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Praca doktorska

**Różnorodność taksonomiczna i funkcjonalna, jako czynniki
determinujące respirację podłoża w płatach spontanicznej
roślinności na zwałach skały płonnej**

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Ewie i Janowi którzy niezmiennie we mnie wierzyli i wspierali

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1. Wstęp

Różnorodność biologiczna, w tym przede wszystkim różnorodność taksonomiczna oraz funkcjonalna, roślin i roślinności jest zależna od abiotycznych i biotycznych warunków siedliskowych, które wpływają również na przebieg procesów ekosystemowych m.in., tak kluczowych jak respiracja gleby. Zagadnienia te pozostają od dawna istotnym przedmiotem badań nauk przyrodniczych (Prach i Walker, 2020; Rotherham, 2017). Pewne aspekty relacji między warunkami siedliskowymi, różnorodnością taksonomiczną i funkcjonalną a procesami oddychania gleby zostały częściowo poznane dla niektórych ekosystemów naturalnych, półnaturalnych oraz w niektórych agrocenozach (Ayub i in., 2014; Atkin i in., 2007; Baker i in., 1997; Grime i in., 1988). Badania nad intensywnością oddychania gleby przeprowadza się w rolnictwie, na polach uprawnych, użytkach zielonych i lasach użytkowych (Xiao i in., 2021).

Dotychczasowy stan wiedzy wskazuje, że warunki abiotyczne (wilgotność, temperatura, tekstura gleby, mikro- i makroelementy, pH gleby, zawartość azotu) mogą wpływać na przebieg procesu respiracji (Fenn i in., 2010; Mo i in., 2008; Heinemeyer i in., 2007; Bouma i Bryla, 2000;). Wilgotność gleby, temperaturę i strukturę granulometryczną uważa się za czynniki wpływające na oddychanie gleby (Bouma i in., 1997a). Badania przeprowadzone na gruntach rolnych wykazały, że zmiany wilgotności gleby mogą wpływać na tempo oddychania w drobnoziarnistej glebie piaszczystej. W siedliskach podmokłych uwalnianie CO₂ z gleby jest ograniczone. Konieczne jest jednak pełne zrozumienie powiązań między architekturą korzenia, teksturą gleby, wilgotnością i oddychaniem (Bouma i Bryla, 2000; Scheurwater i in., 1998; Burton i in., 1997; Bouma i in., 1997b, a; Koizumi i in., 1991). Drobne frakcje w glebie wspomagają zdolność do zatrzymywania wody. Udział dużych frakcji w strukturze granulometrycznej gleby, umożliwia przepływ powietrza i wody, ich wymianę pomiędzy sferą – pedosferą (glebą) i atmosferą. Bouma i Bryla (2000) badali powiązania pomiędzy heterotroficznym i autotroficznym oddychaniem i uwalnianym CO₂ do gleby, w gradiencie wilgotności oraz różnych warunkach tekstury gleby. Tekstura gleby znacząco wpływa na wiązanie wody w glebie (Singer i Munns, 1991). Wykazano, że w pewnym zakresie wartości tempa oddychania gleby jest w dużym stopniu zależne od temperatury i wilgotności gleby (Schlentner i Van Cleve, 1985; Singh i Gupta, 1977). Kolejnymi czynnikami, które mogą wpływać na tempo oddychania gleby jest dostępność substratów węgla dla mikroorganizmów (Seto i Yanagiya, 1983), oraz gęstość korzeni roślin i aktywność fauny glebowej (Ben-Asher i in., 1994), (Singh

i Shukla, 1977), właściwości fizyczne i chemiczne gleby (Boudot i in., 1986) w tym wspomniana wcześniej wilgotność gleby (Lukeno i Billings, 1985; Moore i Knowles, 2011).

Poza wpływem czynników abiotycznych badacze zwracają uwagę na istotną rolę czynników biotycznych w przebiegu procesu respiracji. Rozkład związków węgla w materii organicznej gleby (SOM) jest związany m.in. ze składem gatunkowym roślin, składem mezofauny i aktywnością enzymów glebowych (Sinsabaugh i Antibus, 1991). Różnorodność składu gatunkowego roślin przyczynia się do zwiększenia ilości i różnorodności zasobów pokarmowych w glebie, co stwarza oraz poszerza zakres różnorodności nisz dla saprofitów (Chapman i Newman, 2010; Eisenhauer i in., 2010; Hooper i in., 2005). Zróżnicowany skład gatunkowy roślin zapewnia różnorodną biomasę, co przyspiesza rozkład materii organicznej poprzez komplementarne wykorzystanie zasobów przez mikroorganizmy (Handa i in., 2014). Wśród czynników biotycznych fauna glebowa jest ważnym elementem. Uważa się, że udział nicieni w oddychaniu gleby jest prawdopodobnie mniejszy niż 1%, mogą one jednak odgrywać ważną rolę w obiegu składników odżywczych w glebie, wpływając na wzrost bakterii i dostępność składników odżywczych dla roślin (Eisenhauer i in., 2010; Smulczak i Tracz, 2008). Wszystkie gatunki wazonkowców *Enchytraeidae*, poprzez intensywne procesy oddychania, mają znaczący wkład w mineralizację materii organicznej. *Enchytraeidae* stymulują przebieg procesów mineralizacji i humifikacji, m.in. poprzez spulchnianie gleby, rozbijanie cząstek gleby, ich przemieszczanie się w profilu glebowym oraz katalizowanie aktywności mikroorganizmów. Wkład *Enchytraeidae* w te procesy, a także w kształtowanie prawidłowej struktury i zwiększanie żyzności gleb, jest często znacznie większy niż innych przedstawicieli makro- i mezofauny glebowej (Handa i in., 2014; Kasprzak i in., 1981).

Liczna grupa badaczy wskazuje na rolę składu taksonomicznego i funkcjonalnego roślinności spontanicznej na przebieg procesu respiracji gleby. Przez długi czas różnorodność roślinności charakteryzowana była z wykorzystaniem analiz różnorodności taksonomicznej składu gatunkowego badanych płatów. Ostatnio, różnorodność funkcjonalna stała się dodatkowym obszarem badania różnorodności roślin, który pozwala na poznanie wybranych aspektów funkcjonowania ekosystemów (McGill i in., 2006), takich jak np. produkcja pierwotna netto (Duffy i in., 2017; Liang i in., 2016), sekwestracja węgla (Tilman i in., 2006) oraz obiegu składników odżywczych (Handa i in., 2014). Jednak to, w jaki sposób dynamika różnorodności roślin wpływa na uwalnianie CO₂, w ekosystemach jest wciąż bardzo słabo poznane (Hillebrand i Matthiessen, 2009; Loreau i Hector, 2001). Związek między różnorodnością gatunkową roślinności a respiracją gleby może być bezpośredni lub pośredni i dokonywać poprzez czynniki glebowe (takie jak zawartość wody w glebie, temperatura,

materia organiczna oraz składniki odżywcze) (Metcalf i in., 2011). Przez długi czas badacze skupiali się nad ustaleniem relacji między różnorodnością roślinności a parametrami produktywności i ilością biomasy. Drugim często badanym aspektem funkcjonowania ekosystemu jest obieg składników odżywczych w glebie (Duffy i in., 2017; McKee, 1970). Zarówno produktywność, jak i rozkład materii organicznej są związane z parametrami procesu oddychania gleby (Handa i in., 2014). Jednak do tej pory mniej uwagi poświęcono temu, jak różnorodność roślin wpływa na proces oddychanie gleby.

Wymienione powyżej zależności zidentyfikowane zostały w ekosystemach naturalnych i półnaturalnych. Niewiele uwagi poświęcono badaniom relacji między roślinnością a parametrami funkcjonowania ekosystemów w siedliskach zaburzonych. Aby uzupełnić tę lukę, w niniejszej pracy podjęto próbę ustalenia czynników wpływających na dynamikę uwalniania CO₂ z podłoża mineralnego hałd pokopalnianych porośniętych przez spontaniczną roślinność. Analiza intensywności emisji CO₂ jest kluczowa dla zrozumienia funkcjonowania ekosystemów oraz ich reakcji na zmiany czynników biotycznych i abiotycznych, a także dla poznania wpływu różnorodności roślinności.

Działalność górnictwa surowców mineralnych stwarza nowe wyzwania powstających ekosystemom (Błaszowski i in., 2019; Kałucka i Jagodziński, 2016). Górnictwo powoduje usuwanie istniejącej roślinności oraz zmianę składu i struktury gleby, wpływając na warunki hydrologiczne (Prach i Walker, 2019; Woźniak i in., 2018; Řehouňková i in., 2016; Tropek i in., 2012; Frouz i Jílková, 2008; Kasprzak i in., 1981). Działalność człowieka w niektórych siedliskach i ekosystemach powoduje fundamentalne zmiany wykraczające poza znane dotychczas relacje warunków biogeochemicznych siedlisk. Nieznane dotychczas warunki siedliskowe powodują zrzeszanie się gatunków i kształtowanie się zbiorowisk roślinnych o tzw. *non-analogous species composition*. W konsekwencji, nowe, nieznane wcześniej warunki siedliskowe, prowadzą do powstania różnorodnych płatów roślinności o odmiennym od znanych składzie gatunkowym, co prowadzi do powstania układów typu *Novel Ecosystem* (Zhang i in., 2022; Błońska i in., 2019; Rotherham, 2017; Bradford i in., 2010; Buchmann, 2000). Warunki siedliskowe mineralnych podłoży na hałdach kopalnianych są bardzo zróżnicowane pod względem wilgotności, skład granulometryczny podłoża i zasolenia, nachylenie zboczy, wysokości i kształcie zwału. Rośliny i inne organizmy kolonizują podłoże pozbawione gleby, które stanowi mineralny substrat glebowy o bardzo różnej strukturze frakcji, wilgotności i innych parametrach. Różnorodność siedlisk w obrębie jednej hałdy kopalni węgla kamiennego, jest często znacznie większa niż między dwiema, lub większą liczbą hałd. Mozaika mikrosiedlisk (np. wielkość ziaren, wilgotność, zasolenie) znajduje odzwierciedlenie

w mozaice składu gatunkowego roślinności (Woźniak i in., 2021; Błońska i in. 2019; Mo i in., 2008; Vargas i Allen, 2008; Kasprzak i in., 1981).

Przebieg procesów naturalnych oraz funkcjonowanie układów typu *Novel Ecosystems* jest dotychczas słabo poznane (Doley i Audet, 2013; Hobbs i in., 2013, 2009, 2006). Według tych badaczy układy przyrodnicze typu *Novel Ecosystems* powstają na siedliskach, w których doszło do takiego zaburzenia warunków siedliskowych (*crossing of ecological threshold*), że powrót do poprzedniego stanu nie jest już możliwy. W takich miejscach reguły grupowania się organizmów (*assembly rules*) zostały dostosowane przez działanie procesów naturalnych do zaistniałych, odmiennych, często ekstremalnych warunków siedliska abiotycznego (Evers i in., 2018; Rotherham, 2017 Dooling, 2015; Harris i in., 2006). Oddychanie gleby jest istotną częścią funkcjonowania ekosystemu.

Miejsca eksploatacji surowców mineralnych reprezentują nowo powstałe siedliska, które znacznie różnią się od warunków siedliskowych ekosystemów naturalnych i półnaturalnych. Niektóre badania wykazały, że poza roślinami również zwierzęta intensywnie kolonizują siedliska mineralne zrzeszając, nieanalogiczne zgrupowania gatunkowe fauny (Frouz, 2018; Tropek i in., 2012; Kowarik i in., 2011; Harris i in., 2006). Zainteresowanie tym, jak różnorodność roślinności wpływa na różne parametry funkcjonowania ekosystemu, wzrosło w ostatnich latach (Newbold i in., 2020). Jednak do tej pory mniej uwagi poświęcono zrozumieniu, w jaki sposób różnorodność roślinności może wpływać na przebieg procesu oddychania glebowego. Jednakże wciąż nie jest jasne, w jaki sposób zmiany różnorodności roślinności wpływają na intensywność oddychania gleby w naturalnych i półnaturalnych ekosystemach.

Celem pracy było przetestowanie, w jakim zakresie znane w dużej części mechanizmy funkcjonowania ekosystemów naturalnych i półnaturalnych znajdują zastosowanie w wyjaśnieniu czynników regulujących przebieg i intensywność procesu respiracji w ekosystemach powstałych na zwałach skały płonnej utworzonych po eksploatacji węgla kamiennego. Przeprowadzona zostanie kompleksowa analiza wpływu czynników biotycznych i abiotycznych na procesy uwalniania dwutlenku węgla w płatach roślinności spontanicznej występujących na mineralnym podłożu nowych ekosystemów hałd skały płonnej.

2. Hipoteza i cele pracy

Badania przeprowadzone w ramach niniejszej pracy miały na celu przetestowanie kilku hipotez, które sformułowano w celu ustalenia czynników odpowiedzialnych, za przebieg dynamiki uwalniania CO₂ z podłoża mineralnego hałd pogórnicych, porośniętego przez spontaniczną roślinność. Analiza ilości uwalniania CO₂ jest kluczowa dla zrozumienia funkcjonowania analizowanych ekosystemów oraz reakcji na zmiany biotycznych i abiotycznych warunków siedliskowych, oraz różnych aspektów różnorodności roślinności. Badania przeprowadzone w ramach pracy mają na celu wieloaspektowe poznanie mechanizmów regulujących procesy oddychania gleby, aby móc przewidywać i zarządzać planowanymi zmianami np. w strukturze szaty roślinnej.

Pierwszym istotnym aspektem, który powinien być uwzględniony w ocenie czynników wpływających na przebieg respiracji, jest analiza wpływu czynników abiotycznych na procesy oddychania. Wśród tych parametrów, zawartość węgla wydaje się odgrywać kluczową rolę, ze względu na jego znaczenie dla aktywności biologicznej i zawartości materii organicznej w glebie. Jako, że obieg węgla jest istotą życia i funkcjonowania wszystkich ekosystemów. Jednakże inne czynniki abiotyczne, takie jak tekstura gleby, czy jej pH, również mogą wpływać na procesy oddychania poprzez regulację dostępności substancji odżywczych i wilgotności.

Drugim parametrem, na który należy zwrócić uwagę, identyfikując czynniki decydujące o przebiegu procesu respiracji, są parametry biotyczne mineralnych podłoży zwałów skały płonnej, a wśród nich m.in., takie elementy jak wpływ fauny glebowej i aktywności enzymów glebowych w procesach oddychania gleby. Obecność i aktywność organizmów glebowych mogą znacząco wpływać na tempo oddychania poprzez mineralizację materii organicznej w trakcie procesu dekompozycji.

Trzecim krokiem jest ustalenie związków pomiędzy różnymi aspektami różnorodności roślinności a procesami oddychania, respiracji gleby. Badania wskazują, że różnorodność roślinności pełni kluczową rolę w regulacji ilości wiązanego dwutlenku węgla w procesie fotosyntezy, a następnie uwalnianego dwutlenku węgla w procesie dekompozycji i oddychania gleby lub podłoża. Typy roślinności charakteryzujące się większą różnorodnością i ilością biomasy, mogą wykazywać bardziej intensywny przebieg procesów oddychania. Może to być związane z większym zapotrzebowaniem na energię potrzebną do utrzymania swojego

metabolizmu. Różnorodność roślinności może wpływać na różnice w intensywności oddychania gleby lub podłoża, co może wynikać z różnic w cechach funkcjonalnych gatunków.

Celem niniejszej pracy jest przeprowadzenie kompleksowej analizy wpływu czynników biotycznych i abiotycznych na procesy uwalniania dwutlenku węgla w płatach roślinności spontanicznej występujących na mineralnym podłożu nowych ekosystemów hałd skały płonnej. Wyniki pracy zostały podzielone na trzy części.

W pierwszej części, w pracy “The Abiotic Habitat Factors and Soil Carbon Dioxide Release under Spontaneous Vegetation in Coal Mine Heaps“ głównym celem było zbadanie zależności między czynnikami abiotycznymi mineralnego podłoża zwałów skały płonnej, a intensywnością uwalniania dwutlenku węgla. Skupiono się na analizie poziomów oddychania gleby w kontekście parametrów abiotycznych, takich jak zawartość wody, tekstura gleby, pH, zdolność zatrzymywania wody (WHC), wymienne kationy, zawartość azotu oraz przewodność elektrolityczna. Hipoteza badawcza zakładała, że ilość węgla w podłożu będzie miała największy wpływ na poziom uwalniania dwutlenku węgla z podłoża poszczególnych typów roślinności. Parametry abiotyczne, takie jak tekstura, zdolność zatrzymywania wody podłoża glebowego, przewodność elektrolityczna (EC) i pH, nie będą miały znaczącego wpływu na intensywność przebiegu procesu uwalnianiem CO₂ z mineralnego podłoża zwałów skały płonnej.

W drugiej części, w pracy “The Soil Respiration of Coal Mine Heaps’ Novel Ecosystems in Relation to Biomass and Biotic Parameters” celem było zbadanie wpływu czynników biotycznych podłoża na uwalnianie CO₂. Skoncentrowano się na analizie, aktywności enzymatycznej, obecności nicieni i wazonkowców oraz ilości biomasy roślinności w kontekście różnorodności gatunkowej roślinności, oraz ilości materii organicznej *soil organic matter* (SOM) w mineralnym substracie glebowym. Hipoteza badawcza zakładała, że w typach roślinności z większą ilością materii organicznej procesy respiracji podłoża będą przebiegały intensywniej, oraz że typy roślinności o wyższej aktywności enzymatycznej i większej liczebności fauny glebowej w strefie korzeniowej charakteryzują się większą intensywnością oddychania podłoża mineralnego.

W trzeciej części, w pracy “Factors driving plant diversity in the spontaneous vegetation of the novel ecosystem of post-coal mining heaps and their relationship with soil respiration” celem było zidentyfikowanie gradientów środowiskowych wpływających na różnorodność roślinności oraz analiza zależności między różnymi miarami zróżnicowania roślinności

a parametrami oddychania podłoża glebowego. W pracy tej skoncentrowano się równolegle na identyfikacji czynników środowiskowych, które kształtują różnorodność roślinności, zarówno pod względem taksonomicznym, jak i funkcjonalnym, oraz zbadanie, jak te różnice wpływają na procesy oddychania gleby. Postawiono hipotezę, że różnorodność składu gatunkowego roślinności spontanicznej występującej na podłożu zwałów skały płonnej, kształtuje intensywność oddychania podłoża w nowych ekosystemach siedlisk mineralnych terenów pogórnicych. Założono, że intensywność respiracji podłoża mineralnego różni się znacznie między płatami reprezentującymi różne typy roślinności i jest znacznie większe w płatach roślinności o dużym zróżnicowaniu funkcjonalnym.

3. Materiały i metody

Dla każdego z realizowanych celów zastosowano częściowo odrębne zestawy metod. Część realizowanych analiz była konieczna do wykorzystania w analizach w realizacji kilku stawianych celów. Bardzo istotne w realizowanych celach była kompleksowa analiza różnorodności taksonomicznej i funkcjonalnej szczegółowo odnotowywanych płatów roślinności spontanicznej reprezentujących wszystkie różniące się układy gatunkowe. Na czterech badanych obiektach zlokalizowanych na Wyżynie Śląskiej dokonano szczegółowej charakterystyki różnorodnych typów roślinności, wykorzystując różnorodne metody badawcze. W celu określenia biomasy nadziemnej dominanta i gatunków towarzyszących zastosowano techniki pomiaru masy roślinnej, umożliwiające dokładne wyznaczenie udziału poszczególnych gatunków w strukturze badanego płatu.

Analizę procesów metabolicznych mikroorganizmów, określono poprzez aktywność enzymatyczną, wyrażoną przez aktywność dehydrogenazy, ureazy, fosfatazy kwaśnej i zasadowej, co pozwoliło na ocenę funkcji biologicznych gleby oraz procesów biogeochemicznych. Analiza fauny glebowej, oparta na ilościowej ocenie wazonkowców i nicieni, stanowiła kluczowy aspekt badawczy, pozwalający na zrozumienie dynamiki ekosystemu glebowego oraz relacji między organizmami.

Dodatkowo, przeprowadzono szczegółowe analizy fizykochemiczne podłoża, obejmujące m.in. pomiary pH, zawartości składników odżywczych, przewodności oraz tekstury gleby. Te badania miały na celu zrozumienie fizycznych i chemicznych właściwości gleby, kluczowych dla utrzymania różnorodności biologicznej oraz funkcjonowania procesów ekologicznych.

Metody badawcze wykorzystane w ramach niniejszej pracy stanowiły kompleksowy zestaw narzędzi analizy ekosystemu, umożliwiający jak najpełniejsze zrozumienie jego struktury, funkcjonowania oraz interakcji między organizmami.

