·kg^{-1} to 2.88 cmol(+)·kg^{-1} for the 0.75 kg·ha^{-1} and 2.25 kg·ha^{-1} application rate, respectively. Exchangeable acidity ranged from 2.18 cmol(+)·kg^{-1} to 2.74 cmol(+)·kg^{-1}, for the 0.75 kg·ha^{-1} application rate and the control variant. For acidity, no statistically significant differences were shown. The amount of accumulated nitrogen and carbon showed little variation between doses. The highest C/N ratio was recorded for the 0.75 kg·ha^{-1} dose and the lowest for the 2.25 kg·ha^{-1} dose.

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Table 1.

Mean morphological characteristics of roots and exudation rate [mg C g-1 day-1] in different nitrogen fertilization variant

Series of experiment	Nitrogen dose	Exudation rate	Length [cm]	Diameter [mm]	Weight [mg]	SRA [m^2 kg^{-1}]	SRL [m kg^{-1}]	RTD [kg m^{-3}]
1	0	0.41±0.08ax	353.21±60.49ax	0.21±0.03ax	206.16±36.66ax	11.20±1.19ax	173.48±28.57ax	175.04±27.53ax
	0.75	0.40±0.11ax	402.44±122.54ax	0.22±0.02ax	217.72±50.58ax	12.97±2.94ax	189.22±55.40ax	145.17±31.14ax
	2.25	0.42±0.09ax	331.57±87.36ax	0.22±0.03ax	182.72±47.86ax	12.62±2.24ax	188.52±51.13ax	149.90±28.67ax
	4.50	0.48±0.15ax	389.33±155.66ax	0.21±0.02ax	198.46±80.83ax	13.90±2.96ax	201.71±42.07ax	140.48±17.19ax
2	0	0.21±0.06aby	621.73±389.15ax	0.24±0.02ax	475.24±104.60abx	9.31±3.76abx	127.87±57.26ax	201.94±61.00ax
	0.75	0.13±0.05by	823.03±201.80ay	0.25±0.02ax	798.12±212.92ay	8.19±2.16aby	107.20±34.65ay	207.48±42.53ay
	2.25	0.16±0.06aby	620.64±212.06ay	0.27±0.03ay	723.72±381.85aby	7.69±1.66by	94.00±32.61ay	198.27±18.25ay
	4.50	0.24±0.10ay	602.38±97.27ay	0.27±0.04ay	443.38±156.85by	12.15±3.34ax	144.91±35.08ax	134.44±49.10bx

Mean ±SD; exudation rate [mg C g^{-1} day^{-1}], specific root area (SRA), specific root length (SRL), root tissue density (RTD); different lowercase letters (a, b, c) indicate significant differences in parameters between different nitrogen doses; letters (x, y, z) indicate significant differences in parameters between series; *post-hoc* $p<0.05$)

Table 2.

Basic properties and enzymatic activity [nmol MUB.g-1 dry soil .h-1] of soils in which beech seedlings grew after the completed experiment

Nitrogen dose	pH H_2O	pH KCl	Hh	Hex	N	C	C/N	Al	CB	BG	NAG	PH
0	4.87±0.08	4.01±0.05	2.92±0.36	2.74±0.35	0.79±0.05	46.31±1.23	59.1±5.7	0.17±0.02	19.94±30.68ab	295.04±155.02	339.49±102.07a	3317.38±494.98ab
0.75	4.91±0.18	4.07±0.19	2.30±0.43	2.18±0.41	0.76±0.05	46.93±1.16	62.0±6.0	0.12±0.03	34.54±41.94a	204.95±96.39	254.78±45.45b	2975.89±1028.25b
2.25	4.92±0.06	4.03±0.10	2.88±0.46	2.73±0.41	0.80±0.03	46.82±0.73	58.3±1.6	0.15±0.06	24.36±14.00ab	287.05±93.30	372.72±83.82a	3859.47±619.73a
4.50	4.89±0.12	4.05±0.12	2.69±0.56	2.55±0.54	0.79±0.02	46.59±1.19	59.0±2.5	0.14±0.03	2.24±5.01b	187.56±145.03	232.48±87.40b	2099.90±807.20c

Mean ±SD; Hh – hydrolytic acidity [cmol(+)·kg^{-1}], Hex – exchangeable acidity [cmol(+)·kg^{-1}]; C, N, C/N [%]; CB – cellobiosidase, BG – ß-glucosidase, NAG – N-acetyl-ß-D-glucosaminidase, PH – phosphatase; small letters in the upper index of the mean values (a, b, c) mean significant differences in properties between nitrogen doses