3.1 Charakterystyka terenu badań

Badane obiekty położone są na Wyżynie Katowickiej - centralnej części Wyżyny Śląskiej (południowa Polska). Region ten objęty jest wpływami klimatu umiarkowanym, z rocznymi opadami na poziomie 600-800 mm i najwyższą średnią temperaturą 14-16°C w lipcu. Na Wyżynie Śląskiej dominują wiatry zachodnie. Liczba dni z mgłą waha się od 30 do 100, a zachmurzenie wynosi około 60-80% (Kompała-Bąba i in., 2019). Ten region był poddany wpływom intensywnej działalności przemysłowej, urbanizacji, wpływom rolnictwa, górnictwa oraz hutnictwa. Wpływ działalności człowieka na środowisko naturalne i jego poszczególne składniki jest znaczący. Dotyczy to szczególnie górnictwa mineralnego, które intensywnie przekształciło rzeźbę krajobrazu i wykluczyło duże obszary z innej działalności człowieka, np. użytkowanie rolnicze i leśne. Badania terenowe przeprowadzono w siedliskach mineralnych hałd kopalń węgla kamiennego w Zabrze, 50°16'22" N, 18°44'43" E); w Katowicach (50°11'04" N, 19°00'33" E); w Murckach (50°11'21" N, 19°02'07" E); oraz w Mysłowicach (50°10'28" N, 19°5'44" E) o łącznej powierzchni 170 ha. Produktem ubocznym wydobywania węgla kamiennego są hałdy skały płonnej. Obecnie materiał mineralny deponowany na hałdach pochodzi w wielu kopalni z głębokości około 1 km. Pod względem warunków abiotycznych, obszary poeksploatacyjne hałd charakteryzują się ograniczoną dostępnością wody, niskimi zasobami składników odżywczych, wysoką temperaturą i wysokim zasoleniem. W porównaniu do innych miejsc tego typu (np. hałdy cynkowo-ołowiowe), składowany materiał nie zawiera wysokich stężeń metali ciężkich. (Kompała-Bąba i in., 2019; Woźniak, 2010);

3.2 Dane fitosocjologiczne

Charakterystyka roślinności spontanicznej

Mozaika siedlisk występujących na terenach hałd pokopalnianych wpływa na różnorodność gatunkową płatów roślinności, które tworzone są przez gatunki reprezentujące siedliska łąkowe, szuwarowe, murawowe, a nawet słono-bagniste oraz ruderalne (Markowicz i in., 2015; Woźniak, 2010). W strukturze zbiorowisk roślinnych, które spontanicznie rozwinęły się na hałdach pogórnicych, bardzo istotna jest rola dominantów. Jak wykazały

wcześniejsze badania nad różnorodnością roślinności spontanicznej terenów pogórnich, częstymi dominantami są trawy (m. in., np. *Calamagrostis epigejos*, *Phragmites australis*, *Poa compressa*, *Agrostis stolonifera*, *Puccinellia distans*), rośliny strączkowe (np. *Melilotus alba*, *Medicago lupulina*, *M. sativa*) czy inne rośliny zielne (m. in., np. *Tussilago farfara*, *Centaurea stoebe*, *Chamaenerion palustre*, *Daucus carota*, *Chenopodium botrys*), które nadają im specyficzną fizjonomię (Kompała-Bąba i in., 2020; Błońska i in., 2019; Woźniak 2010).

Metody badań roślinności

W celu określenia różnorodności roślinności badania prowadzono na 324 poletkach badawczych. Poletka badawcze w kształcie koła miały promień 3 m, zostały i zlokalizowane zostały w obrębie jednorodnych wyróżniających się fizjonomicznych płatów roślinności. Współrzędne geograficzne punktu centralnego każdej powierzchni badawczej zostały zarejestrowane za pomocą odbiornika GPS. Na każdej powierzchni spisano skład gatunkowy, a obfitość jako pokrycie każdego gatunku roślin naczyniowych oceniono według 10-stopniowej skali (<1%, 1-5%, 5-10%, 10-20%, 20-30%, 30-40%, 40-50% itd. co 10% - metoda Braun-Blanqueta ze zmodyfikowaną uszczegółowioną skalą wyceny pokrycia). Osobniki gatunków roślin, które pokrywają znaczący obszar powierzchni badawczych w porównaniu z resztą roślinności, zostały zidentyfikowane jako gatunki dominujące. Zebrane dane posłużyły do obliczenia wskaźników różnorodności na analizowanym obszarze łąki pokopalnianej. Obliczono następujące wskaźniki: wskaźnik różnorodności Shannona-Wienera H, wskaźnik równomierności oraz wskaźnik dominacji Simpsona (Woźniak, 2010). Biomasa zebrana została niezależnie dla gatunków dominujących i pozostałych gatunków niebędących dominantami. Biomasa została zważona bezpośrednio po zebraniu, aby uzyskać wartość świeżej biomasy gatunków dominujących i pozostałych gatunków niebędących dominantami. Na poletku testowym wyznaczono reprezentatywny kwadrat, tj. zawierający gatunek dominujący i najlepiej reprezentujący cały płat (pokrycie gatunku dominującego i relacje ilościowe między gatunkiem dominującym a gatunkiem współwystępującymi). Pole testowe miało bok o długości 0,5 m.

3.3 Metody analiz różnorodności gatunkowej

Do określenia różnorodności gatunkowej analizowanych poletek badawczych obliczono następujące wskaźniki: różnorodności Shannon'a-Wienera H' , równomierności Evenness oraz wskaźnik dominacji Simpsona.

Wskaźnik Shannon'a Wienera, znany również jako Indeks Różnorodności Shannona, jest powszechnie stosowanym wskaźnikiem różnorodności biologicznej. Jego wartość określa prawdopodobieństwo, że dwa wylosowane z próbki osobniki będą należały do różnych gatunków. Wskaźnik różnorodności został obliczony zgodnie ze wzorem (Shannon i Wiener 1949)

$$H' = - \sum_{i=1}^R p_i \ln p_i$$

gdzie:

S – liczba gatunków

p_i - udział osobników gatunku i w liczbie osobników wszystkich gatunków / udział

i -tego gatunku w płacie roślinności

Wskaźnik równomierności Evenness obliczono zgodnie ze wzorem (Mulder i in. 2004):

$$E_{max} = \frac{H'}{H_{max}}$$

gdzie:

H' – wartość wskaźnika różnorodności Shannon'a-Wienera

H_{max} – maksymalna możliwa wartość wskaźnika Shannon'a-Wienera, jeśli każdy gatunek był równie prawdopodobny

$$H_{max} = - \sum_{i=1}^S \frac{1}{S} \ln \frac{1}{S} = \ln S$$

gdzie:

S – liczba gatunków

W celu określenia prawdopodobieństwa, że dwa osobniki wybrane z danej próby

losowo będą należąc do tego samego gatunku obliczono wskaźnik Simpsona według poniższego wzoru (Simpson 1949):

$$C = \sum_{i=1}^s p_i^2$$

gdzie:

S – liczba gatunków

p_i - udział osobników gatunku i w liczbie osobników wszystkich gatunków / udział

i -tego gatunku w płacie roślinności

3.4 Metody analiz różnorodności funkcjonalnej

Analizy różnorodność taksonomicznej skupione były na składzie gatunkowym roślin, mierzonym głównie na podstawie liczby i obfitości gatunków, oraz bogactwo gatunkowe. Różnorodność funkcjonalna obejmuje analizy wybranych cechach funkcjonalnych zidentyfikowanych gatunków roślin. Wybrane cechy reprezentują potencjał reprodukcyjny roślin, rozprzestrzenianie się oraz zdolność do konkurencji. Są to:

- Wysokość: Jest to cecha ciągła opisująca wysokość rośliny.
- Powierzchnia liścia: Ta cecha ciągła opisuje powierzchnię liścia rośliny.
- Wysokość pąka: Jest to cecha porządkowa opisująca wysokość pąka rośliny. Porządkowe (0 – T, 0.12 - G, Hy, 0.25 - H, 0.5 - Ch, 0.75 - N, 1 - M).
- Początek kwitnienia i Koniec kwitnienia: Te cechy ogółem opisują okres kwitnienia rośliny.
- Zapylenie przez zwierzęta, Samozapylenie oraz Zapylenie przez wiatr to cechy binarne opisujące różne metody zapylenia roślin.
- Średnia masa nasion: Jest to cecha ciągła opisująca średnią masę nasion rośliny.
- Rozmnażanie przez nasiona oraz rozmnażanie wegetatywne: To cechy binarne opisujące różne metody rozmnażania roślin.
- Obecność mikoryzy: Ta cecha binarna opisuje, czy roślina posiada mikoryzę.
- Anemochoria, Zoochoria, Barochoria: To cechy binarne opisujące różne metody rozprzestrzeniania się roślin.
- Konkurencyjność, Tolerancja na stres oraz Ruderalność: To cechy porządkowe opisujące różne aspekty ekologiczne rośliny.

- Wartości Ellenberga: temperatura (0-9), światło (0-9), wilgotność (0-12), odczyn gleby (0-9), azot (0-9).
- Indeksy ekologiczne Ellenberga są wskaźnikami używanymi do oceny cech ekologicznych gatunków roślin oraz wymagań siedliskowych. (Cornelissen i inni 2003).

Aby zbadać ogólną różnorodność funkcjonalną składu roślin naczyniowych zanotowaną na parcelach, obliczono cztery składowe różnorodności funkcjonalnej: bogactwo (FRic), równomierność (FEve), rozbieżność (FDiv) oraz dyspersję (FDis). Wykorzystano domyślną funkcję dbFD z pakietu FD w środowisku R (wer. 4.2.2, R Core Team 2022, Wiedeń, Austria, <https://www.R-project.org>). Założono, że bogactwo funkcjonalne (FRic) może być interpretowane jako miara niskiego filtrowania siedliskowego. Równomierność funkcjonalna (FEve), może być miarą nakładania się nisz, natomiast rozbieżność funkcjonalna (FDiv), może służyć, jako miara stopnia heterogeniczności funkcjonalnej, mogą być traktowane jako oszacowanie poziomu konkurencji w zbiorowisku.

3.5 Metody badań ilości uwalnianego CO₂ - Respiracji

Gleba jest zasobem ograniczonym i nieodnawialnym. Jest to szczególnie widoczne na terenach miejskich i wiąże się z intensywnym rozwojem gospodarczym powodującym zanieczyszczenie, zaburzenie struktury, zagęszczenie, erozję i zasklepienie gleb oraz uszczelnianie powierzchni (Minixhofer i Stangl, 2021; Rodríguez-Espinosa i in., 2021; Greinert, 2011; Scalenghe i Ajmone-Marsan, 2009).

Oddychanie gleby, zwane również respiracją, to proces aktywnej wymiany gazowej między glebą a powietrzem atmosferycznym. Głównym produktem respiracji glebowej jest dwutlenek węgla, powstający przede wszystkim w cyklu kwasów trójkarboksylowych (Błażka i Fischer, 2017). Współczynnik oddechowy RQ (ang. respiratory quotient) opisuje stan fizjologiczny biomasy drobnoustrojów glebowych (10.1); jest to stosunek uwolnionego CO₂ do wykorzystanego O₂. Ze względu na procesy zachodzące w glebie oraz różne rodzaje fermentacji wartości RQ mogą się wahać, najczęstszy podawany w literaturze zakres wartości wynosi 0,7 do 1,3 (Błażka i Fischer, 2017).

Badania respiracji podłoża mineralnego badanych płatów roślinności wykonano przy użyciu analizatora TARGAS-1 firmy PP Systems. Analizator wykonuje krótkotrwałe pomiary w układzie zamkniętym z wykorzystaniem naziemnej komory oddechowej. Powierzchnia

naziemnej komory oddechowej wynosiła 78 cm², a objętości zamkniętej 1171 cm³. Krawędź komory pomiarowej została umieszczona w podłożu na głębokość około 1–2 cm. Szybkość uwalniania CO₂ oblicza się na podstawie wzrostu ilości CO₂ wewnątrz komory. Charakter zamkniętych komór powoduje, że CO₂ gromadzi się w sposób ciągły, dlatego okresy pomiaru są skracane do minimum, aby osiągnąć wykrywalny liniowy wzrost stężenia, unikając nadmiernego gromadzenia się dwutlenku węgla wewnątrz komory w czasie. Wyniki pięciu pomiarów stężeń CO₂ zostały uśrednione w ramach analizowanego poletka badawczego. Dodatkowo podczas pomiarów emisji CO₂ z podłoża mineralnego równocześnie wykonywano pomiary temperatury (DT-1 termometr) oraz wilgotności z wykorzystaniem (HH2 Delta T Devices).

3.6 Aktywność enzymatyczna

Dehydrogenazy

Dehydrogenazy są grupą enzymów aktywnych tylko wewnątrz żywych organizmów, a po śmierci komórek szybko następuje ich degradacja (Kieliszewska-Rokicka 2001). Aktywność dehydrogenaz jest wskaźnikiem systemu redox i miarą aktywności oddechowej mikroorganizmów glebowych. W związku z tym aktywność dehydrogenaz wskazuje na obecność w glebie fizjologicznie aktywnych mikroorganizmów (Siwik-Ziomek i in., 2006).

Aktywność dehydrogenazy oznaczono poprzez redukcję chlorku 2,3,5-trifenyloctetrazolu (TTC) do trifenyloformazanu (TPF) przy użyciu metody opracowanej przez Schinnera, 1996. Do badanych próbek gleby dodano 1% roztworu TTC. Całą mieszaninę wymieszano, a następnie przeniesiono do komory termicznej w celu inkubacji w temperaturze 37 °C przez okres 24 godzin. Po inkubacji formazan wyekstrahowano z gleby za pomocą acetonu. Intensywność barwy badano za pomocą spektrofotometru DR 5000 firmy Hach Lange. Absorbancję zmierzono przy długości fali 546 nm. Stężenie wytworzonego TPF obliczono następnie na podstawie krzywej wzorcowej. Aktywność dehydrogenazy glebowej określono na podstawie ilości formazanu uzyskanego na jednostkę masy gleby w jednostce czasu (Schinner, 1996).

Ureaza

Ureaza należy do grupy enzymów klasy hydrolaz o bardzo dużej specyficzności substratowej. Enzym ten działa w szerokim zakresie pH: 6-10, przy niskich wartościach pH bakterie odpowiedzialne za produkcję ureazy nie rozwijają się (Stępniewska i Samborska, 2020). Ponadto przeprowadzone testy wykazały, że jest ona odporna na wysokie i niskie temperatury. Całkowitej denaturacji ulega dopiero w temperaturze od -20°C do 105°C , dlatego występuje zarówno w tundrze, jak i glebie saharyjskiej. W wyniku działania ureazy następuje hydrolityczny rozkład mocznika w glebie. Mikroorganizmy wykorzystują powstały w reakcji dwutlenek węgla do szybkiej syntezy substancji organicznych, a jon amonowy silnie wiąże się z kompleksem absorpcyjnym, co chroni glebę przed utratą azotu w formie kationowej (Stępniewska i Samborska, 2020).

Oznaczenie aktywności ureazy wykonano przy użyciu zmodyfikowanej metody Zantua (Zantua i Bremner, 1975). Do szklanych butelek naważono po 10 g przesianej gleby, następnie do każdej butelki dodano 1,5 ml toluenu i 10 ml 10 mM roztworu mocznika. Tak przygotowane próbki inkubowano w temperaturze 37°C przez 18 godzin. Po inkubacji do każdej próby dodano 50 ml 0,03 M kwasu octowego i wytrząsano przez 20 minut. Po przesączeniu oznaczano N-NH_4 metoda Nesslera (2 ml przesączu + 2 ml winianu sodowo-potasowego + 2 ml odczynnika Nesslera + woda destylowana do 100 ml). Intensywność niebieskiego zabarwienia oznaczano przy długości fali 410 nm za pomocą spektrofotometru DR 5000 firmy Hach Lange (Jezińska-Tys i in., 2004).

Fosfatazy

Określenie “fosfatazy” używa się w odniesieniu do szerokiej grupy enzymów, które katalizują hydrolizę estrów i bezwodników kwasu ortofosforowego. Fosfatazy odgrywają istotną rolę w glebie, gdyż stymulują przemiany organicznych związków fosforu w nieorganiczne fosforany (HPO_4^{2-} i H_2PO_4), bezpośrednio dostępne dla roślin i organizmów glebowych. Określone nazwy tych enzymów wynikają ze specyficznych substratów, lecz są albo hydrolazami monoestrów fosforanowych, albo hydrolazami diestrów fosforanowych. Kwaśny odczyn (pH 4-6) jest optymalny dla kwaśnej fosfomonoesterazy zwanej potocznie fosfatazą kwaśną zaś alkaliczny (pH 8-10) dla alkalicznej fosfomonoesterazy, zwanej potocznie fosfatazą alkaliczną (Bielińska 2005).

Oznaczanie aktywności fosfatazy kwaśnej i fosfatazy alkalicznej oznaczono przez pomiar p-nitrofenolu (PNP) uwalnianego przez aktywność fosfatazy po inkubacji gleby

z buforowanym (pH 6,0 dla fosfatazy kwaśnej i pH 11,0 dla fosfatazy alkalicznej) roztworem fosforanu sodu p-nitrofenylowego (115 mM). Absorbancję zmierzono za pomocą spektrofotometru DR 5000–Hach Lange przy długości fali 400 nm. Następnie wykonano krzywą kalibracyjną, do której użyto roztworu p-nitrofenolu (Bierza i in., 2023; Burmeier, 1995).

3.7 Fauna glebowa

Aby rośliny mogły prawidłowo rosnąć i rozwijać się, konieczne jest poznanie warunków biologicznych podłoża glebowego, w wypadku badanych płatów roślinności mineralnego podłoża zwałów skały płonnej. Jednym z czynników decydujących o parametrach biologicznych siedlisk na terenach pogórnicych, jest ilość i aktywność mezofauny glebowej (Hanus-Fajerska i in., 2015). Głównym zadaniem mezofauny jest rozdrabnianie, mineralizacja oraz humifikowanie materii organicznej. Organizmy glebowe wpływają na przestrzenny ruch substancji w glebie, zarówno mineralnych, jak i organicznych, przyczyniając się tym samym do rozprzestrzeniania się mikroorganizmów, bakterii i grzybów (Radosz i in., 2019).

Na badanych 324 poletkach pobrano po trzy próby mineralnego podłoża (około 1,5 kg). Pobierając mineralne podłoże z losowych miejsc poletek uzyskano właściwy obraz liczebności gatunków fauny glebowej, czyli liczbę występujących w podłożu mineralnym nicieni i wazonkowców na konkretnym płacie roślinności. Zebrany materiał przechowywano w workach strunowych w niskiej temperaturze (6°C).

3.8 Analizy fizykochemiczne

Z 324 poletek badawczych pobrano próbki podłoża (każda próbka była mieszana z 5 losowych punktów podłoża poletka) podłoża do badań fizykochemicznych suszono w laboratorium na powietrzu do stałej masy w temperaturze pokojowej i przesiano (w zależności od analizy przez oczko 2 mm lub 0,25 mm). W celu pomiaru pH mineralnego podłoża, przeprowadzono pomiary po 24 godzinach w stosunku substrat/roztwór 1:2,5, zarówno w wodzie, jak i w 1 M roztworze KCl, przy użyciu elektrody szklanej. Natomiast przewodność elektryczną (EC) podłoża zmierzono w stosunku substrat/woda 1:5 (Hristov i in., 2016).

Zawartość węgla organicznego (SOC) w glebie została oznaczona metodą Tiurina zmodyfikowaną przez Simakova (Hristov i in., 2016).

Azot całkowity (TN) został zidentyfikowany metodą Kjeldahla. Całkowity azot Kjeldahla jest sumą organicznych związków azotowych i azotu amonowego (Rutherford i in., 2008).

Zawartość przyswajalnych form fosforu (P_2O_5) oszacowano zgodnie z Polską Normą PN-R-04023:1996 metodą Egnera-Riehma.

Stężenie dostępnego magnezu (MgO) zostało zmierzone metodą Schachtschabela poprzez ekstrakcję w 0,0125 M roztworze chlorku wapnia (Staugaitis i Rutkauskienė, 2010). Wymienne kationy (K^+ , Na^+) zostały ekstrahowane przy użyciu 1 M octanu amonu przy pH 7,0 (López-Marcos i in., 2018). Stężenia dostępnego magnezu i kationów wymiennych zostały oznaczone za pomocą spektrometrii absorpcyjnej (Thermo Scientific ICE 3500, Thermo Fisher Scientific, Waltham, MA, USA).

3.9 Analizy Statystyczne

3.9.1. Analiza wpływu czynników biotycznych w odniesieniu do respiracji gleby.

Analizy statystyczne i wizualizacje zostały przeprowadzone za pomocą języka i środowiska oprogramowania R (wer. 4.2.2, R Core Team 2022, Wiedeń, Austria, <https://www.R-project.org>). Analizę skupień danych dotyczących roślinności (324 powierzchnie badawcze) przeprowadzono przy użyciu odległości Manhattan i metody Warda jako metody grupowania. Miara odległości została wybrana na podstawie rankingu testów korelacji między danymi dotyczącymi roślinności a danymi dotyczącymi oddychania (cztery powtórzenia dla sezonu wegetacyjnego). Kryterium Calińskiego-Harabasa posłużyło do wskazania najwłaściwszej liczby grup roślinności. Klasyfikację gatunków wskaźnikowych do odrębnych zbiorowisk, a następnie dla dwóch i więcej grup roślinności przeprowadzono za pomocą statystyki wartości wskaźnikowej, według zmodyfikowanej metody IndVal (De Cáceres i in., 2010). Istotność statystyczną tej zależności zbadano testem permutacji (999 powtórzeń). Przedstawiono jedynie gatunki wskaźnikowe grup stanowisk o statystycznie istotnym IndVal.