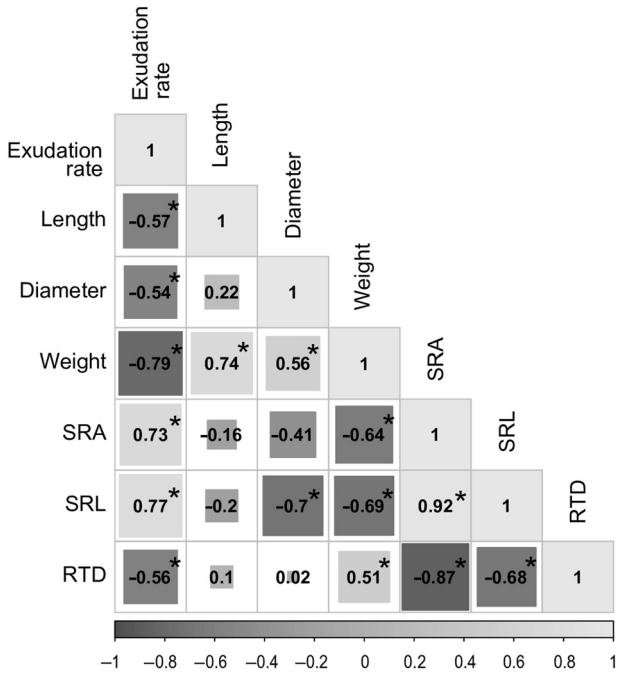

Fig. 2.

Correlation between different root parameters, exudation rate and enzyme activity

RTD – root tissue density, SRA – specific root area, SRL – specific root length, CB – cellobiosidase, BG – β-glucosidase, NAG – N-acetyl--β-D-glucosaminidase, PH – phosphatase; *p<0.05

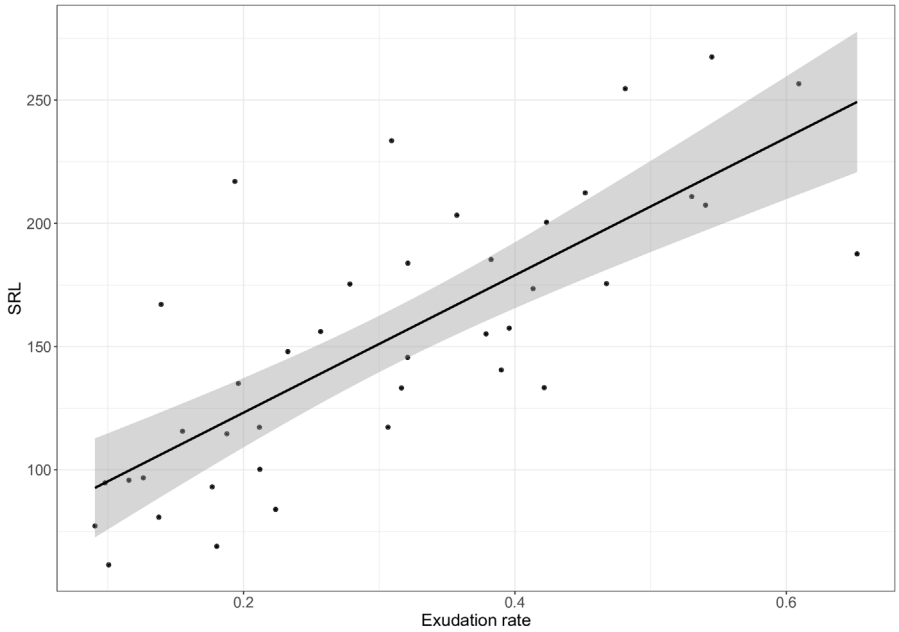

Fig. 3.

The relationship between root exudation rate (mg C g^{-1}·day^{-1}) and specific root length (SRL, m^2·kg^{-1})

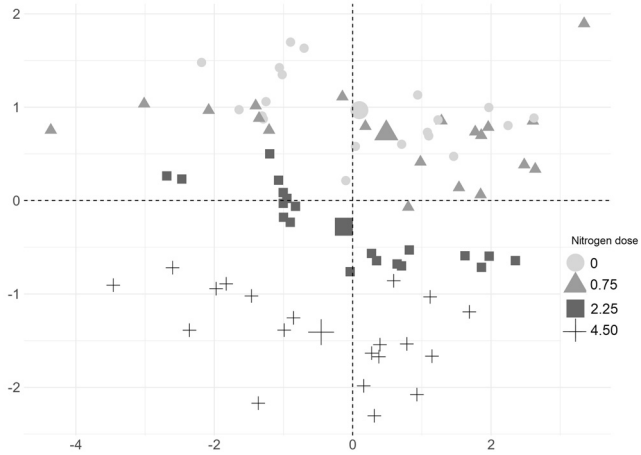

Fig. 4.

Cluster analysis carried out taking into account exudation rate, root tissue density (RTD) and specific root length (SRL)

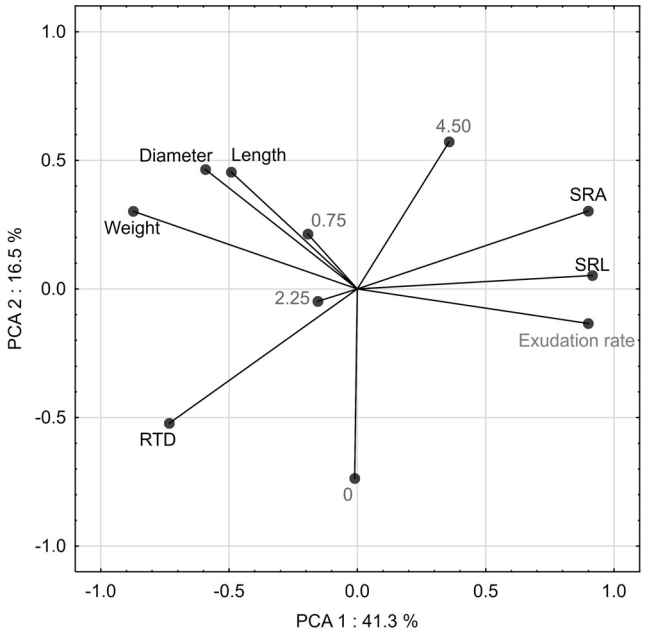

Fig. 5.

Projection of variables on the plane of the first and second PCA factors (root tissue density (RTD), specific root area (SRA), specific root length (SRL), exudation rate, nitrogen doses (0, 0.75, 2.25, 4.5)

In the case of the activity of the enzymes tested, differences were noted between the variants of the experiment. The lowest activity of the four analyzed enzymes was found at the application rate of 4.5 kg·ha^{-1}. In the case of CB, significantly higher activity was recorded in the variant with the application rate of 0.75 kg·ha^{-1}, and the lowest activity was recorded at the application rate of 4.5 kg·ha^{-1} (Table 2). In the case of BG, there was no effect of the applied fertilization variants.

Table 3.
Content of basic elements in leaves, roots and substrate depending on the variant of nitrogen fertilization

	Nitrogen dose	N	C	C/N	Ca	K	Mg	Na	P
Leaves	0	0.82±0.1b	46.60±0.3	57.2±5.2a	8682.5±647.3a	1804.3±361.8b	2600.7±197.0a	453.5±37.6	267.0±58.2b
	0.75	0.86±0.2b	46.97±0.7	58.1±15.7a	8148.0±1100.1ab	1886.1±958.2b	2509.9±239.8a	482.1±102.3	271.9±67.3b
	2.25	1.31±0.2a	46.34±0.7	36.1±5.5b	7034.1±122.5ab	3243.4±359.9a	2238.8±88.2b	479.2±63.9	387.2±63.9a
	4.5	1.21±0.2a	46.37±1.3	39.0±4.9 b	6413.8±766.5b	3405.7±837.1a	2159.5±159.5b	416.0±51.4	414.0±42.9a
Roots	0	0.59±0.2ab	44.98±1.6	80.4±18.0ab	1534.5±229.5b	2958.4±322.4	1237.3±205.9b	357.8±118.5	365.6±31.8
	0.75	0.58±0.1b	46.12±0.6	82.4±17.8a	1969.7±133.5a	3064.8±382.5	1488.3±201.7ab	468.8±49.7	407.2±45.0
	2.25	0.73±0.6ab	45.80±1.2	63.8±10.8ab	1926.6±383.1a	3298.7±125.8	1531.6±177.9a	467.3±47.2	366.0±47.8
	4.5	0.82±0.6a	45.45±0.9	57.5±12.5b	1967.4±277.2a	3193.9±287.7	1423.5±204.4ab	441.2±116.2	359.0±38.9