Zastosowano pośrednią analizę gradientową – *Detrended Correspondence Analysis* niedendycyjną analizę korespondencji (DCA) – w celu wykazania zmienności gatunkowej pod wpływem oddychania gleby (średnia wartość roczna), całkowitego pokrycia roślin, całkowitej biomasy i wskaźników różnorodności biologicznej. Pasywne odwzorowanie tych zmiennych

przeprowadzono za pomocą testu Monte Carlo z 999 permutacjami. Wzajemne korelacje między wskaźnikami różnorodności biologicznej, biomasa i pokryciem obliczono za pomocą macierzy korelacji rang Spearmana. Test Kruskala-Wallisa, a następnie test Conovera do porównań parami, zostały wykorzystane do sprawdzenia istotności różnic między wyróżnionymi płatami roślinności pod względem wskaźników różnorodności biologicznej i pokrycia, biomasy i respiracji.

3.9.2. Analiza wpływu czynników abiotycznych w odniesieniu do respiracji gleby

Obliczono współczynniki korelacji rang Spearmana oraz istotności statystycznej w celu analizy zależności między ilością uwalnianego dwutlenku węgla z mineralnego podłoża, a poszczególnymi zmiennymi, czynnikami abiotycznymi. Aby ustalić główne gradienty, zbadano zależności między parametrami fizykochemicznymi i przeprowadzono analizę głównych składowych (PCA). Przed analizą dane były standaryzowane, aby uniknąć wpływu różnych zakresów i rozkładów zmiennych. PCA umożliwiło nam wybranie najbardziej krytycznych czynników odpowiedzialnych za zmienność mineralnego podłoża. Zmienne o największym wpływie poddano analizie korelacji rang Spearmana z macierzą roślinności. Macierz roślinności została obliczona na podstawie surowych danych dotyczących roślinności i odmienności Manhattan dla danych dotyczących liczebności i odległości Manhattan jako metryki dla danych dotyczących mineralnego podłoża. Funkcja `vegan::bioenv()` została użyta do znalezienia najlepszego podzbioru zmiennych środowiskowych w celu uzyskania maksymalnej (rangi) korelacji z czynnikiem odmienności analizowanego zbiorowiska.

Zastosowano również inne podejście oparte na ordynacji zbiorów rozmytych (*Fuzzy set ordination*, Boyce, 1998; Roberts, 1986) w celu znalezienia czynników środowiskowych odpowiedzialnych za kształtowanie roślinności. Po analizie FSO przeprowadzono wielowymiarową ordynację zbiorów rozmytych (MFSO) z krokowym wyborem zmiennej za pomocą funkcji `fso:fso()` i `mfsso()`. FSO jest narzędziem do bezpośredniej ordynacji, alternatywą dla kanonicznej analizy korespondencji (CCA) i analizy redundancji (RDA). W przypadkach FSO przypisywane są wartości częściowego członkostwa (rozmyte) z zakresu od 0 do 1, co oznacza ich stopień członkostwa w zbiorze (Roberts, 2008). W metodach ordynacyjnych takich jak CCA i RDA, analiza redundancji jest oparta na odległości db-RDA, bazującej na konfiguracji punktów, jest najpierw obliczana jako analiza korespondencji CA lub PCA, odpowiednio analiza współrzędnych głównych PCO, która jest następnie poddawana regresji ważonej względem zmiennych środowiskowych lub eksperymentalnych, zachowując dopasowane wartości regresji jako współrzędne. Analiza FSO jest pierwszą techniką, która

bezpośrednio włącza dane środowiskowe do obliczeń konfiguracji. Podobnie, reakcje poszczególnych gatunków na czynniki abiotyczne nie ograniczają się do określonej funkcji. W naszym badaniu FSO wykonano w celu oceny związku między poszczególnymi czynnikami środowiskowymi, a różnorodnością roślinności. Z kolei MFSSO dostarczyło kryterium dopasowania, które wyraża się korelacją między odległościami wszystkich poletek badawczych w przestrzeni ordynacji składu gatunkowego roślinności, a ich pierwotnymi zmianami. Zmienne środowiskowe znacząco wpływają na skład gatunkowy (Roberts, 2008). W przypadku analiz FSO, jak i MFSSO wykonano 1000 permutacji na podstawie odległość Manhattan w odmienności roślinności za pomocą *vegan::vegdist()*. W MFSSO po metodzie domyślnej zastosowano procedurę krokową, w ramach której wartość respiracji (SRL) została włączona do modelu ze względu na jej duże znaczenie w początkowych wynikach MFSSO oraz w celu znalezienia innych istotnych czynników środowiskowych, które wpływają na zmienność składu gatunkowego.

Aby wskazać gatunki roślin najbardziej zależne od SRL, wybrano gatunki z częstotliwością co najmniej 15% (tj. 50 zajętych powierzchni), a następnie skorelowano je z SRL.

3.9.3. Analiza czynników wpływających na różnorodność roślinności i ich związek z respiracją gleby

W celu określenia zależności między czynnikami środowiskowymi a atrybutami funkcjonalnymi gatunku zastosowano ordynację RLQ, a następnie ulepszoną metodę *fourth-corner* (Dray i in., 2014), ponieważ łączy ona trzy tabele macierzy danych: Tabela L z wartościami liczebności odnotowanymi dla gatunków rosnących na badanych poletkach badawczych, tabela R zawiera zmienne opisujące czynniki siedliskowe, oraz Tabela Q zawierająca wybrane cechy poszczególnych gatunków. W tabeli L przedstawiono 192 gatunki roślin naczyniowych wraz z ich liczebnością na 324 badanych powierzchniach. Tabela R zawierała zmienne środowiskowe, głównie cechy gleby (właściwości fizykochemiczne, skład granulometryczny, pH, obecność enzymów glebowych, SRL). Tabela Q zawierała 23 cechy roślin dla 192 gatunków roślin naczyniowych znalezionych w tym badaniu.

Analizą RLQ domyślna opcja, tj. standaryzowana analiza głównych składowych (PCA), była obliczana na macierzy zmiennych środowiskowych według miejsc (tabela R), a także na macierzy cech według gatunków (tabela Q). Na potrzeby analizy RLQ przeprowadzono analizy statystyczne z wykorzystaniem statystyk *fourth-corner* (Dray i in., 2014). Analizę przeprowadzono w celu oceny związku między każdą zmienną środowiskową a dwiema

pierwszymi osiami RLQ, a także między cechami i osiami RLQ. To narzędzie statystyczne pozwoliło na wykorzystanie zarówno zmiennych ilościowych, jak i jakościowych. Na koniec przeanalizowaliśmy dwuwymiarowe powiązania między zmiennymi dwóch macierzy, R i Q. Istotność została przetestowana za pomocą procedury permutacji. Do tej analizy wykorzystano *fourth-corner*. Aby zbadać ogólną różnorodność funkcjonalną składu roślin naczyniowych odnotowaną na wykresach, obliczono cztery składowe różnorodności funkcjonalnej: bogactwo (FRic), równość (FEve), dywergencję (FDiv) i dyspersję (FDis). Użyto domyślnej funkcji `dbFD()` w pakiecie FD. Założono, że bogactwo funkcjonalne (FRic) może być interpretowane jako miara niskiego filtrowania siedlisk. Równość funkcjonalna (FEve), miara nakładania się nisz i dywergencji funkcjonalnej (FDiv) jako stopień niejednorodności funkcjonalnej, może być traktowana jako oznaki konkurencji w społeczności. Oprócz czynników funkcjonalnych obliczono zróżnicowanie taksonomiczne: bogactwo gatunkowe (S), indeks Shannona-Wienera (H), równomierność (E) oraz wskaźnik dominacji Simpsona przy użyciu pakietów “*vegan()* i *abdiv()*”. Do oceny zależności między składnikami FD i SRL w stosunku do różnorodności gatunkowej zastosowano analizę korespondencji DCA z projekcją pasywną (999 permutacji testu Monte Carlo). Test korelacji rang SRL Spearmana został wykorzystany do sprawdzenia, czy istnieją zależności między parametrami różnorodności funkcjonalnej.

4. Wyniki

4.1 The Abiotic Habitat Factors and Soil Carbon Dioxide Release Under Spontaneous Vegetation in Coal Mine Heaps.

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The Abiotic Habitat Factors and Soil Carbon Dioxide Release under Spontaneous Vegetation in Coal Mine Heaps

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ABSTRACT

This research is focused on examining the link between the abiotic conditions of coal mine heaps (specifically, the type of spontaneous vegetation) and their respiration rates. The hypothesis is that there is a significant correlation between the carbon content of the soil substrate and the respiration rate of the coal mine heap among the abiotic factors studied. The investigation was carried out on the mineral material found in coal mining heaps, which consisted of Carboniferous mineral rock material. The fieldwork spanned the vegetation seasons from 2018 to 2022. Various physicochemical parameters of the substrate samples were analyzed, including soil organic carbon content, electrical conductivity (EC), pH, total nitrogen (TN), available forms of phosphorus (P_2O_5) content, available magnesium (MgO) concentration, exchangeable cations (K^+ , Na^+), and moisture. Soil respiration measurements were taken using the TARGAZ -1 analyzer. The amount of carbon dioxide released at the sites studied ranged from 0.00158 to 1.21462 [g $CO_2/m^2/h$]. It was found that the carbon content and all the environmental factors tested had a significant impact on soil respiration ($p = 0.001$), except total nitrogen ($p = 0.893$). The factors most strongly correlated with soil respiration were potassium (K), alkaline phosphatase, and SRL (soil respiration). Of the taxa analyzed, only the below-ground conditions provided by the vegetation communities dominated by *Centaurea stoebe* showed a significant correlation with SRL. Three dominant plant species influenced the development of below-ground conditions, leading to negative effects. On the other hand, the below-ground conditions associated with vegetation patches dominated by *Daucus carota* showed the strongest negative correlation.

Keywords: soil respiration, vegetation types, novel ecosystems, abiotic factors, coal mining heaps.

INTRODUCTION

The basis of ecology explains that the composition of vegetation trees and herbaceous species, along with the associated heterotroph species and saprotrophic organisms, is strictly dependent on habitat conditions. In this way, the ecosystem process reflects the relationship between habitat conditions. In all the ecosystem matter and energy flow functioning processes, soil respiration indicates various ecosystems and vegetation (Chen and Chen, 2019; Xiao et al., 2021). The release of carbon dioxide is among the crucial ecosystem

functional processes. Soil respiration oxidizes organic carbon into inorganic CO_2 and releases energy. The CO_2 captured by plants is photosynthesized and transformed into organic compounds. This process is the basis of ecosystem functional processes in all ecosystems (Baral et al., 2016; Bark et al., 2016; Washbourne et al., 2020).

Photosynthesis and the autotrophic and heterotrophic carbon dioxide release are fundamental processes responsible for the carbon cycle in ecosystems. Respiration activity is releasing carbon as carbon dioxide from the soil's organic matter. Soil respiration is strictly related to the

biochemistry of the composition of vegetation species. The composition of plant species influences vegetation and ecosystem biomass. In the natural and semi-natural ecosystems, it is known that both the vegetation biomass and the physical-chemical parameters of soil influence the carbon dioxide release processes (Bond-Lamberty and Thomson, 2010; Le Quéré et al., 2015). Soil carbon release, respiration is part of the global carbon cycle, releasing annually approximately ten times more carbon dioxide from all the habitats and ecosystems to the atmosphere than the fossil fuel used for heating per year (Bond-Lamberty and Thomson, 2010; Le Quéré et al., 2015).

Many factors influence carbon cycling and, as a consequence, soil respiration. It is challenging to distinguish the interactions between the factors. In the physiological processes of microbes and plants, soil respiration is sensitive to the most limiting or stress factors (Luo and Zhou, 2006). The soil microorganisms in the plant root zone are attracted by the selected chemical compounds, root exudates released by plant species in response to disturbances drought, salinity, or disturbances caused by mining (Luo and Zhou, 2006; Wolińska, 2019; Wolińska et al., 2014).

Estimating the below-ground respiration components is complicated and remains unresolved (Bouma and Bryla, 2000). The soil moisture, temperature, and texture are among the factors influencing soil respiration (Bouma et al., 1997a). The study conducted in agricultural lands found that soil moisture changes may influence respiration rates in fine sandy soil. In the wetland habitats, the soil CO₂ release was reduced; the more, the finer the texture, and the higher the clay content in the soil. However, the links between the root's architecture, soil texture, moisture, and respiration must be fully understood (Bouma and Bryla, 2000). The fine particles support the ability of water to hold well. The large particles interconnect, allowing air and water to exchange and move between the sphere - pedosphere (soil) and atmosphere. Bouma and Bryla (2000) tested the links between heterotrophic and autotrophic CO₂ released for soils, characterized by drying and wetting cycles in varied soil texture conditions. The soil carbon dioxide amount has been identified to influence the respiration rates of the soil microbial communities (Koizumi et al., 1991) and the respiration level of plant species roots (Bouma et al., 1997b, a; Burton et al., 1997; Scheurwater et al., 1998). The soil texture

condition influences the soil water links considerably (Singer and Munns, 1991); the changes in CO₂ amount may also be modified along the soil texture gradient.

In agriculture, studies compare the influence of the soil CO₂ concentrations, soil water uptake, water content, soil texture (clay, silt, sand content), and root respiration on citrus species seedlings' growth (Bouma et al., 1997a, 1997b; Bouma and Bryla, 2000) The temperature, moisture, nutrient content, and level of oxygen, are factors that control the soil respiration rates. Human activity, such as mining, can influence the amount of soil respiration by altering the parameters. The above conditions can influence the rates of the soil's global respiration. The following should be listed: the agricultural increase of nitrogen and phosphorus fertilization over time and space scales.

Many abiotic factors (e.g., moisture, temperature, soil texture, micro-, macro-element, soil pH, nitrogen deposition (Fenn et al., 2010; Radosz et al., 2023) affect soil respiration (Heinemeyer et al., 2007; Mo et al., 2008). Temperature influences many aboveground and below-ground processes. Soil respiration is a fundamental ecosystem functional process (Atkin and Tjoelker, 2003; Moyano et al., 2008, 2007). The temperature-respiration models indicate the links between temperature and soil respiration rates (Davidson and Janssens, 2006; Zheng et al., 2009). The extreme of soil moisture (low or high) are factors that affect strongly the soil carbon release (Ilstedt et al., 2000; Borcken et al., 2003; Wang et al., 2003). Physico-chemical factors, such as soil pH, impact some soil respiration parameters. The soil pH can modify the activities of soil microorganisms and, later, the respiration of soil organic matter and plant biomass (Ilstedt et al., 2000; Sitaula et al., 1995). Photosynthesis indirectly influences soil respiration (Zhang et al., 2013). In many studies' the role of abiotic habitat factors on soil respiration is analyzed separately from the other factors. Some parameters are synergic and do not act independently but interact with each other, e.g., due to feedback relations and affect soil respiration (Yu et al., 2015).

The abiotic and biotic links might become complicated when the spontaneous vegetation patches and the ecosystems are developing on novel ecosystem habitats (Hobbs et al., 2006), e.g., mining post-mineral excavation sites. The *de novo* established mineral sites allow research on the relations among the individuals of the dominant plant species and the biotic and abiotic

mineral soil substrate characteristics along with the colonizing best-adapted plant species individuals, richness value, and biomass amount of the spontaneously developing vegetation. In novel ecosystems, the biotic and biotic relationships might be shaped by the effect of previously unknown relations resulting from the human impact on the ecosystems during the so-called Anthropocene Epoch (Zalasiewicz et al., 2016). The post-mineral excavation places represent the newly artificial habitats that are significantly different from the natural and semi-natural ecosystems in the neighborhood landscape. Some studies have shown that the living organisms intensively colonized these places through spontaneous succession, providing new non-analogous species compositions of flora and fauna (Hobbs et al., 2006; Kowarik et al., 2011; Frouz, 2018). The varied chemical and physical conditions of post-mineral mining habitats resulted in the development of new unknown, non-analogous species compositions of the spontaneous vegetation and other organisms (Woźniak, 2010; Helingerová et al., 2010; Keith et al., 2009). The non-analogous species vegetation composition growing on novel ecosystems post-mining heaps presents a mosaic of vegetation patches that are dominated by different species best adapted to the range of available microhabitats (Rawlik et al., 2018a, 2018b). The observed mosaic reflects the variety of biotic and abiotic habitat conditions (Woźniak, 2010). The influence of the early-successional stages of vegetation communities on the harsh mineral soil habitat site conditions is not understood (Woźniak, 2010; Lamošová et al., 2010; Orwin et al., 2014). The presented research aims to analyze the vascular plant species composition assembled on various mineral materials of post-mining sites.

Some studies on soil respiration (R_s) and temperature sensitivity (Q_{10}) concentration are focused on humidity, soil temperature, carbon and nitrogen, and root biomass (Arevalo et al., 2010; Wang et al., 2018). The soil microorganisms' composition shapes most of the soil functions (Xiao et al., 2021). The extreme habitat conditions of the coal mine heaps of novel ecosystem mineral material are crucial to how the abiotic site parameters and the plant species composition of developed vegetation types impact the soil substrate respiration parameters of the studied ecosystem. This study aims to analyze the links between the abiotic factors of the post-coal mining heaps (spontaneous vegetation types) and the

carbon dioxide release rates of the soil substrate. We compared the respiration levels to the studied habitat field water content, the texture EC, pH, the water holding capacity (WHC), basic abiotic N_p , C_p , and exchangeable cations Mg, Ca, Na, K and soil CO_2 release of coal mine heaps novel ecosystems soil substratum under the recorded spontaneous vegetation types.

In particular, we tested which abiotic habitat conditions influence the carbon dioxide release rates most. We hypothesized (assuming) that the C content will influence the carbon dioxide release level most significantly. The abiotic parameters, like the WHC texture of the soil substratum, EC, and pH, will not significantly impact; only texture will significantly influence mineral soil substrate material, as it influences moisture and indirectly the other parameters.

MATERIAL AND METHODS

Site description

Several 324 study plots have been studied. The stratified random sampling method was used to select the plots for the study. The sampling method was based on research conducted by Woźniak, 2010, in the same habitats. The Silesian Upland is in a temperate climate zone, transitioning between continental and oceanic climates. Its weather is predominantly shaped by polar maritime air masses originating from the Atlantic (60% of the time) and polar-continental air masses originating from Eurasia (30% of the time).

The post-coal mine has heaps of Carboniferous rock mineral material. The heaps are sites with habitat conditions different from those known from natural or semi-natural ones. The new man-created landscape forms are unique sites because they provide oligotrophic conditions (very poor in nutrients, such as phosphorus, nitrogen, sulfur, and carbon) mineral substrates; after many years of study, it has been shown that plants and animals have colonized them despite unfavorable conditions (Woźniak, 2010; Radosz et al., 2019). During the field research, the object was analyzed: “Makoszowy” (Zabrze, Sosnica; 50°16'22" N, 18°44'43" E); “Kostuchna” in Katowice (50°11'04" N, 19°00'33" E); “Murcki Boże Dary” in Murcki (50°11'21" N, 19°02'07" E); and “Wesoła” in Mysłowice (50°10'28" N, 19°5'44" E).

Vegetation sampling and vegetation diversity analysis

Land cover data were made on study plots of inhomogeneous vegetation patches dominated by the dominant species studied of a circular shape of 3 m radius. Each study plot's geographic coordinates of its center point were recorded using a GPS receiver. In each plot, the species composition was written down, and the coverage of each vascular plant species was valued according to a 10 – grade scale (< 1%, 1–5%, 5–10%, 10–20%, 20–30%, 30–40%, 40–50%, etc. in 10% increments – Method Braun-Blanquet). The plant species individuals that cover the most significant area of the research plots compared to the rest of the vegetation were identified as the dominant species. The collected data were used to calculate diversity indices in the analyzed area of the post-mining heap. Based on the phytosociological records, the following indices were calculated: Shannon-Wiener H's diversity index, Evenness uniformity index, and Simpson's dominance index (Woźniak, 2010).

Biomass sampling

In the analyzed vegetation study plots of a circular shape (3 m radius) dominated by an identified dominant plant species (the species that covers the most significant area within the studied plot compared to the accompanying species, the vegetation biomass was sampled. The collected plants were stored in string bags. The samples were weighed in the field, and data were obtained on the fresh biomass of the dominant species and the other (rest) plant species separately. A representative biomass of 0.25 square meters was selected in the test plot, i.e., including the dominant plant species and best representative of the entire vegetation patch. The test plot frame is 0.5 m of one-side length.

Respiration measurement - CO₂ gas analyzer

Soil respiration values were taken using the TARGAZ-1 analyzer. Five measurements were taken on the vegetation patches analyzed to average the results and check the level of CO₂ emissions over the entire analyzed area. The design of the instrument ensures calibration stability. The soil carbon dioxide efflux level is obtained based on increased CO₂ in the chamber. CO₂ continues to accumulate within the closed chambers. The

measurement periods are reduced to obtain a detectable linear concentration increase without an excessive build-up of carbon dioxide inside the chamber over time. During the measurement, the soil respiration chamber covers a surface area of 78 cm² and a volume of 1171 cm³. The edge of the measurement chamber was placed into the heap soil substrate to a depth of about 1–2 cm.

Laboratory analysis – physicochemical analyses of soil substrate

The soil mineral substrate samples collected for the abiotic physicochemical analyses were air-dried. Later, the soil mineral substrate samples were ground and sieved to a fraction smaller than 2 mm. Substrate mineral samples were analyzed to assess the following physicochemical parameters: electrical conductivity (EC), pH, C loss of ignition, soil organic carbon content (SOC), total N (TN), available forms of phosphorus (P₂O₅) content, available Mg (MgO) concentration, exchangeable cations (K⁺, Na⁺) and moisture. The mineral soil substrate samples of about 1 Kg were taken to analyze soil abiotic physicochemical characteristics data. The soil substratum mineral samples were obtained from five subsampling points in each plot. The samples were collected at 0–15 cm profile depth at each plot sample site. The following have been collected and measured among the measured soil substratum parameters: the total soil porosity and maximum (WHC) water holding capacity. The concentration of bioavailable Mg (MgO) was determined using the Schachtschabel method; 0.0125 M calcium chloride was used as the extraction solution. Based on the Egner-Riehm method, the bioavailable phosphorus (P₂O₅) content was assessed following the Polish Standard PN-R-04023:1996. The amount of soil organic carbon (SOC) was determined using the Tiurin method. The total nitrogen amount was assessed using the Kjeldahl method. pH of the substrate was measured after 24 hours of equilibrium at a ratio of 1:2.5 substrate/solution (Bierza et al., 2023).

Data analysis

All statistical tests and visualization were performed in R language and environment ver. 4.2.2 (R Core Team 2022) and implemented therein packages. The Spearman rank correlation coefficients and probability were computed to analyze the relations between soil carbon dioxide

release and particular abiotic mineral soil substratum variables. To explore overall relationships among physicochemical soil data, biotic variables of soil, and soil respiration, Principal Components Analysis (PCA) was done. Before the analysis, data were standardized to avoid impacts of different ranges and distributions of the variables. The PCA enabled us to select the most critical factors responsible for soil variation. The most contributing variables were subjected to Spearman rank correlation analysis with the vegetation matrix. The vegetation matrix was calculated based on raw vegetation data and Manhattan dissimilarity for abundance data (vegan: vegdist) and Manhattan distance as a metric for soil data. The function bioenv () was used to find the best subset of environmental variables to obtain maximum (rank) correlation with the factor of community dissimilarity.

We also used another approach based on fuzzy set ordination (Boyce, 1998; Roberts, 1986) to find environmental factors responsible for shaping vegetation. The FSO was followed by multidimensional fuzzy set ordination (MFSO) with step-wise variable selection using fso:fso() and mfso() functions. FSO is a direct ordination tool, an alternative to canonical correspondence analysis (CCA) and redundancy analysis (RDA). In FSO cases, partial membership (fuzzy) values are assigned ranging from 0 to 1, which denotes their degree of membership in a set (Roberts, 2008). In eigen-based ordinations methods like CCA and RDA, distance-based redundancy analysis db-RDA, the configuration of points is first calculated as a correspondence analysis CA or PCA, principal coordinates analysis PCO respectively, which is then subjected to weighted regression against environmental or experimental variables, keeping the fitted values of the regression as coordinates. Thus, CCA and db-RDA are sometimes referred to as “constrained ordinations” as they constrain the values of the underlying ordination to achieve what is typically referred to as their “canonical” axes. The FSO analysis is the first technique that directly incorporates the environmental data into the configuration calculation. Likewise, the responses of individual species to abiotic factors are not limited to a specific function. For example, the species’ responses can be discontinuous or nonlinear (Zaharescu et al., 2017). Sometimes, FSO is treated as a constrained version of polar (Bray-Curtis) ordination. In our study, FSO was done to assess

the relationship between particular environmental factors and vegetation dissimilarity. In turn, MFSO provided goodness of fit criterion that is expressed by the correlation between Euclidean distances of all samples in the ordination space of vegetation plant species composition and their original dissimilarities. The high value of the correlation indicates an effective ordination. Environmental variables significantly affect species composition (Roberts, 2008). Both in FSO and MFSO, 1000 iterations of permutations were done based on Manhattan distance in the dissimilarity of vegetation using vegan: vegdist(). In MFSO, the default method was followed by a step-wise procedure where SRL content was included in the model due to its high significance in the initial results of MFSO and to find other significant environmental factors that influence species composition variation. To indicate the most affected plant species by SRL, the species with at least a frequency of 15% (i.e., 50 plots occupied) were selected and then correlated with SRL. The Spearman rank correlation was done with pairwise complete observations.

RESULTS

The preliminary analysis is focused on the diversity of spontaneous vegetation types. The diversity of vegetation types reflects habitat conditions and enables the measured biomass vegetation/ecosystem unit to be identified clearly. The analysis revealed different vegetation types. The studied sites were established from Carboniferous mineral material. The fieldwork was carried out in the 2018–2022 vegetation seasons. During this research, 210 plots were studied, vascular plant species were recorded, and detailed soil substratum analysis was performed. Substrate samples were analyzed for the following physicochemical parameters: pH, electrical conductivity (EC), soil organic carbon content (SOC), total N (TN), available forms of phosphorus (P_2O_5) content, available Mg (MgO) concentration, exchangeable cations (K^+ , Na^+) and moisture. Soil respiration values were taken using the TARGAZ -1 analyzer. Five measurements were taken on the vegetation patches analyzed to average the results and check the level of CO_2 emissions over the entire analyzed area. The range of carbon dioxide released at the analyzed sites was 0.00158 - 1.21462 [g $CO_2/m_2/h$] (Fig. 1).

The four environmental factors, i.e., acid phosphatase and pH, were positive, and the content of potassium and potworms were negatively significantly correlated with SRL (Figure 1). According to PCA based on standardized variables, the high contribution to the presented gradients

was revealed by Mg, K, Ca, pH aqua, pH KCL, Basic phosphatase, and NT (Fig. 2). The soil respiration level responding to the identified gradients. Nitrogen and carbon did not influence the intensity of soil respiration. The indirect phosphorus measure, the acid phosphatase, and pH (aqua)

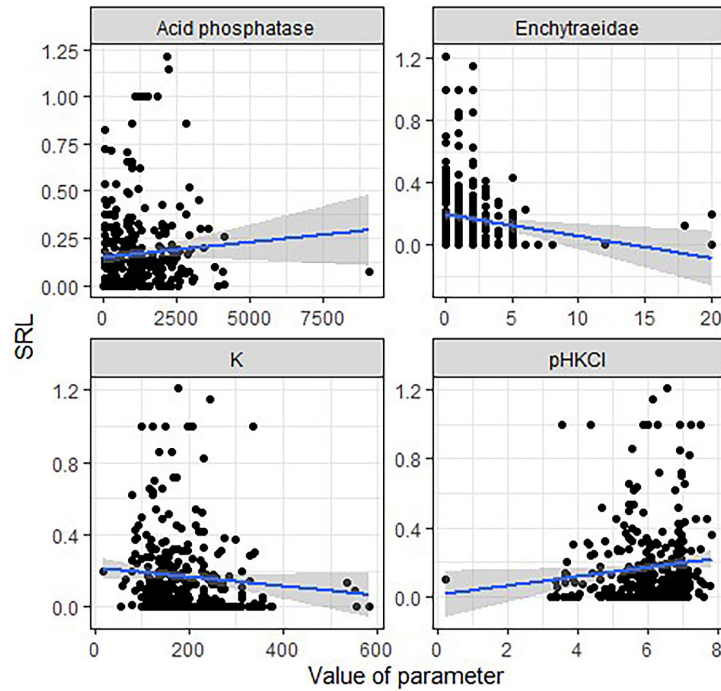


Figure 1. The Spearman correlation between SRL and soil variables (only significant factors are shown)

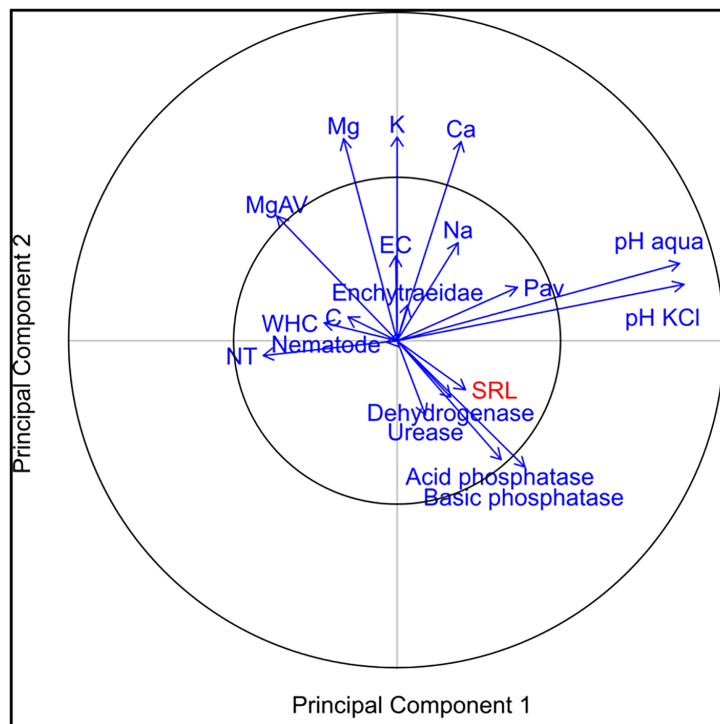


Figure 2. The principal component analysis of soil data and their relations with soil respiration SRL

were excluded due to the correlation with other variables. The Bioenv function revealed that the best fitting model had 9 parameters (with max. 13 allowed): WHC, pHKCl, NT, C, Mg, MgAV, K, and the indirect including Dehydrogenase, and Urease, with a correlation of 0.20. The function excluded SRL, either.

Figure 3 shows the links between vegetation dissimilarity and values of chosen environmental factors based on FSO. All studied abiotic factors were recognized as significant ($p = 0.001$) apart from the total nitrogen ($p = 0.893$). The potassium (K) content, the indirectly available phosphorus (essential phosphatase), and the soil respiration level revealed the highest correlation. The correlations among particular variables in FSO are shown in tab. 1. The relation has been identified between soil respiration level and total nitrogen, followed by pH, and negative ones with calcium and magnesium. In the MFSO, the relation between ordination distance and the matrix dissimilarity of vegetation is relatively ($r = 0.748$, Fig. 4). In the results of MFSO analysis, samples of vegetation are arranged mainly through soil respiration (especially in essential phosphatase,

potassium) and further by other environmental factors what was confirmed by p-value (Table 2). In all cases most of samples are concentrated at lower value of SRL (Fig 4). Some vegetation plots present the association with higher values along axes represented by SRL. In the case of Mg and Ca, samples are also grouped similarly, suggesting that these variables are correlated.

Both in MFSO and more robust step-wise, MFSO soil respiration significantly improved the model of the impact of environmental parameters on vegetation patterns (Table 2). When SRL was included in the model, the other significant vital variables that explain species variation were magnesium, potassium, pH and essential phosphatase, while remaining factors (total nitrogen, calcium) were not significant (Table 2). Mapping the distributions of the sample point from the fuzzy topological space of fuzzy set ordination (FSO). Using the Euclidean space for the distributions of the sample points enables analysis using a broad range of parametric statistical methods. The results obtained by Roberts (2009) show direct interpretability, as each axis in an MFSO reflects a single environmental variable and is orthogonal

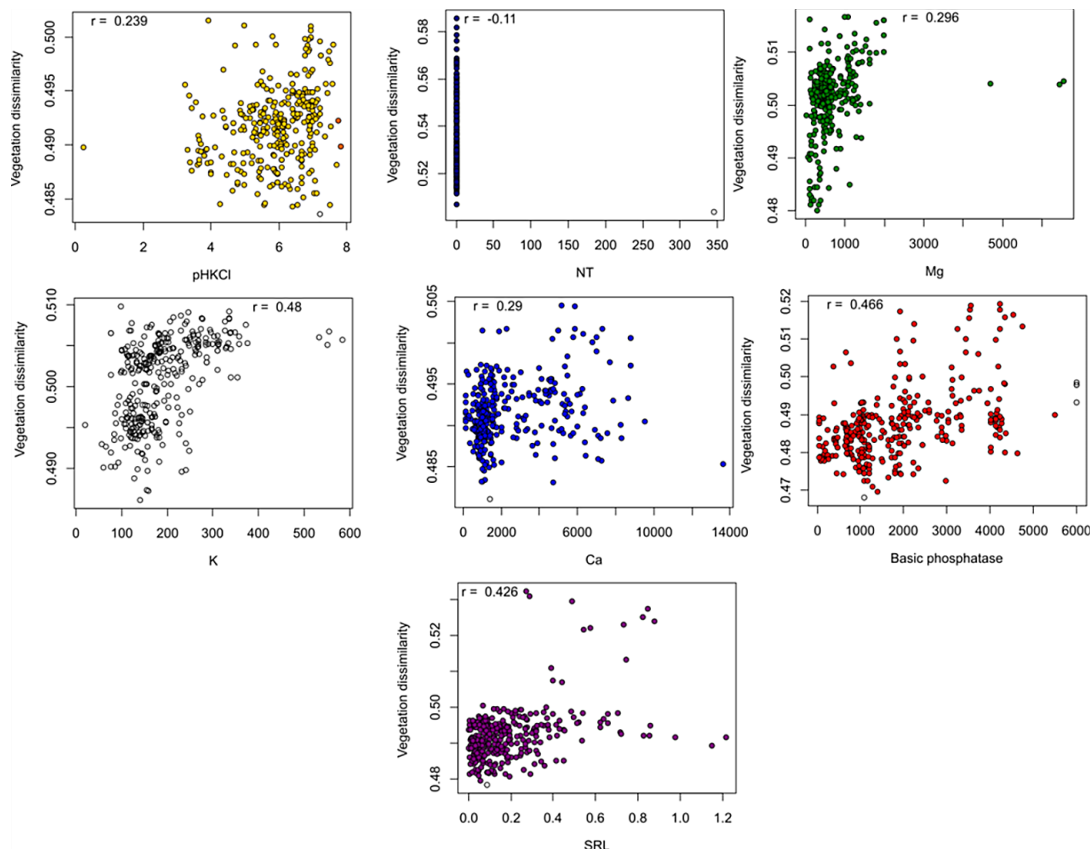


Figure 3. The relation linking the soil respiration level and particular soil variables on the background of vegetation based dissimilarity on the fuzzy ordination

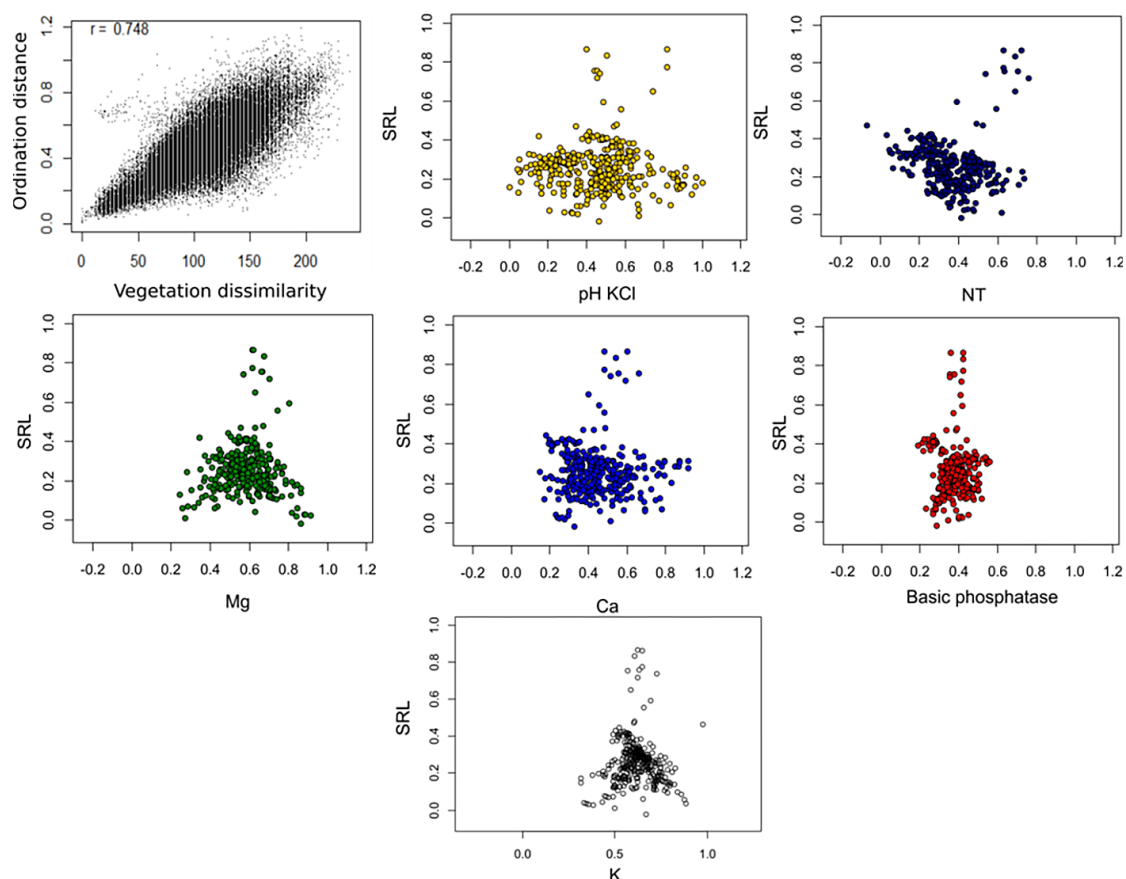


Figure 4. The plots of multidimensional fuzzy set ordination diagrams of coal mine heaps. The vertical axis represents soil respiration, and the horizontal axis – represents other soil variables

Table 1. The correlation of fuzzy set ordination between the soil substrate characteristics and the soil (substrate) respiration parameters

Soil substrate	pHKCl	NT	Mg	K	Ca	Basic phosphatase
pHKCl		-0.672142	-0.755854	-0.5754234	0.1957003	0.8574878
NT	-0.672142		0.4782322	0.6274184	-0.2250911	-0.5791621
Mg	-0.755854	0.478232		0.7848299	0.0708542	-0.8873671
K	-0.575423	0.627418	0.7848299		0.2511016	-0.7128900
Ca	0.195700	-0.225091	0.0708542	0.2511010		0.0671225
B. phosphatase	0.857488	-0.579162	-0.8873671	-0.7128900	0.0671225	
SRL	0.151741	0.169597	-0.1434519	-0.0024395	-0.1338680	0.1436176

Table 2. The results of multi-fuzzy set ordination (MFSO) and step-wise MFSO

MFSO			And step-wise MFSO			
Variable	Cumulative_r	Increment	P-value	Gamma	Delta_cor	P-value
pHKCl	0.3677557	0.36775571	0.670	1.0000000	0.07768200	0.01
NT	0.4408519	0.07309617	0.348	0.5482247	-0.14407976	0.59
Mg	0.5464880	0.10563616	0.379	0.4270644	0.22695488	0.01
Ca	0.6265471	0.08005902	0.280	0.8388148	0.04924796	0.40
K	0.6539962	0.02744911	0.837	0.1953713	0.12357905	0.01
Basic phosphatase	0.6610047	0.00700857	0.984	0.1281119	0.17304420	0.01
SRL	0.7477256	0.08672083	0.004	0.8264381	Included in the model	

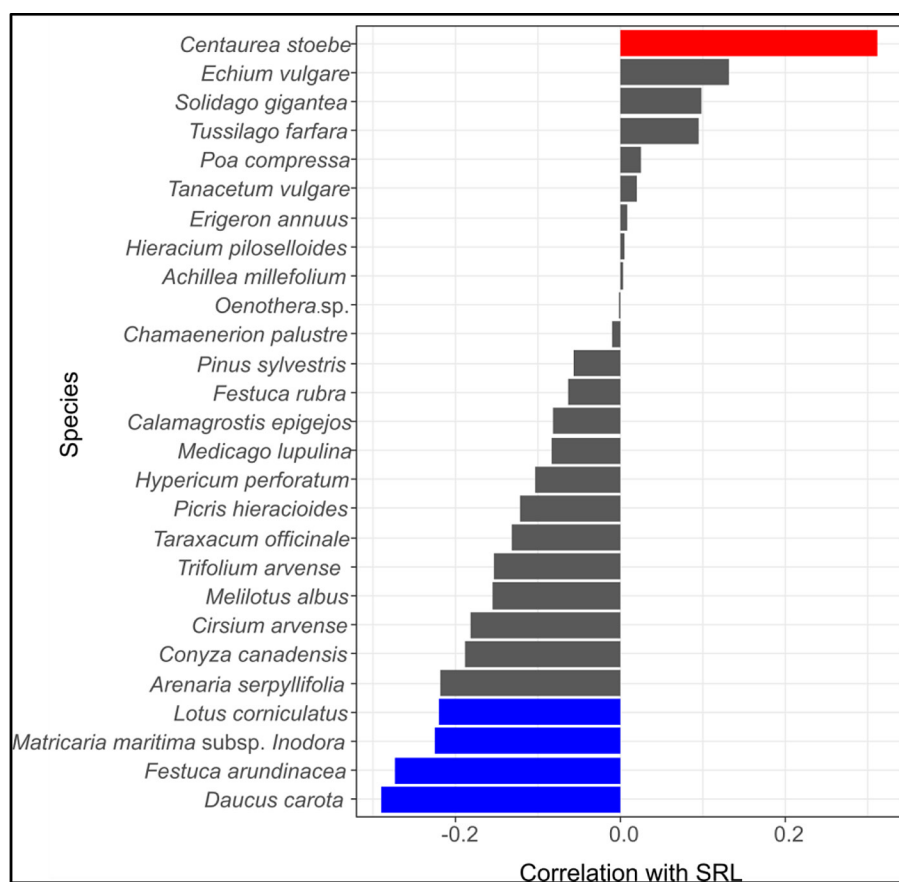


Figure 5. The analysis was performed using Spearman correlation coefficient values based on complete pairwise observations of the most frequently occurring plant species and soil carbon dioxide release values in coal mine heaps. Red indicates – positive significant relationships, and blue indicates significant negative correlations

to all other axes by design. Among 27 taxa (26 species), only the conditions associated with the patches of vegetation communities dominated *Centaurea stoebe* were correlated with the value of soil respiration level. The patches of vegetation communities of three other plant species dominants provide conditions that negatively impact the release of carbon dioxide. The most robust negative relation was provided by habitat conditions developed along with the vegetation patches dominated by *Daucus carota* and, to a lesser extent, *Festuca arundinacea* and *Matricaria maritima* subsp.-*inodora* (Figure 5).