Mean ±SD; C, N [%]; Ca, K, Mg, Na, P [mg/kg]; small letters in the upper index of the mean values (a, b, c) mean significant differences in properties between nitrogen doses

In the case of NAG, significantly the highest activity was recorded in the variant with a dose of 2.25 kg·ha^{-1}. PH activity varied significantly between the variants tested. Significantly the highest PH activity was recorded in the experimental variant at 2.25 kg·ha^{-1}. The lowest PH activity was recorded in the variant with a dose of 4.5 kg·ha^{-1}.

The elements content of the leaves and roots of the seedlings differed significantly depending on the nitrogen dose applied (Table 3). In the case of leaves, the lowest nitrogen concentration was recorded in the control variant, and the highest in the 2.25 kg·ha^{-1} variant. Significantly the lowest nitrogen content in roots was recorded in the 0.75 kg·ha^{-1} variant, and the highest in the 4.5 kg·ha^{-1} variant. The amount of carbon accumulated in leaves and roots shows little variation. Statistically significant differences in leaves and roots were recorded for the C/N ratio. For leaves and roots, significantly the highest C/N values were recorded in the control variant and in the variant with a dose of 0.75 kg·ha^{-1}. Ca content in leaves ranged from 6413.8 mg/kg for the dose of 4.5 kg·ha^{-1}, to 8682.5 mg/kg for the control variant. For leaves, the highest Ca contents were noted in the control variant and the variant with the lowest nitrogen rate. In the case of roots, the lowest Ca content was recorded in the control variant. The nitrogen doses used in the experiment resulted in changes in the Mg content of the leaves and roots of the tested seedlings. Leaves and roots in variants with higher nitrogen doses, *i.e.*, 2.25 kg·ha^{-1} and 4.5 kg·ha^{-1}, were characterized by significantly higher Mg content. The highest K and P contents in leaves were recorded in the 4.5 kg·ha^{-1} variant, and the lowest in the control variant (Table 3). No significant differences were noted for K and P contents in roots. Na content in leaves and roots was not statistically significantly different between the variants of the experiment (Table 3).

Discussion

The study confirms the effect of deposition of different doses of nitrogen on the root systems of beech *Fagus sylvatica* seedlings and their exudates. The amount of carbon secretion in our experiment ranged from 0.13 to 0.48 mg C g^{-1}·day^{-1} (Table 1).

Similar values of root exudates for the genus *Fagus* were shown in the study by Brzostek *et al.* (2013), the values ranged from 0.35-1.10 mg C g^{-1}·day^{-1}. Higher availability of N in the soil may affect the release of more C by root exudates, which consequently leads to stimulation of microbial activity and acceleration of N transformation (Wang *et al.*, 2021). In our study, there were no significant changes in the physical and chemical properties of the substrate in which beech seedlings grew. As a result of the applied nitrogen doses, changes in the enzymatic activity were observed. It is known that nitrogen directly or indirectly modifies the composition of soil microorganisms, affecting enzymatic activity (Klironomos *et al.*, 2011; Wang *et al.*, 2019). As a result of long-term nitrogen fertilization, there is an accumulation of C in the soil, which leads to a decrease in the rate of decomposition of organic matter and thus to a decrease in the activity of enzymes involved in the decomposition of lignin (Morrison *et al.*, 2018). From a physiological point of view, in the era of climate change, the acceleration of soil N cycling driven by root exudates may be a mechanism regulating forest productivity (Phillips *et al.*, 2012; Wang *et al.*, 2021). Authors of other studies repeatedly point to root exudates as having a significant impact on nutrient cycling and microbial activity (Brzostek *et al.*, 2013; Sun *et al.*, 2021). The differences in the amount of secreted C with exudates between the first and second series of the experiment may be the result of seasonal variation in the rate of C secretion. At the end of the growing season, the rate of physiological processes taking place inside the plant changes due to the slow transition into dormancy caused by lower temperatures (Heide, 1993; Liu *et al.*, 2015). In our study, we showed a strong positive correlation between the amount of root exudates and root parameters such as SRA and SRL. Previous studies also demonstrated that the amount of root exudates is strongly correlated with root morphological characteristics (Yin *et al.*, 2013; Sun *et al.*, 2017; Staszel *et al.*, 2022a, b). Root systems exhibiting higher SRL parameters tend to have more fine roots, which directly translates into root exudates (Ma *et al.*, 2018). Since N is one of the most important components necessary for plants, it directly affects their growth characteristics by increasing shoot and root biomass (Harper, 1974; Zhao *et al.*, 2008). In our study, we found higher SRA and SRL with lower RTD as an effect of increasing the nitrogen rate. Similar relationships were shown by Costa *et al.* (2002) and Makita *et al.* (2012), the higher the nitrogen availability the higher the root area.