DISCUSSION

This study analyses the links between the abiotic conditions of the mineral site material of the coal mine heaps (spontaneous vegetation-type patches) and the respiration rates. We have tested the hypothesis that the soil substrate carbon content is significantly related to the coal mine heap

spontaneous vegetation respiration rate among the studied abiotic factors. The habitats and vegetation types with higher carbon content are releasing more CO₂. Contrary to our expectations, soil carbon content did not significantly influence soil respiration intensity. There might be a few reasons why the results contradict our hypothesis. The carbon (C) cycle, together with nitrogen, is the primary nutrient cycle in ecosystems, and it has only sometimes been considered in detail (Cusack et al., 2011; Sinsabaugh et al., 2005). Carbon, phosphorus, and nitrogen cycles are frequently interlinked in ecosystems (Fahey et al., 2013; Zarif et al., 2020). In some ecosystems, and probably should also be considered in mineral soil substratum material of new coal mine ecosystems, the C: N ratio in the soil (subsoil) supports the available N uptake by plants (Eberwein et al., 2017; Zarif et al., 2020). In our study, some of the analyzed abiotic habitat factors have been revealed to be significant ($p = 0.001$). The exception has been stated for total nitrogen content ($p = 0.893$). The content of Potassium (K), the indirect measurement of the

alkaline phosphatase, presented the highest correlation with the soil expiration level. The total nitrogen amount, followed by pH, presented the highest positive correlation with soil expiration level, while the carbon release was negatively related to magnesium and calcium.

The phosphorus content influencing the SRL

The phosphorus, particularly the available forms of phosphorus in soil or mineral soil substratum, is, to some extent, related and linked to the activity of the phosphatases. In our study, alkaline phosphatase was linked to the amount of carbon dioxide released by the mineral soil substratum. Chemically, the phosphorus component in soils is a low-mobility element. Plant roots are available only from the close vicinity of the roots and are highly pH- and temperature-dependent. The P cycle can be significantly connected with the N cycle (De Groot et al., 2001; Aber et al., 1989). In the presented study, the base phosphatase activity is a proxy for the phosphorus content parameter mineral soil substratum in the coal mine heaps novel ecosystems. The study showed a positive correlation between base phosphatase activity and the amount of carbon dioxide released.

Most of the previous studies analyzing the soil respiration levels have paid attention to the role of nitrogen in the first place. The phosphorus content has been considered as an element that interacts with nitrogen (Guo et al., 2016; Helfenstein et al., 2020; Zhang and Zhang, 2016; Zhang et al., 2020). The mineral inorganic phosphorus (Pi) changes to organic phosphorus (Po) and can take part in the soil P bioavailability (Helfenstein et al., 2020, 2018; Rosling et al., 2016). Some studies show phosphorus increases litter nitrogen, limiting nitrogen mineralization (Homeier et al., 2017; Mao et al., 2017). The constant nitrogen uptake, e.g., the inorganic nitrogen from the atmosphere is reducing the soil acidity, which can lead to the buffering effect caused by the additional phosphorus that keeps the soil pH at a stable level (Mao et al., 2017; Yang et al., 2015; Zarif et al., 2020). What is a factor that stabilizes soil respiration in developing ecosystems? This can be the possible interpretation of the results obtained in our research on mineral soil substratum respiration rate level, measured in the habitat from under the different spontaneous vegetation types.

The carbon dioxide released in the carbon life cycle

The carbon cycle through the inorganic and organic forms and the dynamic interaction with various nitrogen and phosphorus is the fundamental functional process in each ecosystem (Cusack et al., 2011; Sinsabaugh et al., 2005). Including the base cations and soil pH impact in the C, N, and P cycles in the biotic abiotic transition process are good practical eco-chemical indicators of soil health in a dynamic approach (Futa et al., 2016; Małek, 2009). The soil or mineral soil material development is dependent on the vegetation plant species composition in the ecosystem vegetation patches mosaic (Binkley et al., 1999; Rhoades, 1996), and likewise, the modifications in the local and global habitat conditions may impact the diversity and composition of plant species composition in the communities (Bardgett, 2005; Zarif et al., 2020; Bolan, 1991). The additional phosphorus content significantly increased by 60% in the soil exchangeable cations (Yang et al., 2015). The weathering of the selected base cations, including $\text{Ca} > \text{Na} > \text{Mg} > \text{K}$, can cause the reduction of the base cation amount and some metal ion imbalances in the mineral soil substratum, as has been presented by Lucas et al. (2011).

The understanding of the mechanisms of the mineral soil substratum carbon dioxide release level about the abiotic factors along the feedback relation of the plant species composition is crucial for a range of reasons (Bouma and Bryla, 2000; Šimůnek and Suarez, 1993; Skopp et al., 1990). Many studies conducted on soil respiration rate assessments are performed in agriculture, cropland, grasslands, and managed forests (Xiao et al., 2021). More research needs to be focused on studying soil respiration parameters in other, e.g., natural ecosystems and spontaneous ecosystems developed in, e.g., human-established habitats, such as the post-mining mine heaps providing specific habitat conditions of mineral soil material. Some studies show that the carbon dioxide released from soil declines from summer to winter. Additionally, this study has presented that soil temperature and moisture are crucial factors that support the explanation of the results obtained. The mean soil respiration in grassland ($3.68 \mu_{\text{mol}} \text{m}^{-2} \text{s}^{-1}$) and tree stands or woodlands ($3.81 \mu_{\text{mol}} \text{m}^{-2} \text{s}^{-1}$) appear higher than in abandoned agricultural land and cropland. In the projection values of the variable importance from the regression

model, it has been presented that the soil temperature, pH, soil moisture, available nitrogen, microbial biomass carbon, nitrogen, and bacterial abundance were the crucial factors that have an impact on the soil respiration level in the studied under different land-use types habitats (Xiao et al., 2021). In our study, we could not include the measurements for root respiration. There are studies in which the importance of root respiration has been underlined. There are assessments that in some habitats, the roots can respire (use energy and release carbon dioxide to an extent more than 50% of plant daily photosynthesis efficiency, the produced biomass (Lambers et al., 2002).

The overall soil respiration includes two main components: the respiration of the microorganisms and plant roots and respiration both based on soil organic matter, plant biomass, soil animals, and litter (Scott-Denton et al., 2006; Bond-Lamberty et al., 2004; Hanson et al., 2000; Rodeghiero and Cescatti, 2006). The measurement of both elements of total respiration is methodologically challenging. For this reason, a relatively low number of studies have attempted to perform the separated measurement of the two above respiration components (Butler et al., 2013; Jiang et al., 2017; Kooijman, Welschen and Lambers, 1988; Bouma, Broekhuysen and Veen, 1996). The equipment most frequently used is the surface chambers or chambers buried with carbon dioxide sensors. Soil respiration is generally measured over a restricted area (< 1 m²). The soil has a limited diffusivity, and carbon dioxide is more concentrated than in the atmosphere (Phillips and Nickerson, 2015). In the presented study, we have used one of the most frequently and commonly used soil respiration measurements. We have measured the total carbon dioxide released from the soil, including all the soil sources of carbon dioxide released from the habitat type, reflecting the specifics of particular vegetation types.

Soil respiration elements in managed forests

In the environmental studies, the research ecosystems are likely to be compared with forests. A study was also conducted on soil respiration in two forest types (Butler et al., 2012; Yu et al., 2015). This study revealed that respiration of litter was the primary source of heterotrophic respiration (88%). The heterotrophic respiration occurred after some time, as the soil litter carbon used by microorganisms was not immediate. The

use of carbon by the microorganisms and high carbon amount in organic matter respiration of litter is characterized by a hysteresis phenomenon (Yu et al., 2015). The study of carbon dioxide release from the dead roots presented that the dead roots provide new soil organic material for microorganisms, and in this way, the heterotrophic respiration rate is increased (Bond-Lamberty et al., 2004; Lee et al., 2003; Yu et al., 2015). The other study presented that the low carbon/nitrogen ratio leads to the site conditions in which the respiration and carbon dioxide release levels are growing due to the increase of the soil microbe organic matter decomposition (Grant et al., 2001; Liu et al., 2011; Yang et al., 2015). Some studies performed in tree stands and managed woodlands have shown that soil substrate respiration can have a linear positive correlation when compared with the young tree stands (Chen et al., 2010; Franzluebbers et al., 2001).

The components of soil respiration to total soil respiration

The heterotrophic and autotrophic respiration is divided into different ratios to the total soil respiration in different ecosystem types, e.g., forests. In general, root respiration reveals 10–60% (–90%) of the overall soil carbon dioxide release in many forest ecosystems (Kuzyakov, 2004; Shen et al., 2011; Satomura et al., 2006). Some of the research sites were located in a nature reserve area with forest vegetation patches of abundant soil organic matter and litter layer, resulting in a high relative humidity level (Yang et al., 2015).

The study by Subke et al. (2006) indicated that the heterotrophic part of carbon dioxide releases 27–86% of total soil respiration in forest ecosystems. The results of the study referred to above are consistent with our assumptions. In our study, we have assumed that the total respiration amount will be related to the species' biochemical, organic carbon compounds, and composition, which is dependent on the plant species' spontaneous of the herbaceous vegetation type that has developed in response to the abiotic mineral material of the post-coal mine heaps novel ecosystem.

The effect of texture on the abiotic parameters on the soil respiration level

In our study, the mineral soil substrate texture characteristics are irrelevant and must be

presented in the paper results section. Such a result was surprising because it is known that the finest soil texture material can impact soil water-holding capacity, influencing many other functional processes, including respiration intensity. The soil texture structure is known to be able to impact the nitrogen cycle in the ecosystem. The weak binding to the soil particles and nitrate leaching can be the reason for the decrease in soil pH and the base cations (Araujo et al., 2017).

The strong relation between the soil organic matter content and the amount of clay in the soil structure has been presented in most of the studies (Jenkinson, 1990; Parton et al., 1987; Franzluebbers et al., 2001; Wang et al., 2003; Müller and Höper, 2004). When the first clay particles appear, the process of clay particle aggregation can start, and the other particles can come together (Tisdall and Oades, 1982). The clay particle aggregation process can change the water holding capacity and, as a result, the soil moisture parameters, influencing the input of C into the soil using the photosynthetic plant species composition productivity and, as a result, the soil organic matter distribution (McLauchlan, 2006; Six et al., 2000).

The study performed in the way in which humidity and temperature were controlled revealed how the results of soil respiration rate can vary (Giardina et al. 2001). In the study conducted by Giardina et al. (2001), it has also been revealed that clay participation only partially influences the level of net N mineralization (Giardina et al., 2001; Côté et al., 2000; Schimel, 1995; McLauchlan, 2006). Opposite to our expectations, no significant relation between the mineral soil material texture and the respiration rate parameters has been revealed, and in our study, stones, gravel, and sand-sized particles influenced the texture of the mineral soil substratum material. This might be the reason for the unexpected results.

Soil water content and soil respiration

Identifying the environmental factors that control soil CO₂ release from the ecosystems is an essential step in assessing the potential effects of environmental changes (Schlentner and Van Cleve, 1985; Singh and Gupta, 1977). When modeling soil respiration (e.g., how the soil carbon dioxide release rates are dependent on moisture or temperature), the models have shown that an exponential equation best describes the temperature soil respiration relation (Borken et al.,

1999; Kutsch et al., 2001; Rochette et al., 2013). The precipitation amount and soil moisture are crucial in addition to temperature in the soil respiration models (Savage and Davidson, 2003; Tang et al., 2005; Tüfekçioğlu and Küçük, 2004). Some additional factors are considered in the models, such as pH (Reth et al., 2005), land use (Ardö and Olsson, 2003), carbon amount (Kutsch et al., 2001; Rodeghiero and Cescatti, 2005), and the plant traits parameters such as maximum leaf area index (Skopp et al., 1990). In our above study, we analyzed pH and the amount of carbon.

Effect of salinity on SRL

The mineral material of the coal mine heaps can be frequently characterized as the habitat of high salinity. The salty waters and salt are associated with coal excavation and the geological layers. Soil salinity is regarded as a significant factor in forestry and agriculture, particularly with high evapotranspiration and low rainfall (Bossio et al., 2007; Rengasamy, 2006. Beltrán and Manzur, 2005; Pannell and Ewing, 2006). As Zeng et al. (2014) indicated, soil denitrification / nitrification and, consequently also, soil respiration depend on soil salinity. The stress related to salinity and drought are conditions to which the plants building the vegetation of natural and semi-natural ecosystems are susceptible. Some researchers understand that plant individuals are unable to adapt quickly to those stresses (Jarvis, Lane and Hijmans, 2008; Mittler, 2006). It is less frequently studied how the adaptation processes that can result in salt and drought adaptation might differ in the biochemistry of the plant individuals. The changes in plants' biochemistry influence the character of the soil organic matter and, as a consequence, the decomposition process, which is directly related to the release of carbon dioxide. The salinity can be changed by the occurrence of exchangeable cations and the conditions that might influence the exchange. The presence and amount of the exchangeable cations Ca²⁺, Mg²⁺, Na⁺, and K⁺ are essential in the soil functioning in the natural and semi-natural ecosystems. Exchangeable cations can become exchanged by a cation of an added salt solution (Ramos et al., 2018). In the presented study, the magnesium Mg, potassium K, and calcium Ca content and the pH value in coal mine mineral soil material are significantly related to the carbon dioxide release.

CONCLUSIONS

This study aims to analyze the links between the abiotic conditions of the coal mine heaps habitat variety identified as spontaneous vegetation types and the CO₂ respiration rates. We hypothesized that the soil substrate carbon content is significantly related to the coal mine heap respiration rate among the studied abiotic factors. The vegetation types of habitats with higher carbon content might release more CO₂. The post-black coal mining heaps mineral material soil substratum samples were analyzed for the following physicochemical parameters: pH, electrical conductivity (EC), soil organic carbon content (SOC), total N (TN), available forms of phosphorus (P₂O₅) content, available Mg (MgO) concentration, exchangeable cations (K⁺, Na⁺), and soil substratum moisture. The results revealed that the carbon dioxide range from the studied vegetation types varied from 0.00158–1.21462 [g CO₂/m²/h]. The FSO analysis showed that contrary to the hypothesized expectations, the carbon content and all the analyzed habitat factors were significant (p = 0.001), apart from the total nitrogen. Potassium (K) and soil respiration levels presented a significant correlation among the identified vegetation community types dominated by 26 species, only the habitat conditions provided by the vegetation communities dominated by *Centaurea stoebe* significantly correlated with soil respiration level. Three plant species dominants caused the development of habitat conditions, resulting in a negative impact. In contrast, below-ground conditions associated with the vegetation patches dominated by *Daucus carota* demonstrated the strongest negative correlation.

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4.2 The Soil Respiration of Coal Mine Heaps' Novel Ecosystems in Relation to Biomass and Biotic Parameters.

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Article

The Soil Respiration of Coal Mine Heaps' Novel Ecosystems in Relation to Biomass and Biotic Parameters

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Abstract: The biodiversity, including the diversity of autotrophic organisms of mostly plant species, assembled in vegetation patches and its impact on the course of ecosystem processes is still a key subject of research in natural sciences around the world. Certain aspects of the relationship between biodiversity and CO₂ release processes have been studied only in some natural and semi-natural ecosystems (semi-natural ecosystems such as meadow or grasslands). In contrast, very little is known about the biotic parameters related to natural processes and the functioning of novel ecosystems. This study was performed on post-black coal mining heaps. The studied sites were established on carboniferous mineral material. Among the considered biotic parameters, the vegetation plant species composition, soil organic matter, soil enzymatic activity, soil fauna presence, and the plant species biomass were studied. The aim of the research was to analyse the influence of the selected biotic factors on the CO₂ release from the mineral material of black coal mining heaps' novel ecosystems. The range of CO₂ release at the analysed sites was 0.00158–1.21462 [g CO₂/m²/h]. The activity of soil enzymes such as dehydrogenase, acid phosphatase, and basic phosphatase was positively correlated with the amount of CO₂ released, however, there was no correlation between urease activity and CO₂ emissions from the soil. In our study, a comparison of the soil organic matter developed under the vegetation types studied and CO₂ release (rate) showed a dependence on vegetation type. The amount of biomass was not linearly correlated with CO₂ release from the soil. The presence of soil fauna displayed a positive effect on CO₂ release.

Keywords: soil respiration; biodiversity; biomass; soil organic matter; black coal mining heaps; novel ecosystems; soil fauna; soil enzyme activity



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1. Introduction

The basic process of ecosystem functioning is the flow of matter and energy. Each ecosystem starts with the habitat colonization by autotrophic organisms and later with a heterotrophic organisms' food chain along with the appropriate microorganisms, and saprophyte decomposition activity, leading to, e.g., CO₂ release as the side effect of the energy acquisition [1–4]. Depending on the environmental conditions, the best adapted plant species colonize and grow in particular microhabitat places. As a result of the processes of the above-ground biomass development, the foundation of the biochemical characteristics of the soil biomass, soil organic matter (SOM), is established. The biochemical composition of the biomass and the condition of the respiration processes influence the amount of CO₂ released to the atmosphere. Apart from the biochemical composition of the biomass, the microbial ability to decompose the complex chemical carbon compounds determines the respiration process [5–7].

The sites that are significantly transformed (or those that are established de novo) due to human activities such as mineral resource mining, provide unusual habitat conditions

for the organisms that are successfully colonizing such sites. The previous field study and conceptual works revealed that extreme habitats are colonized by a non-analogous species composition assembling spontaneously and leading to the development of a novel ecosystem [8–11], such as the mineral post-coal mining habitats. The non-analogous species composition assemblage is comprised of the species best adapted to the unusual, extreme habitat conditions. The extreme habitat conditions are a trigger to push the living organisms (primary producers, heterotrophs, and saprophytes) to evolve and adapt. The harsh, complex system of the habitat conditions are caused by the lack of initial soils in the sites where the novel ecosystems develop. The term soil is strictly defined in soil sciences. In many post-mining sites, the mineral material substrate without organic matter of varied texture or structure (referred to as the soil substrate further in the text) is colonized by vascular plant species.

Both the untransformed ecosystems and novel ecosystems are dependent on the biomass quality and quantity which in turn depend on the plant species' taxonomic and functional composition and diversity. High diversity enables complementary habitat resource use among competing species in species-rich ecosystems [12,13]. A larger amount of biomass in species-rich ecosystems enhances Rs primarily by increasing root, microbial communities', and rhizo-microbial respiration [14]. The diversity of plant species composition increases the quantity and multiplicity of plant exudates-derived food resources in the soil, providing and expanding a variety of niches for saprophytic microbes. The diversity of plant species composition influences microenvironment variety and habitat complexity including the soil enzyme activity [12,15,16]. Diverse plant species composition provides a litter mixture, which accelerates the decomposition of organic matter via complementary resource use among microbes, resulting in more effective Rs [17]. The carbon compound decomposition of the soil organic matter (SOM) is related to, e.g., the plant species composition, mesofauna composition, and soil enzyme activity [18]. Biomass carbon compounds are broken down quickly during decomposition, providing an energy source for microbes [19]. Plant species composition plays a crucial role in vegetation and ecosystem development and the regulation of soil respiration (Rs) because the autotrophs are the prevalent synthesising organisms through which carbon enters the soil [10].

The magnitude of the soil respiratory metabolism is due to four factors: (1) CO₂ release by plant roots, (2) soil fauna, (3) soil microorganisms, and (4) the chemical oxidation of carbon compounds. The rate of the soil metabolic processes depends on a number of factors, which includes the microclimatic conditions (temperature and humidity), the structure of the soil and the content of organic matter—living and SOM. Soil nematodes, whether they are hosts of bacteria, fungi, plants, omnivores or predators, affect the populations of the organisms they feed on. Although the contribution of nematodes to soil respiration is probably less than 1%, they can play an important role in soil nutrient cycling by influencing bacterial growth and nutrient availability to plants [20]. All species of Enchytraeidae, through intensive respiration processes, have a significant contribution to the mineralization of organic matter. Enchytraeidae stimulate the course of mineralization and humification processes, including by loosening the soil, breaking up soil particles, their movement in the soil profile, and catalysing the activity of microorganisms. The contribution of Enchytraeidae to these processes, and to shaping of the proper structure of and increasing the fertility of soils, is often much greater than that of other representatives of soil macro- and mesofauna [21].

The knowledge of the biotic factors influencing the nature of the functional respiration processes in the coal mine heaps' novel ecosystem is very limited and only includes limited information on mechanisms related to aspects of the biotic parameters and respiration that are a key part of the functioning of the ecosystem [22].

It can be hypothesized that (a.) the vegetation types with a larger amount of biomass will respire more intensively, while the vegetation types with a smaller amount of biomass will respire less intensively; (b.) the respiration will be less intense in less diverse vegetation

types; and (c.) the vegetation types with higher enzyme activity and a greater presence of soil fauna are characterized by greater respiration intensity.

The aim of this research was to analyse the influence of the selected biotic factors on the CO₂ release from the mineral material of black coal mining heaps' novel ecosystems. Among the biotic habitat parameters, the following have been recorded and analysed: (i.) the diversity of plant species composition of the studied vegetation types; (ii.) the amount of SOM; (iii.) the enzyme activity; (iv.) the presence of nematodes and vases; (v.) soil respiration measurements; and (vi.) the biomass amount recorded for the studied vegetation types.

2. Materials and Methods

2.1. Study Site Description

The study site's area is located in the central part of the Silesian Upland. The Silesian Upland lies in a moderate climate zone, transitional between oceanic and continental. It is mainly influenced by polar maritime air masses from the Atlantic (60% of days) and polar–continental air masses from Eurasia (30% of days). This region has been subject to intense industrial human activity, such as urbanization, agriculture, mining, smelting, open sandpits, and quarries. The impact of human activity on the natural environment and its individual components is significant. This is particularly true of mineral mining, which has intensively transformed the relief of the landscape and excluded large areas of other human activity, e.g., agricultural and forest land use, degrading the landscape's value. Field studies were carried out in the mineral habitats of the mine heaps of the coal mines at “Makoszowy” (Zabrze, Sosnica; 50°16'22" N, 18°44'43" E); “Kostuchna” in Katowice (50°11'04" N, 19°00'33" E); “Murcki Boże Dary” in Murcki (50°11'21" N, 19°02'07" E); and “Wesoła” in Mysłowice (50°10'28" N, 19°5'44" E) with a total area of 170 ha and an altitude of approximately 310–339 m a.s.l. p.m. The by-products of the black hard coal extraction in the coal mine heaps are carboniferous rocks—quarried at a depth of about 1 km. In terms of abiotic conditions, the post-mining heap areas are characterized by limited water availability, low nutrient reserves, high temperature, and high salinity. Compared to other sites of this type (e.g., zinc–lead heaps), the deposited material does not contain high concentrations of heavy metals.

2.2. Vegetation Sample Collection

In order to determine the relationship and potential impact of species diversity on soil carbon emissions, a number of species diversity indices were calculated for the vegetation patches in the areas analysed and these were compared and analysed. On the basis of phytosociological studies, the following indices were calculated: the Shannon–Wiener H' diversity, Evenness uniformity, and Simpson's dominance index. The Shannon–Wiener index (H'), which takes into account evenness and species richness, was used to determine the species diversity of the vegetation patches studied. To determine the probability that two individuals selected at random from a given sample would belong to the same species, Simpson's index was calculated as provided in the next section. During the field work, GPS devices were used, 324 plots were established on the analysed site (Figure 1), and vegetation and soil substrate samples and data were collected.

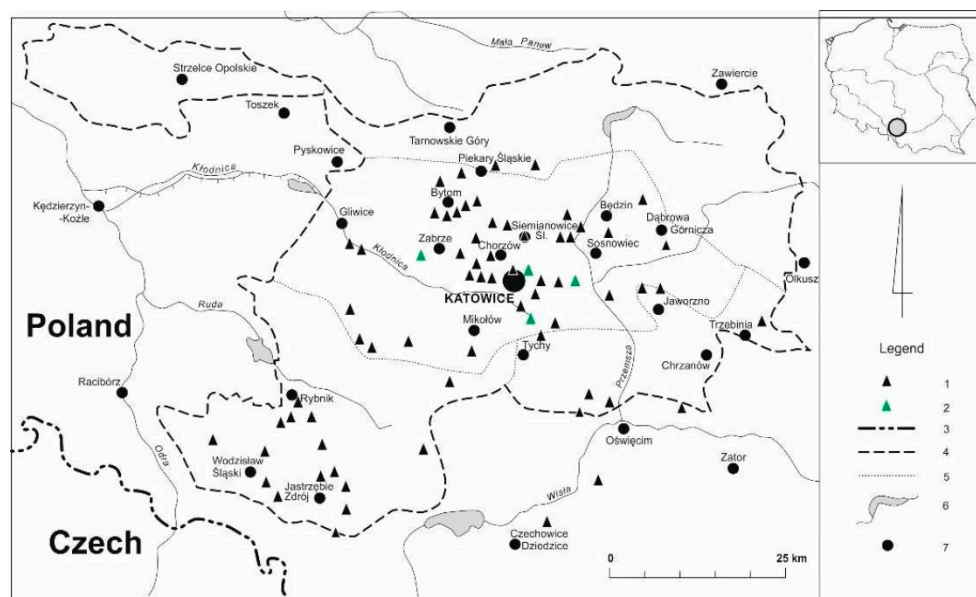


Figure 1. A map of the location of post-coal mine heaps in the area of the Upper Silesia (South Poland). 1—black coal mine heaps; 2—the studied black coal mine heaps; 3—country border; 4—the Silesia Upland border (according to Woźniak 2010); 5—minor country border; 6—lakes and rivers; 7—towns, cities.

2.3. Vegetation Diversity Analysis

This research uses the most commonly used indicators calculated on the basis of the frequency, abundance, and population density of individual species and facilitates the assessment of similarities and differences between vegetation patches. The analysed indicators were selected on the basis of previous studies conducted in such areas. In order to determine the relationship and potential impact of species diversity on CO₂ release, a number of species diversity indices were calculated for vegetation patches in the analysed area of the post-mining heap, and results were compared and analysed. On the basis of phytosociological studies, visual estimates of the cover of individual species, using a percentage scale, were determined. For each plot the total cover of plants and total biomass were also determined. To measure biodiversity, the following biodiversity indices were implemented: number of all species present— S , Shannon–Wiener index (H), Shannon evenness ($H/\log(S)$), evenness uniformity, and Simpson’s dominance index.

The Shannon–Wiener index (H'), was calculated according to the formula [9].

$$H' = \sum_{i=1}^R p_i \ln p_i$$

R —number of species;

p_i —share of individuals of species i in the cover of individuals of all species;

i —cover of this species in the vegetation patch.

The evenness index for vegetation patches occurring in the analysed area was calculated using the following formula [9]:

$$H_{max} = \frac{H'}{H_{max}}$$

H' —the value of the Shannon–Wiener diversity index;

H_{max} —the maximum possible value of the Shannon–Wiener index if each species was equally probable.

In order to determine the probability that two individuals randomly selected from a given sample would belong to the same species, Simpson's index was calculated according to the following formula [9]:

$$H_{max} = - \sum_{i=1}^s \frac{1}{s} \ln \frac{1}{s} = \ln S$$

S —number of species;

p_i —share of individuals of species i in the cover of individuals of all species;

i —share of that species in the vegetation patch.

2.4. Soil Sample Collection

The research was conducted on 324 study plots. The sites were selected using stratified random sampling in which the study population is divided into qualitatively different parts, and then a certain number of units are drawn from each distinguished part (layer).

The sampling method was based on research conducted by Woźniak, 2010, in the same type of habitats [9].

During the field work, research plots with a radius of 3 m were established. The test plots were established in patches of vegetation comprised of various dominant plant species that had been identified during the field work. The control plot was a section of the heap area not covered by vegetation. Substrate was taken from each plot at 3 points, from a depth of 0–10 cm (the root zone). After transporting the samples to the laboratory, they were sieved through 2 mm mesh sieves and then frozen until further analysis [9].

2.5. Soil Biotic Parameters Analysis

Soil Fauna Samples

The plots were delineated on heaps with similar conditions, which was necessary due to the different age of the heaps, size, method of dumping, nature of the surroundings, and high variability of abiotic factors. In order to present the fullest possible range of variability, a network of systematically delineated study plots was used [9]. Three soil samples (approximately 1.5 kg each) were taken from the test plots. By taking soil from different locations, a proper picture of the abundance of soil fauna species was obtained, i.e., the average number of vascular flies occurring on the study plot. The material was stored in string bags at low temperature (6 °C). The *Nematode* and *Enchytraeidae* numbers and their dispersal procedure was carried out using a Tullgren apparatus. Quantitative analysis of the biological material obtained was carried out using a Delta Optical SZH-650T stereo microscope, Delta Optical, Mississauga, ON, Canada.

2.6. Enzyme Activity Measurement

Dehydrogenase activity was determined via the reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) using the method developed by Schinner, 1996. The TTC solution was added to the soil samples being studied. The whole mixture was mixed and then transferred to a thermal chamber for incubation at 37 °C for a period of 24 h. After incubation, the formazan was extracted from the soil with acetone. The colour intensity was examined using a DR 5000 Spectrophotometer–Hach Lange, Loveland, CO, USA. Absorbances were measured at 546 nm. The concentration of TPF produced was then calculated based on the standard curve. Soil dehydrogenase activity was determined based on the amount of formazan obtained per unit weight of the soil per unit time [23]. Urease activity was determined according to the Alef and Nannipieri protocol based on incubating soil substrate samples in urea solution. The activity of this enzyme was determined using a spectrophotometric method that used urea as a substrate. To maintain the appropriate pH, lemon buffer was added to the test samples. The samples were then transferred to a thermal chamber, where they were incubated at 37 °C for a period of 3 h. After the incubation period, the samples were filtered through a hard tissue filter, and 0.75 mL of the

filtrate was collected, to which a solution of sodium hypochlorite and sodium phenolate was added to induce a blue colour. The colour intensity was analysed using a DR 5000 Spectrophotometer–Hach Lange at 580 nm. The assays of acid phosphatase and alkaline phosphatase activity were determined by measuring the p-nitrophenol (PNP) released by phosphatase activity after soil incubation with buffered (pH 6.0 for acid phosphatase and pH 11.0 for alkaline phosphatase) sodium p-nitrophenyl phosphate (115 mM) solution. Absorbances were measured using a DR 5000–Hach Lange spectrophotometer at 400 nm. This was followed by producing a calibration curve, for which a p-nitrophenol solution was used [24].

2.7. Biomass Samples (Cover) Collection

Field studies were conducted in 2018/2019, during one growing season. Plant samples were collected on four post-mining waste dumps in the area of the Katowice Upland, i.e., the “Sośnica” dump in Gliwice, the “Wesoła” dump in Mysłowice, and the “Murcki” and the “Kostuchna” dumps in Katowice. Research plots were delineated in a circle shape with a radius of 3 m, in a homogeneous vegetation patch dominated by a given dominant species. The species that occupied the largest area within the established plot, compared to the rest of the vegetation, was taken as the dominant species. The samples were then packed into string bags. After collection, the samples were immediately weighed using a field scale so that the weight of the fresh biomass of the dominant species and other plants was obtained. In the test plot, a representative square was determined, i.e., containing the dominant species and best representing the entire patch (the coverage of the dominant species and quantitative responses between the dominant and co-occurring species). The test field had a side length of 0.5 m (Figure 2).



(A)

Figure 2. Cont.



(B)



(C)

Figure 2. (A) An example of a disturbed site with initial plant species assembled into vegetation patches. (B) A patch of *Calamagrostis epigejos* vegetation. (C) A distant view of a colonised site (photo. G. Woźniak).

2.8. Respiration Measurement—CO₂ Gas Analyzer

Respiration tests were performed using a Targaz-1 analyser. The analyser performs short-term measurements in a closed system using a ground breathing chamber. The area of the ground breathing chamber was 78 cm² and the closed volume was 1171 cm³. The edge of the soil breathing chamber was pushed into the substrate to a depth of 1–2 cm. The rate of soil CO₂ efflux is calculated on the basis of the CO₂ increase inside the chamber. The nature of the closed chambers causes CO₂ to continue to accumulate, and therefore

measurement periods are reduced to a minimum to achieve a detectable linear concentration increase, avoiding an excessive build-up of CO₂ inside the chamber over time. The results of five measurements of CO₂ concentrations have been averaged within the research field (for a certain site). The range of CO₂ release at the analysed sites was 0.00158–1.21462 [g CO₂/m²/h].

2.9. Data Analysis

All statistical analyses and visualizations were carried out by means of R software (ver. 4.2.2, R Core Team 2022, Vienna, Austria, <https://www.R-project.org>) using the chosen libraries “vegan”, “labdsv”, “corrplot”, and “ggplot2”. Cluster analysis of vegetation data (324 plots) was performed using Manhattan distance and the Ward method as a grouping method. The distance measure was selected based on the ranking of correlation tests between vegetation data and respiration data (four repetitions for vegetation season). The Caliński–Harabasz criterion was used to indicate the most appropriate number of vegetation groups. The classification of indicator species into separate communities and then the merging of two or more vegetation groups was carried out using the statistics of the indicator value, the IndVal method [25], which was modified [26]. The statistical significance of this relationship was tested with a permutation test (999 repetitions). Only the indicator species of site groups with a statistically significant IndVal were presented.

An unconstrained ordination method—Detrended Correspondence Analysis (DCA)—was undertaken to demonstrate the species variation under the influence of soil respiration (the mean annual value), the total cover of plants in a plot, the total biomass, and biodiversity indices. The passive projection of these variables was performed using the Monte Carlo test with 999 permutations. The inter-correlations among biodiversity indices, biomass, and cover were calculated using the Spearman rank correlation matrix. A Kruskal–Wallis test, followed by a Conover test for pair-wise comparisons, was used to check the significance of differences among distinguished vegetation patches in terms of biodiversity indices and cover, biomass, and SRL.

3. Results

The studied vegetation patches, based on the species composition, have been arranged into five groups (Figure 3A). The distinguished vegetation patch groups can be characterized as 1—*Arenaria serpyllifolia*; 2—*Phragmites australis*; 3—*Tussilago farfara*; 4—*Hieracium pilosella*; and 5—*Solidago gigantea*.

In total, 47 species were significant indicator species for at least one vegetation group. The most common indicator species for each one of five groups are presented in Table 1. The transitory species, i.e., indicator plants for at least two vegetation groups are shown in Table 2. The first group has no indicator species while group no. 2 has 14 species. The species *Daucus carota*, *Hieracium piloselloides*, and *Medicago lupulina* were significant indicator species for four of the five distinguished vegetation groups (Table 2).

Table 1. The list of the vegetation types (dominant plant species) with their individual IndVal value, calculated for the group vegetation patches, based on the distance measure selected on ranking of correlation tests between vegetation plant species composition data and the data on respiration (four repetitions for vegetation season).

Group	IndVal Value	p-Value
Group 2 <i>Phragmites australis</i>	0.994	0.001
<i>Bidens frondosa</i>	0.378	0.001
<i>Lathyrus sylvestris</i>	0.378	0.002
<i>Puccinellia distans</i>	0.365	0.006
<i>Plantago major</i>	0.363	0.006
<i>Phalaris arundinacea</i>	0.339	0.009
<i>Urtica dioica</i>	0.33	0.011

Table 1. Cont.

Group	IndVal Value	p-Value
<i>Chelidonium majus</i>	0.267	0.041
<i>Eleocharis palustris</i>	0.267	0.033
<i>Polygonum hydropiper</i>	0.267	0.033
<i>Rorippa sylvestris</i>	0.267	0.033
Group 3 <i>Tussilago farfara</i>	0.969	0.001
Group 4 <i>Hieracium pilosella</i>	0.35	0.03
Group 5 <i>Solidago gigantea</i>	0.901	0.001
<i>Tanacetum vulgare</i>	0.661	0.001
<i>Hypericum perforatum</i>	0.637	0.001
<i>Melilotus albus</i>	0.512	0.015
<i>Vicia hirsuta</i>	0.48	0.001
<i>Carex spicata</i>	0.471	0.001
<i>Carex hirta</i>	0.457	0.002
<i>Deschampsia caespitosa</i>	0.437	0.002
<i>Astragalus glycyphyllos</i>	0.426	0.003
<i>Melandrium album</i>	0.399	0.002
<i>Lathyrus pratensis</i>	0.333	0.005
<i>Linaria vulgaris</i>	0.329	0.011
<i>Rosa canina</i>	0.316	0.012
<i>Leucanthemum vulgare</i>	0.252	0.047

Table 2. The indicator species analysis, ISA, based on species abundance and frequency.

Group	Stat	p-Value
	Group	1 + 4
<i>Arenaria serpyllifolia</i>	0.509	0.004
	Group	2 + 5
<i>Poa palustris</i>	0.515	0.001
<i>Agrostis gigantea</i>	0.483	0.001
<i>Elymus repens</i>	0.423	0.001
	Group	4 + 5
<i>Calamagrostis epigejos</i>	0.923	0.001
<i>Erigeron annuus</i>	0.574	0.003
<i>Achillea millefolium</i>	0.564	0.001
<i>Cirsium arvense</i>	0.509	0.023
<i>Vicia tetrasperma</i>	0.458	0.006
<i>Centaurea jacea</i>	0.287	0.036
	Group	1 + 2 + 4
<i>Lotus corniculatus</i>	0.529	0.023
	Group	1 + 3 + 4
<i>Chamaenerion palustre</i>	0.619	0.008
<i>Echium vulgare</i>	0.585	0.021
	Group	1 + 4 + 5
<i>Poa compressa</i>	0.659	0.001
<i>Centaurea stoebe</i>	0.603	0.015
	Group	2 + 3 + 4
<i>Pinus sylvestris</i>	0.425	0.036
	Group	2 + 4 + 5
<i>Epilobium</i> sp.	0.356	0.024
	Group	1 + 2 + 3 + 4
<i>Daucus carota</i>	0.772	0.002
<i>Hieracium piloselloides</i>	0.728	0.001
	Group	1 + 2 + 4 + 5
<i>Medicago lupulina</i>	0.607	0.003

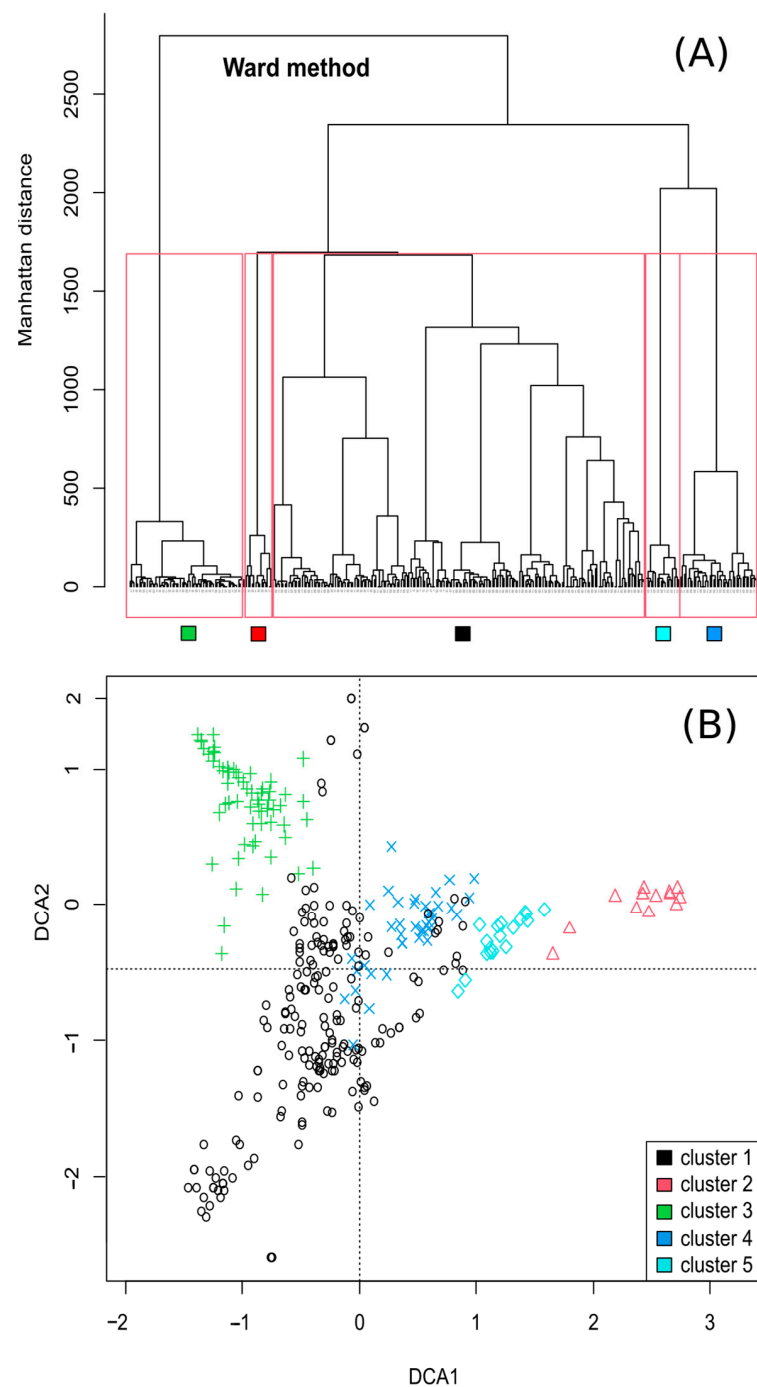


Figure 3. Cluster analysis based on Manhattan distance (A) and Ward method. The biplot of DCA with ordination of distinguished vegetation groups (B). Explanation of abbreviations: cluster 1, *Poa compressa*; cluster 2, *Tussilago farfara*; cluster 3, *Daucus carota*; cluster 4, *Chamaenerion palustre*; cluster 5, *Phragmites australis*.