The applied fertilization with different doses of nitrogen led to an increase in its content in both leaves and roots of beech seedlings. Significantly higher contents of this element were associated with the highest fertilization doses. According to Zhu *et al.* (2016), N deposition can modify nutrient availability in soils, which consequently is reflected in plant health. In our study, we confirmed the importance of N fertilization on the content of basic components such as Ca, K, Mg and P. In the leaves of the studied seedlings, there was an increase in the content of K and P as a result of fertilization with higher doses of nitrogen. Higher concentrations of N, P and K and, at the same time, lower concentrations of Ca in the leaves of seedlings, were observed in the study of Lambers and Oliveira (2019). For Ca and Mg content in roots, higher values were observed in the fertilized variants compared to the control. Leaves and roots differ in function, but are most commonly used to monitor the effects of nutrient availability and long-term environmental changes on tree nutrition (Adams and Hutchinson, 1992; Duquesnay *et al.*, 2000). According to Vesala *et al.* (2021) nitrogen deposition can affect nutrient cycling. Nutrient allocation in roots can depend on the morphological characteristics of the roots and on their extent (Zhao *et al.*, 2020). In our study, we noted differences in the characteristics of root systems depending on the nitrogen dose which, consequently, may affect the differences in nutrient content in the roots of beech seedlings. In the leaves and roots of seedlings treated with the highest nitrogen

doses, there was a reduction in the value of the C/N ratio. The C/N ratio in leaves can be linked to important ecological processes and the ability to adapt to environmental stresses (Woods *et al*., 2003). C/N ratios in leaves depend on differences in plant physiology (Reich and Oleksyn, 2004). According to Sheng *et al*. (2021) human-induced nitrogen addition can reduce the C/N ratio of leaves by increasing soil nitrogen availability. Zhao *et al*. (2022) highlight the differential responses of fine root traits to N deposition. A global increase in nitrogen (N) deposition affects the underground allocation of plant photosynthesis and the formation of rhizosphere-associated roots and symbionts, as well as the availability of nutrients in the soil, thereby affecting nutrient acquisition by trees (Ma *et al*., 2021).

Conclusions

- The study showed that the applied doses of nitrogen fertilization influenced the characteristics of the root systems and, consequently, the amount of carbon secreted by the root systems with their exudates.
- In research a strong positive correlation was noted between the amount of C from exudates and SRL (specific root length) and SRA (specific root area).
- Our study confirmed the importance of nitrogen fertilization in shaping the nutrition of beech seedlings. There was a significant increase in N content in the leaves and roots of the tested seedlings as a result of higher doses.
- The results indicate that beech can be used as a plastic species that adapts well to conditions of increased nitrogen deposition.

Supplementary materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

Authors' contributions

K.S., E.B., J.L. – conceived and designed the investigation; analysed and visualised the data; E.B., J.L., M.K. – concepts research methodology; K.S., E.B., J.L., M.K. – preparation of manuscript.

Conflicts of interest

The authors declare the absence of potential conflicts of interest.

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STRESZCZENIE

Wpływ depozycji azotu na systemy korzeniowe i wydzieliny siewek buka *Fagus sylvatica* L. w klimacie umiarkowanym