The five groups are distinctively distributed along the strongest gradients revealed in the detrended correspondence analysis (Figure 3B). The distribution of the studied vegetation patches, in the ordination space of the detrended correspondence analysis, reveals the distance between cluster 2, cluster 3, and cluster 1, while the vegetation patches grouped in the clusters 4 and 5 and part of cluster 1 are in between the middle crossing of the main gradients (Figure 3B).

The unconstrained ordination DCA biplot of species scores along the first two DCA axes, while the passive projection of significant explanatory variables (Figure 4A) showed

that cover and the biomass of species are strongly correlated, mostly along the first axis, whereas the biodiversity indices are correlated with the second axis of the DCA. The main factors explaining the variability of the studied patches are soil respiration and H. The vector representing the SRL index is parallel to the DCA 1 axis along which species belonging to clusters 4 and 5 are differentiated (Table 2). The vector representing the H index is the second most important factor differentiating the studied species, around which the species represented by cluster 1 are clustered.

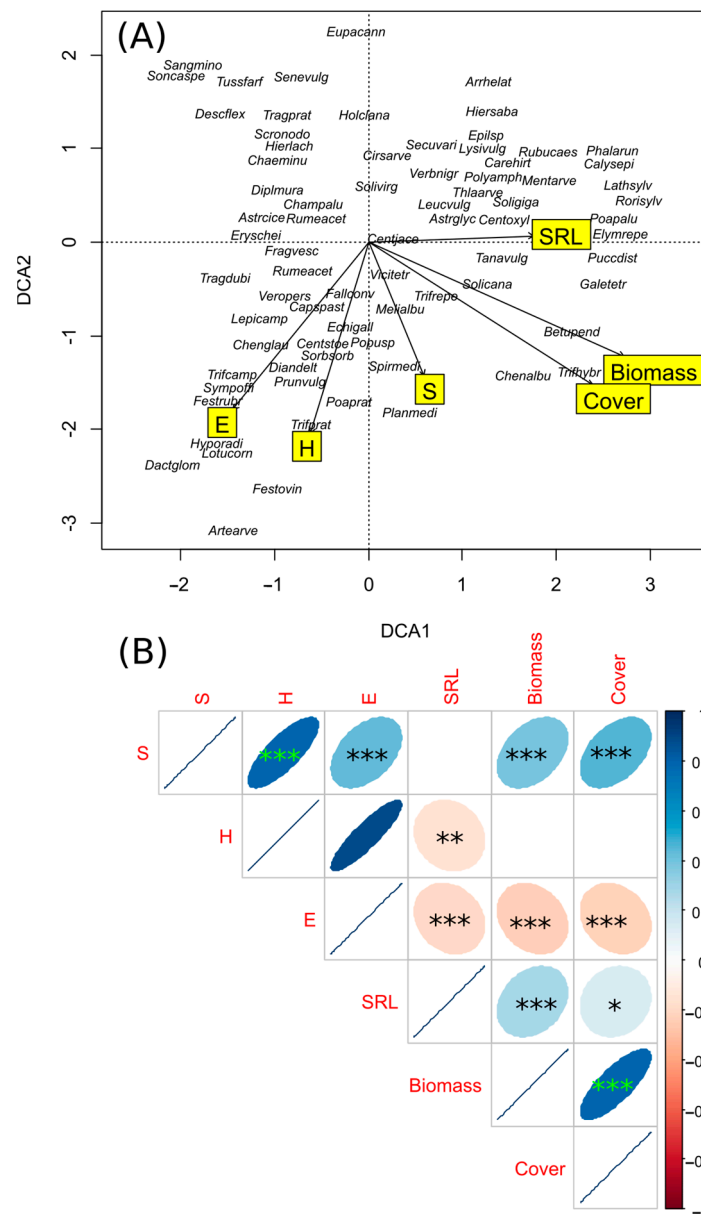


Figure 4. The unconstrained ordination DCA biplot of species scores along the first two DCA axes, with the passive projection of significant explanatory variables (A) and matrix correlation (Spearman rank correlation) of the studied diversity indexes and biomass measurements’ variables (B). E—Evenness index; H—Shannon–Wiener diversity index; S—Simpson’s index; SRL—CO₂ release. *— $p < 0.05$, **— $p < 0.01$, ***— $p < 0.001$.

Spearman analysis showed a positive correlation between the SRL respiration index and H and E, biomass and E, and cover and E. There is a strong positive correlation between the CO₂ release and the biomass of all the studied vegetation patches. However, a strong negative correlation has been revealed by the evenness diversity index and Shannon–

Weiner diversity index values. Additionally, the Spearman rank correlation showed a strong correlation between the biomass measurements and the cover value (Figure 4B).

The significance of the relationship between the respiration parameters and the part of the biotic parameters related to varied diversity measurements (diversity indexes) and abundance measurements (cover and biomass) was the inspiration to perform a more detailed analysis (Figure 5).

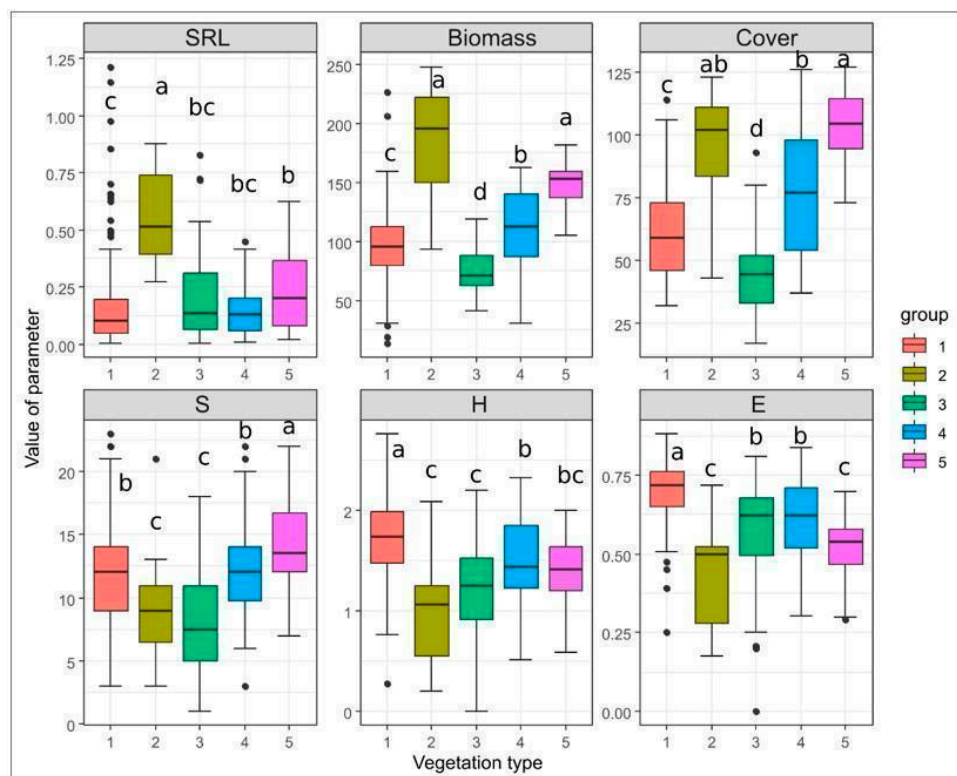


Figure 5. The comparison of the five distinguished vegetation types in terms of the six analysed parameters (SRL soil respiration; Biomass; Vegetation cover; S—species richness (number of species in a patch); H—Shannon–Weiner; E—Evenness); (Kruskal–Wallis test and Conover test for multiple comparisons). The groups with the same letters do not differ significantly at $p < 0.05$. Abbreviation explanation of the vegetation type coding: 1—*Arenaria serpyllifolia*; 2—*Phragmites australis*; 3—*Tussilago farfara*; 4—*Hieracium pilosella*; 5—*Solidago gigantea*.

The graph shows the results of statistical analyses in terms of the differences among distinguished vegetation groups in terms of diversity, biomass, cover, and respiration. These four biotic parameters are shown to be significantly higher in group 2. In terms of the vegetation diversity indexes such as the analysed S-index, H-index and E-index, the values are significantly higher in group 1. The group of vegetation also presenting the largest deviation from all analysed groups, compared with the other groups, was group 5 in particular.

The significant Spearman rank correlation between the soil respiration CO₂ release and biomass and urease is positive, while the correlation is negative with Enchytraeidae (Figure 6).

The analysis of the impact of the biotic variables showed (Figure 7) that all of the analysed vegetation groups are significantly statistically different in terms of the soil enzymes studied. In the quantitative index of Enchytraeidae, there were significant statistical differences between groups 1, 2, and 4, but groups 3 and 5 are not statistically significantly different from each other. In terms of nematode quantitative index, significant statistical differences were shown between groups 1, 3, and 4, while groups 2 and 5 were not statistically significantly different from each other.

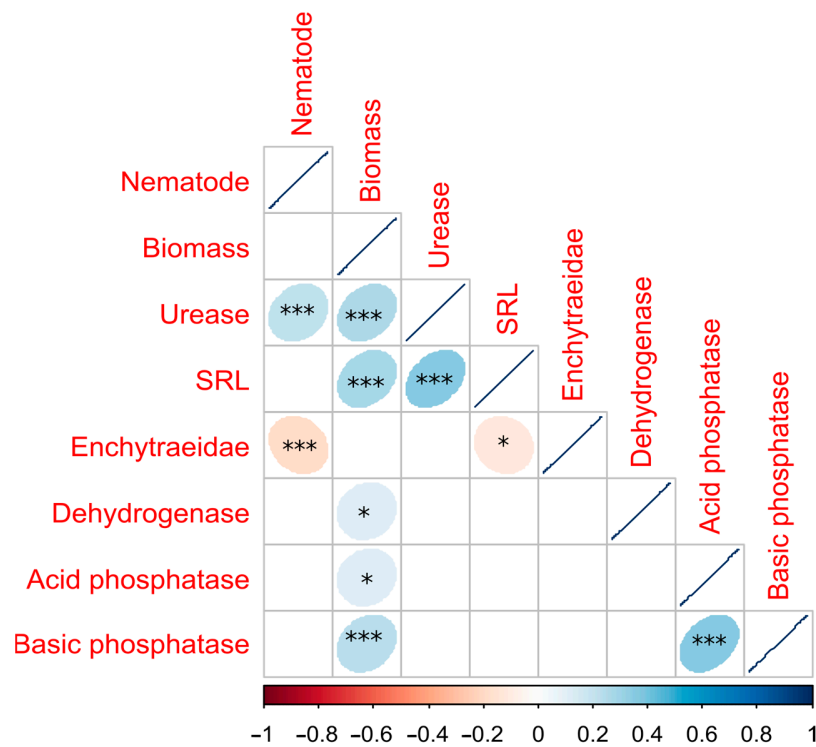


Figure 6. The graph indicates the relationships among the analysed biotic variables including the amount of biomass, the presence of nematodes, the analysed soil enzyme activity, and soil respiration. *— $p < 0.05$, ***— $p < 0.001$.

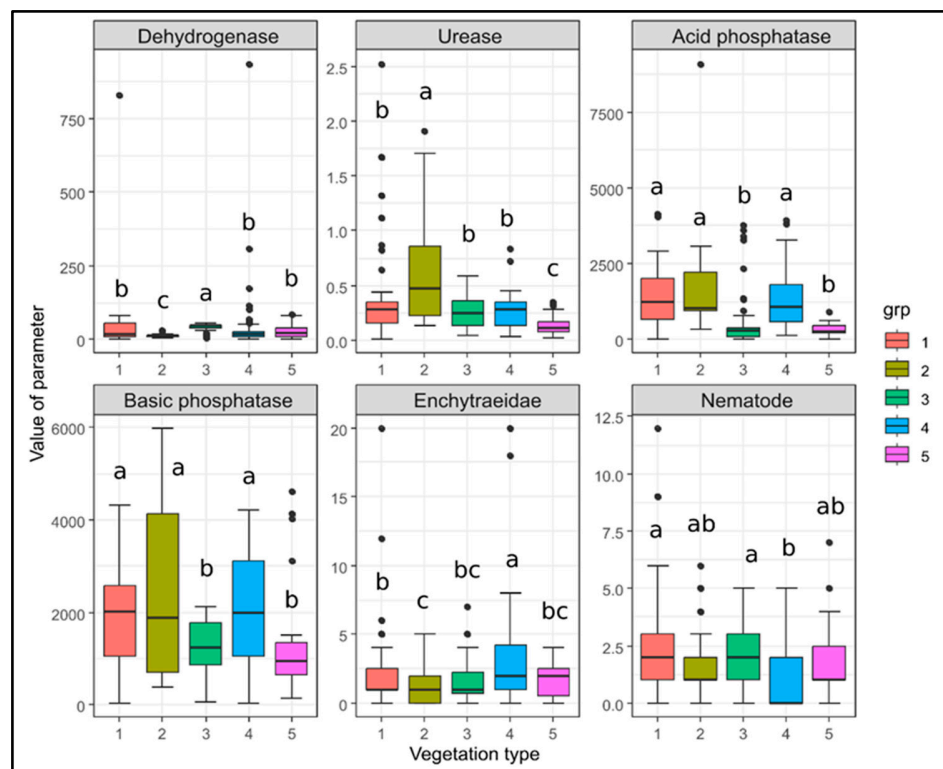


Figure 7. The comparison of selected biotic variables among distinguished vegetation types (Kruskal–Wallis test and Conover test for multiple comparisons) is provided. The groups with the same letters do not differ significantly at $p < 0.05$. Abbreviation explanation of the vegetation type coding: 1—*Arenaria serpyllifolia*; 2—*Phragmites australis*; 3—*Tussilago farfara*; 4—*Hieracium pilosella*; 5—*Solidago gigantea*.

4. Discussion

In our study, we analysed a variety of biotic habitat parameters concerning the soil respiration rate. Among the biotic site characteristics, the diversity indexes of the vegetation species composition of the studied vegetation types, the SOM content, the enzyme activity, the mesofauna occurrence, and the cover percentage, along with the amount of biomass in the recorded vegetation types, were analysed in the novel post-coal mine ecosystems. It is known that natural or anthropogenic disturbances (e.g., logging, agriculture, urbanization, land-use changes, mineral resource excavation) often alter the soil profile, and the habitat conditions change in terms of carbon stocks and carbon fluxes [27]. The magnitude of change in the soil or soil substrate CO₂ flux depends on the amount and quality of litter and organic layers, disturbance by roots, or the admixture of mineral soil horizons that are exposed to oxygen. When organic matter, which is almost pure organic carbon, is exposed to oxygen, the organic carbon is oxidized to CO₂ and an adequate flux of energy is released.

4.1. The Unusual Conditions of Novel Ecosystems

Mineral extraction significantly transforms the natural landscape. Complicated habitat conditions are the basis for the implementation of research focused on understanding the new ecosystems, the complexity of the relationships between bacteria, plants, and abiotic conditions, and the broadening of the limited understanding of these relationships in natural and semi-natural ecosystems. At the same time, human industrial and mining activities are creating entirely new challenges [2,28]. Open-pit mining and the storage of minerals from underground mining are causing the removal of the existing vegetation and a change in soil composition and structure, affecting hydrological conditions [21,29–33]. Human activities in some places cause fundamental changes beyond the particular biogeochemical thresholds of habitats. These new habitats with different plant compositions appear to be examples of the new ecosystems [10,19,22,34,35]. Living conditions in coal mine heaps vary significantly in terms of moisture content, grain size, and salinity, not to mention differences in slope, elevation, and shape. The diversity of habitats within a coal mine heap is often much greater than between two or more heaps. The mosaic of microhabitats (e.g., grain size, moisture content, salinity) is reflected in the mosaic of plant composition [21,36–38]. Studies of the different species composition of vegetation on the mineral material of mining sites reveal new ecosystems that differ from the surrounding non-industrial areas [39,40]. New ecosystem habitats offer a unique opportunity to study the primary succession processes in broad and specific habitat conditions [7,19,34,37,41,42].

4.2. Diversity of Plant Species Composition and Soil Respiration

In natural and semi-natural non-disturbed ecosystems, the plant species composition of the studied communities plays a crucial role in controlling soil respiration. Vegetation plant species composition is the only way carbon enters the soil and determines the microbial communities' composition. The variety of different plant species assemblages enriches soil autotrophic respiration by intensifying metabolic rates and fine root biomass [10]. In our study the recorded vegetation type diversity has been grouped into five clusters based on the similarities in plant species composition and the background soil substrate respiration parameters. The identified vegetation type groups revealed significant differences. The respiration is significantly higher in the vegetation type cluster in group 2 in comparison to the much lower respiration rate in the vegetation type clustered in groups 1, 3, 4, and 5.

The autotrophic diversity promotes soil heterotrophic respiration due to a greater variety of nutrient and carbon resources available for soil microorganisms [43]. The structural complexity of the vegetation can indirectly influence soil respiration by changing environments (e.g., light intensity, high cover, and shading of the lower layers), plant diversity [44], and soil temperature variability [45]. Except for the differences in root respiration, plant species communities' diversity mainly influences soil respiration via the quality and quantity of the plant input on the remaining biomass biochemistry and, as a result, the attraction of the available microbes [46]. It is possible that soil microbial diversity and variety, derived

from plant species diversity, has a crucial impact on the cycling of carbon and nutrients in the soil in terrestrial ecosystems. Some empirical studies have revealed that a loss of microbial diversity caused higher velocities of soil microbial respiration [35,39,47]. Soil microbial diversity and community composition is closely related to the diversity of plant species composition [48]. How above- and below-ground diversity collectively shapes seasonal differences in soil respiration at local scales is unknown [35]. The vegetation percentage cover, the biomass amount, and soil organic carbon (SOM) provide comparative indices. Our respiration measurements were conducted at the same time of year (August). In late summer, the vegetation percentage cover and the above-ground biomass is the highest in group 2 and the lowest in group 3. The recorded differences are statistically significant.

The seasonal changes in soil respiration are also related to the seasonality of the vegetation plant species composition in the temperate zone [49–51].

4.3. Soil Enzyme Activity and Respiration in the Studied Vegetation Types

The temperature increases could increase extracellular enzyme reaction rates and, consequently, the decomposition process [14,52–55]. If warming increases decomposition, soil microbial communities could drain soil C pools [56–59]. Soil enzyme activity is suggested to be the indicator of soil quality, which can reflect the changes in the ecosystem's health. Ecosystem health can be characterized by soil enzymes closely related to crucial soil-quality parameters such as biomass, soil organic matter, the resulting microbial activity, and soil physical properties [60].

Theoretical models suggest that increased soil respiration results in a rapid decomposition of the carbon pool—soil respiration typically returns to pre-warming levels [6]. Two hypotheses explaining the observed decline in respiration after warming are substrate depletion and the thermal adaptation of soil microbial communities [56,61,62]. The substrate depletion hypothesis suggests that energy-intensive enzyme production should only begin when the substrate inhibits microbial growth and is ready for degradation [63,64]. Soil respiration reaches a steady state if unstable carbon is rapidly consumed in response to warming, and carbon resists degradation at the same rate [15,65–67]. With enzymatic changes, respiration will increase until the labile substrate C is depleted, and then decrease, or return to the initial temperature, before the bacteria have switched from a labile substrate to resistant substrate. The thermal adaptation hypothesis suggests that microbial communities will adapt to warming and change their structure to utilize non-catalytic substrates after labile substrate depletion [68]. Heat-adapted microbial communities can maintain enzyme production even with reduced soil respiration, allocating more C for growth [14]. Enzyme activity is likely closely related to the overall demand for C, N, and P and is not regulated by the availability of a single target enzyme [64].

The SOM quality and biochemistry affect the energy supply for microbial growth and therefore enzyme release. Some studies show positive correlations between enzyme activities and SOC and T to N in human-disrupted areas [69,70]. Baldrian et al. (2008) [71] found that during spontaneous succession on heaps, established after brown coal extraction, the presence of SOC and T to N in the topsoil layer significantly influenced enzyme activities. In the few studies performed on the mineral material of deep post-black coal mine heaps, results show no significant correlations between the amount of SOC in the mineral soil substrate and dehydrogenase activity, or between SOC and the activity level of acid or alkaline phosphatases [72]. However, some studies showed a negative correlation between urease activity and SOC [72–74]. The SOM in the mineral soil substrate of the post-coal mine heaps might be rich in carbon related to organic matter of recent or geogenic origin [37,75–78]. The black, hard, geogenic coal is not available to microorganisms, regardless of its high quantity of organic carbon (loss of ignition analysis of 10–18%) [79]. The mineral post-coal mine substrate had low carbon sources for microorganisms [76,78]. Typical for mineral habitats, the limited amount of carbon available for microorganisms in the total habitat pool of SOC may influence the lack of correlation between SOC and the activity of the studied soil enzymes [72]. The results of some studies have revealed that soil pH

influences enzyme activity and soil microbial community structure [73]. Some studies have detected positive correlations between dehydrogenase and alkaline phosphatase activity, soil functional diversity, and substrate pH. In our study, the activity of soil enzymes such as dehydrogenase, acid phosphatase, and the alkaline phosphatase is positively correlated with the amount of CO₂ released. There was no positive correlation between urease activity and soil respiration.

Tests of soil respiration have shown that the respiration is seasonally specific, i.e., there is a decrease in summer [80] or an increase in winter [81]. The soil respiration changes with long histories of N deposition [82,83]. In forest ecosystems the herbaceous understory plants are often make up the largest part of plant diversity in deciduous forests. In this respect the herbaceous plants in forests can contribute significantly to total forest net primary productivity (NPP) and consequently contribute significantly to the soil organic matter (SOM) [84,85]. In this way the forest understory should be considered as an important focus for understanding the effect of warming on soil C storage.