Wielkość systemu korzeniowego wpływa na pobieranie wody i ilości składników pokarmowych, od czego zależy stan odżywienia sadzonek. Systemy korzeniowe, zwłaszcza korzenie drobne, odgrywają ważną rolę w kształtowaniu właściwości gleb. Celem badań było określenie wpływu różnych dawek azotu na systemy korzeniowe siewek buka zwyczajnego *Fagus sylvatica* L. i ich wydzieliny. Próbowano wyjaśnić, w jaki sposób różne dawki azotu mogą wpływać na przyswajanie składników pokarmowych niezbędnych do wzrostu siewek, a także na morfologię drobnych korzeni. Doświadczenie przeprowadzono w szkółce w Kopciowej na terenie Leśnego Zakładu Doświadczalnego Uniwersytetu Rolniczego w Krakowie. W badaniach uwzględniono 4 warianty doświadczenia: 3 różne dawki azotu (0,75 kg·ha^{-1}, 2,25 kg·ha^{-1}, 4,5 kg·ha^{-1}) oraz wariant kontrolny bez azotu. Doświadczenie prowadzono od maja do września 2022 r. Siewki wraz z systemami korzeniowymi oraz ich wydzielinami zostały pobrane dwukrotnie: na początku i na końcu doświadczenia. W wydzielinach korzeniowych oznaczono zawartość węgla organicznego. Systemy korzeniowe poddano szczegółowej analizie z wykorzystaniem programu WinRhizo. Na podstawie uzyskanych parametrów korzeni określono gęstość systemów korzeniowych RTD [kg·m^{-3}], właściwą powierzchnię korzeni SRA [m^2·kg^{-1}] i właściwą długość korzeni SRL [m·g^{-1}]. W nadziemnej i podziemnej części siewek oznaczono zawartość mikro- i makroskładników. Ponadto określono podstawowe właściwości chemiczne oraz aktywność enzymatyczną substratu, w którym wzrastały siewki (tab. 2). W badaniach wykazano, że wyższa dawka azotu wpływała na większą ilość węgla uwalnianego z wysiękami z korzeni drobnych, co było związane z ogólną morfologią korzeni (ryc. 1). Stwierdzono silną dodatnią korelację między ilością węgla z wysięków a SRL i SRA (ryc. 2). Dodatkowo analizy statystyczne potwierdziły istotną zależność pomiędzy ilością wysięków a parametrami korzeni (ryc. 3). Badania wykazały, że zastosowanie różnych dawek azotu miało wpływ na charakterystykę systemów korzeniowych, a co za tym idzie na ilość węgla wydzielanego przez systemy korzeniowe wraz z ich wysiękami (ryc. 4-5). Ponadto badania potwierdziły znaczenie nawożenia azotem w kształtowaniu odżywienia sadzonek buka (tab. 1). W wyniku stosowania wyższych dawek nastąpił znaczny wzrost zawartości N w liściach i korzeniach badanych sadzonek (tab. 3). Uzyskane wyniki wskazują, że buk może być wykorzystany jako gatunek plastyczny, który dobrze adaptuje się do warunków zwiększonej depozycji azotu.

Oświadczenie o udziale autorów w publikacji / Declaration of author contribution

Staszel K., Błońska E., Lasota J. 2022a. Fine root morphology and soil properties under influence of different tree stands along an altitudinal climosequence in the Carpathian mountains. Forest Ecosystems, 9, 100066.

Oświadczam, że mój wkład polegał na sformułowaniu problemu badawczego, zaprojektowaniu eksperymentu, przeprowadzeniu doświadczenia w terenie oraz zebraniu próbek, przygotowaniu próbek do analiz laboratoryjnych, opracowaniu i interpretacji wyników, przygotowaniu wstępnej wersji manuskryptu i edycji ostatecznej wersji manuskryptu, nadzór nad procesem publikacyjnym.

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Udział / Contribution: 60%
mgr inż. Karolina Staszel-Szlachta .....

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Udział / Contribution: 30%
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Oświadczenie o udziale autorów w publikacji / Declaration of author contribution

Staszel K., Lasota J., Błońska E. 2022b. Soil Organic Matter Fractions in Relation to Root Characteristics of Different Tree Species in Altitude Gradient of Temperate Forest in Carpathian Mountains. Forests, 13(10), 1656.

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Oświadczenie o udziale autorów w publikacji / Declaration of author contribution

Staszel-Szlachta K., Lasota J., Szlachta A. Błońska E. 2024. The impact of root systems and their exudates in different tree species on soil properties and microorganisms in a temperate forest ecosystem. BMC Plant Biol 24, 45 doi.org/10.1186/s12870-024-04724-2

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mgr inż. Karolina Staszel-Szlachta .....

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Oświadczenie o udziale autorów w publikacji / Declaration of author contribution

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Oświadczenie o udziale autorów w publikacji / Declaration of author contribution

Staszel-Szlachta K., Lasota J., Kempf M., Błońska E. 2022d. Effect of nitrogen deposition on root systems and exudates of seedlings of beech Fagus sylvatica L. in a temperate climate. Sylwan, 166(12).

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