4.4. Soil Organic Matter and Respiration in the Studied Vegetation Types

The majority of the study focused on the relation between the soil organic matter and the soil respiration process, considering the temperature and moisture conditions influencing the process. The example of *Calamagrostis epigejos* protein quality and quantity composition has shown significant differences between individuals of the same species in response to severe habitat factors [86].

Soil organic matter (SOM) derives mainly from above and below-ground organic matter synthesized by plants. The foundation of SOM establishment is well studied, but it is still unknown how the litter's biochemical structure and composition influence the formation of new SOM and the decomposition of already existing SOM [11,84]. There are few studies on the separation of the effect of plant litter composition on carbon transference from different plant tissues into specific SOM fractions, and the determination of the magnitude of the effect on already existing SOC caused by litter amendments could be very informative. The effect of the different litter types, such as bark, leaves, twigs, and roots in the soil rhizosphere zone, has been studied [87].

The origin and amount of organic carbon influence the presence, metabolic activity, and functional diversity of the microbial assembly in mineral habitats, such as organic carbon availability in poor mineral habitats in, for example, sandy soils [35].

However, it remains difficult to separate root respiration (including rhizosphere microorganisms) from microbial respiration under field conditions. To date, various in situ approaches have been used, ranging from crude soil exposure to very specific requirements such as changing the carbon isotopic signatures of the two components of respiration (as a result of altering the photosynthetic pathways of vegetation or manipulating isotopic tracers). The autotrophic portion of respiration in intact roots has been measured in the field using root cuvettes [88,89]. A different approach has been used when the autotrophic part of the respiration has been measured with excised roots in the laboratory [90]. In another study the method of trenching labelling with ¹⁴C, ¹³C, or ¹⁸O has been applied [91], inhibiting one respiratory component [92,93] has been used to separate the root from microbial respiration. However, the ratio between the two respiration components is generally quite site-specific and varies between 1:9 and 9:1 [94].

4.5. The Amount of Biomass and Respiration in the Studied Vegetation Types

The plant species composition in the vegetation patch can be assessed by estimating the percentage cover of the whole plant community and each of the plant species separately. Apart from the percentage cover of the vegetation, the biomass weight (dry and wet) is measured. The biomass can be divided into the dominant plant species weight and the weight of the rest of the non-dominant plant species. All of the biomass, and thus the amount of carbon that accumulates in the vegetation types in each ecosystem, is established due to the balance of photosynthesis (the whole primary production, (P), and the plants'

(autotrophs) respiration, the energy needed for the plant growth and living processes). The difference between these fluxes is the net primary production (Pn) [95,96].

In our study we have performed a comparison of the identified vegetation group types, vegetation cover, and the amount of biomass in relation to the respiration parameters. The results revealed that the vegetation biomass was the highest in vegetation group cluster 2, and is significantly higher and different from the 1, 3, 4, and 5 vegetation group clusters.

In our study, we conducted a comparison of the identified vegetation groups, types, vegetation cover, and the amount of biomass in relation to respiration parameters. The amount of biomass is not linearly correlated with CO₂ release from the soil.

Based on the metabolic scaling theory, the hypothesis suggests that respiration should scale with biomass [97]. According to some studies [98,99] respiration scales with whole-plant carbon (C) or nitrogen (N) content. In this approach, the scaling is similar within and among different species, irrespective of environmental and climatic conditions, which might influence the normalization constant, but not the exponent. The traditional view of forest dynamics assumed the isometric scaling of respiration with biomass set out, for example, by Kira and Shidei (1967) [100] and Odum (1969) [101]. In the absence of significant disturbances, if respiration increases in parallel with biomass, primary productivity (Pn) necessarily declines because primary productivity (Pn) cannot increase indefinitely but becomes relatively stable at canopy closure in forest ecosystems [102].

For forest ecosystems, many vegetation models simulate plant respiration considering respiration R to be a fixed fraction of (phosphorus) P. Others more explicitly relate respiration (R) to the amount of biomass, and thus only indirectly to P [96]. The calculation based on the theoretical approach of the two above hypotheses produces quite different results. The differences in the obtained results caused by both hypotheses (and their supposed underlying mechanisms) have been subject to criticism [102–105].

This study was performed on the comparison of the identified vegetation group types and vegetation cover in relation to the respiration parameters. The results revealed that vegetation percentage cover was the highest in vegetation group cluster 5, and is significantly higher and different from the other three vegetation group clusters. No significant differences have been identified between the recorded vegetation cover in the vegetation group clusters 2 and 4. In our study, we conducted a comparison of identified vegetation groups, types, vegetation cover, and the amount of biomass in relation to respiration parameters. The amount of biomass is not linearly correlated with CO₂ release from the soil.

The vegetation percentage cover, and the biomass quantity and quality determine a considerable CO₂ flux within the soil or soil substrate respiration in terrestrial ecosystems and between the biosphere and the atmosphere [103]. Soil CO₂ fluxes include autotrophic root respiration and heterotrophic microbial respiration in the soil. Information on soil CO₂ fluxes and on factors that govern these fluxes are needed to constrain the ecosystem carbon cycling and to decide whether terrestrial ecosystems are carbon sinks or sources [104,105]. Other factors, such as land-use change, can also enhance or reduce soil CO₂ fluxes. Changes in precipitation (moisture) and temperature, as well as changes in habitat conditions or management practices, will impact soil respiration fluxes and the carbon budget of terrestrial ecosystems [18,106].

4.6. Soil Fauna and Soil Respiration in the Studied Vegetation Types

The soil fauna improves litter decomposition at the global biome and ecosystem scales (average enhancement of 27%) [107,108]. The soil fauna is a vital element of ecosystems because of its functional position in biogeochemical processes in accelerating the efficiency of biomass and litter decomposition and nutrient conversion [109].

The data collected on species composition, mesofauna abundance, and biomass allowed us to look for evidence of interrelationships between the biotic elements of the study sites. Relatively strong interactions were detected between the biomass and abundance of the studied soil organisms. The abundance of Vasonomads and nematodes on vegetated

sites is relatively low compared to natural ecosystems. The formation of the first links of succession on a substrate almost completely devoid of life, or on one where it has been forming for a short time, is one of the most interesting issues of environmental biology. Succession processes can be observed, among other things, on various types of post-industrial heaps. Research on succession has been carried out for many years. However, most of the studies mainly concern plants. Studies on the groupings of soil fauna at different stages of succession are rare [31].

As indicated by Nielsen et al., 2014, local nematode abundance was related to soil characteristics, and no relationships were noted between colony richness and environmental or climatic variables. Family composition was related to the average annual precipitation and temperature, suggesting that weather conditions (climate) are a good indicator of local assemblage structure. As a result, climate change could have a significant impact on nematode assemblages, with potential impacts on ecosystem functioning [110].

Soils or soil substrate in below-ground ecosystems are composed of diverse organisms including invertebrate fauna that are responsible for the global turnover of biomass dead organic matter [1,111,112]. The soil fauna provide key ecosystem processes significantly influencing the decomposition of organic matter and the matter and energy flow, enabling the recycling of nutrients [40,111,113]. Some groups of soil macrofauna represent saprophage organisms and contribute to litter decomposition, for example the litter-feeding nematodes [110,114]. Macro- and mesofauna are mostly saprophagous whereas some mesofauna and some larvae are fungal and bacterial feeders [40,113]. The diversity in soil fauna composition changes the feeding activity and alters the environmental conditions in the topsoil and thus influences the composition and diversity of soil microorganisms [112,113].

Some of the diversity might be partly related to methodological differences [115]. Soil temperature and soil moisture are among the most critical factors controlling the CO₂ flux [18,103,106]. Soil substrate quantity and quality, and soil texture, have also been shown to have an effect [116]. There are many studies conducted on how to model the influence of these factors on soil respiration [18]. The variability of soil CO₂ fluxes and some of the underlying processes are well known, but they still involve uncertainties that need to be resolved.

4.7. The Environmental Novelty of the Coal Mine Heaps—Why Understanding the Novel Ecosystems Processes Is Important

Many discussions have focused on the definition of novelty in novel ecosystems. As a result, some concepts of the description of the novelty are presented [117].

According to some researchers, novel ecosystems represent new ecological entities, and they should be identified and enhanced according to their own independently developed biological, ecological, and environmental rules [34]. Following the novel ecosystems concept and the pathways to the establishment of the best adjusted and adapted organisms, and their relationships, will bring more benefits to the functioning of the environment (ecosystem services and human quality of life). The establishment of novel ecosystems can be considered better than taking the risk of losing time and financial resources to restore, for example, post-mineral excavation sites to their previous state [117,118].

Understanding of the processes and the functioning and the nature of novel ecosystems needs further study. Apart from the non-analogous species composition of vascular plants, biological analyses of the biotic parameters of the mineral substrate, such as the activity of soil enzymes, the functional diversity of bacteria, and the primary producers have been performed. The studies of vascular plant species' functional traits (morphological, biochemical, or physiological) identified that the adaptations that enable them to colonize harsh habitats are necessary [86].

Apart from studying the natural processes occurring in the post-coal mine heaps, there are also studies presenting an alternative approach. There is a study regarding the strategy for restoring the potential for the use of metal-leaching technologies. There are studies focused on completely different points of view, underlining that most ore processing

by-products can be used in production. The results of studies on bringing non-extracted metals into the solution are presented in some studies [119]. That study does not consider any biological processes but presents the advantages of leaching in disintegrator mills and identifies the possibilities of implementing the techniques for resource-saving in order to obtain economic benefits [119]. In a study by Golik et al., (2023) no environmental aspects were considered. The biological potential of the oligotrophic habitat of the mineral material is not mentioned in similar papers. The quantitative strength parameters of the hardening mixture, as a result of the alternative methods, were presented [119].

On some post-industrial sites, reclamation is performed. It is crucial to understand the mechanisms influencing the response of vegetation species composition, and soil or soil substratum, to reclamation procedures [120]. Ren et al., (2023) in their study, used *Medicago sativa* L. (alfalfa), as an indicator. In this study a total of 41 soil and 70 vegetation sampling points were analysed. In the Ren et al. (2023) study the habitat records including soil environmental factors (soil temperature, ST; soil water content, SWC; soil organic carbon, SOC; total nitrogen, TN; available phosphorus, AP; available potassium, AK; bulk density, BD; pH) and the alfalfa growth indicator (above-ground biomass, AGB) were recorded [120].

5. Conclusions

Novel ecosystems such as those developing on post-black coal mine heaps are fulfilling all the criteria used as the prerequisite of the novel ecosystem definition. The ecosystems developing, based on the non-analogous spontaneous vegetation species composition, can provide significantly different systems for functioning. Some of the relationships between the biotic parameters and soil respiration intensity are different in comparison to those occurring in natural and semi-natural vegetation types and their dependent ecosystems.

The diversity of vegetation species composition, measured by the use of varied indexes, decreased with the mineral soil substrate respiration intensity.

Our results revealed that, in accordance with our expectations, the vegetation types with a higher amount of plant biomass respire more intensively. The study has shown that this relationship in post-coal mining novel ecosystems is positively strong and statistically significant between the vegetation biomass and spoil substrate respiration parameters. In the vegetation types with a lower amount of plant vegetation biomass present in the mineral soil substratum respiration is less intense. In the studied vegetation types, the spontaneous patches of *Phragmites australis* presented the highest biomass in the dominant vegetation patches.

The activity of soil enzymes such as dehydrogenase, acid phosphatase, and alkaline phosphatase does not increase significantly with the amount of CO₂ released. Only urease activity is increased with the mineral soil substrate respiration of coal mine heaps.

Only the number of Enchytraeidae increases with the release of CO₂. In the other soil fauna, the number of nematodes showed an increase with the release of CO₂ from the mineral soil substratum of the coal mine heaps' novel ecosystems.

The study illuminates the intricate relationships between abiotic conditions and CO₂ respiration rates in coal mine heap habitats. The findings underline the necessity of considering a multitude of environmental factors in predicting CO₂ release accurately.

As ecosystems continue to evolve in response to the disturbances caused by anthropogenic activities, the study's insights are crucial for informed ecological management. Understanding the complex interplay of factors impacting CO₂ respiration can guide strategies to mitigate carbon emissions and foster sustainable land management practices.

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4.3. Factors driving plant diversity in the spontaneous vegetation of the novel ecosystem of post-coal mining spoil heaps and their relationship with soil respiration

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Factors driving plant diversity in the spontaneous vegetation of the novel ecosystem of post-coal mining spoil heaps and their relationship with soil respiration

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Abstract: The subject of the paper is the analysis of the relationship between spontaneous vegetation diversity and soil respiration in novel post-coal mine ecosystem. In the natural and semi-natural ecosystems, soil respiration process (R_s) is a crucial ecosystem function regulating terrestrial ecosystems' carbon cycle. Soil respiration depends on the quality and quantity of the soil organic matter (SOM), the soil microbes' activity, and root metabolism. The listed factors are directly related to the composition diversity of vegetation plant species (biochemistry). For many years, soil respiration parameters have been studied in natural and seminatural vegetation communities and ecosystems. However, there still need to be a greater understanding of the relationship between vegetation plant species diversity and soil respiration as a crucial ecosystem function. Plant species diversity has to be analysed through both the taxonomic diversity and the functional diversity. These approaches reflect the composition, structure, and function of plant species communities. We hypothesise that the diversity of the spontaneous vegetation species composition shapes the amount of soil respiration in a post-coal mine novel ecosystem. The soil respiration differs significantly along the vegetational types driven by habitat gradients and is significantly higher in highly functional richness and dispersion vegetation patches. Contrary to our expectation, soil respiration was the highest in the less diverse vegetation types – both taxonomical and functional evenness were non-significant factors. Only functional dispersion is weakly negative correlated with soil respiration level (SRL).

Keywords: coal mining heaps, disturbed sites, functional diversity mineral habitats, non-analogous species composition, soil respiration, spontaneous vegetation species composition, taxonomic diversity

INTRODUCTION

Soil respiration (R_s) is a crucial ecosystem function, regulating terrestrial ecosystems' carbon cycle (Chen and Chen, 2019). It depends on the quality and quantity of the soil organic matter (SOM), soil microbes' activity, and root metabolism. The above-ground and below-ground ecological processes are connected by soil respiration (Wang *et al.*, 2021). Soil respiration, as one of the key processes in ecosystems, is related to ecosystem productivity,

soil fertility, and the regional and global carbon cycle. As the global carbon cycle regulates climate change, soil respiration is also relevant to climate change, carbon trading, and environmental policy. In the natural and semi-natural ecosystems, soil respiration is now a multidisciplinary issue of interest to ecologists, soil scientists, microbiologists, agronomists, and climate scientists. Soil is one of the largest carbon reservoirs on Earth, storing more carbon than the atmosphere and terrestrial vegetation combined. Therefore, the mechanism of soil respiration

is of great importance to the Earth's carbon balance and ultimately to the rate of climate change. It is also important to understand to what extent the factors influencing, and the relations driving, soil respiration are different in novel ecosystems.

The interest in how plant diversity influences ecosystem function (Newbold *et al.*, 2020) has increased recently. For a long time, many ecosystem functions have focused on productivity and biomass quantity (McKee, 1970). The second commonly studied aspect of ecosystem functioning is soil nutrient cycling (Duffy, Godwin and Cardinale, 2017). Both productivity and decomposition are related to respiration parameters (Handa *et al.*, 2014). However, until now, less attention has been paid to understand how plant diversity can affect soil respiration amount. The relationship between plant species diversity and soil respiration can be direct or indirect through soil factors (such as soil water content, temperature, SOM parameters, and nutrients) (Metcalfe *et al.*, 2011).

The direct effects of plant species diversity on soil respiration in natural and near-natural vegetation communities is still limited, however the understanding of the relationship between plant species diversity and ecosystem function such as soil respiration is crucial (Loreau and Hector, 2001; Hillebrand and Matthiessen, 2009). Plant species diversity has to be analysed in a few dimensions. Plant species diversity has to be analysed through both the taxonomic diversity and the functional diversity perspective. The varied diversity aspects are reflected in plant species communities' composition, structure, function, and relations with the associated and heterotrophic and saprophytic organisms.

However, for different species, the taxonomic diversity in a community often exhibits different physiological-ecological and adaptive processes, so considering taxonomic diversity alone does not reveal the full spectrum of plant diversity and its impact on R_s (Arnan, Cerdá and Retana, 2015). An additional aspect of plant diversity is the variety of functional traits. The functional plant attributes are closely related to species growth, reproduction, and competition and can better represent the direct influence of species on ecosystem function (McGill *et al.*, 2006). In recent years, functional diversity has become a mainstream approach in studying plant diversity and ecosystem function (McGill *et al.*, 2006). Recently, the understanding of the negative impacts of plant diversity loss on ecosystem functions has increased, including the understanding of net primary production (Liang *et al.*, 2016; Duffy, *et al.*, 2017), carbon sequestration (Tilman, Hill and Lehman, 2006) and nutrient cycling (Handa *et al.*, 2014). However, how plant diversity loss affects R_s and its components remains uncertain. Regardless of the uncertainty in natural and seminatural ecosystems in human-disturbed habitats, additional factors influence crucial processes, including respiration.

Human activity have very strong impact on environment especially when it is area which was it was heavily exploited and destroyed as a result of his activities. Habitat transformations are sometimes so substantial (e.g., on sites of post-mineral excavation) that the emerging system resulting from natural succession processes meets the criteria set for defining novel ecosystem (Hobbs, Higgs and Harris, 2009). Vegetation composition also undergoes significant changes. The abiotic and biotic parameters are unknown from natural or seminatural systems, and the main feature of the biotic background is non-analogous species composition (Keith, Mackey and Lindenmayer, 2009; Morse *et al.*, 2014; Rotherham, 2017).

This study aims to identify the environmental gradients that influence the diversity expressed as taxonomical and functional vegetation community composition and, based on the identified gradients, analyse the relationships between different measures of vegetation diversity and soil substratum respiration parameters.

We hypothesise that the diversity of the spontaneous vegetation species composition shapes the amount of soil respiration in a post-coal mine novel ecosystem. The soil respiration differs significantly along the vegetational gradient and is significantly higher in vegetation patches of high functional diversity. In particular, evenness and divergence and low functional richness and dispersion.

MATERIAL AND METHODS

STUDY AREA

The study area is in the Katowice Upland – a central part of the Silesian Upland (southern Poland). This region is under a temperate climate, with annual rainfall of 600–800 mm and the highest mean temperature of 14–16°C in July. In the Silesian Upland, western winds dominate. The number of days with mists ranges from 30 to over 100, and cloud cover is around 60–80%. Field studies were carried out in the mineral habitats of the spoil heaps of the coal mines at (Zabrze, 50°16'22"N, 18°44'43"E; altitude: 255 m); "Kostuchna" (50°11'04"N, 19°00'33"E; altitude: 324 m); Murcki (50°11'21"N, 19°02'07"E; altitude: 314 m); and "Wesoła" in Mysłowice (50°10'28" N, 19°5'44" E; altitude: 281 m). The sites analysed are areas with environmental conditions that differ from natural ones. The sites were created by human activity during fossil fuel extraction, and the mounded material comes from a depth of 0.5–1.0 km. These new anthropogenic forms are unique habitats because they are depleted in nutrients such as nitrogen, carbon, sulphur, phosphorus, etc., and are devoid of organic matter.

CALCULATION OF SPECIES DIVERSITY

To measure biodiversity, the following biodiversity indices were implemented: the number of all species present (S), Shannon–Wiener index (H), Shannon evenness ($H/\log(S)$), evenness uniformity, and Simpson's dominance index. The detailed measurement procedure is described in Radosz *et al.* (2023).

SPONTANEOUS VEGETATION ON COAL MINE SPOIL HEAPS

The mosaic of habitats occurring on coal mine sites affects the species diversity of vegetation patches that are created by ruderal, grassland, meadow, psammophilous, rush, and even saltmarsh species (Woźniak, 2010; Markowicz *et al.*, 2015). In the structure of plant communities that developed spontaneously on coal mine spoil heaps, the role of dominants and co-dominants is played by expansive, perennial grasses (e.g., *Calamagrostis epigejos*, *Phragmites australis*), legumes (e.g., *Melilotus alba*, *Medicago lupulina*, *M. sativa*) or other herbaceous plants (*Tussilago farfara*, *Centaurea stoebe*, *Chamaenerion palustre*), which give the patches a specific physiognomy (Błońska *et al.*, 2019; Kompała-Bąba *et al.*, 2020). In further stages of succession, alien species (e.g., *Solidago gigantea*) can also be found in the composition of patches.

FUNCTIONAL DIVERSITY

During the fieldwork, GPS devices were used, 324 plots were established on the analysed site, and vegetation and soil substrate samples and data were collected. In the test plot, a representative square was determined to contain the dominant species and best represent the entire patch (the coverage of the dominant species and quantitative responses between the dominant and co-occurring species). The test field had a side length of 0.5 m.

The taxonomy focuses on plant species composition, measured primarily by species richness. The functional diversity has been based on some selected plant species' functional traits. The chosen traits represent the plants' productivity potential, propagation, and competition ability. **Height:** this is a continuous characteristic that describes the height of the plant. **Leaf area:** this continuous characteristic describes the plant's leaf area. **Bud height:** this is an ordinal trait that describes the height of the plant's bud. Ordinal (0 – T, 0.12 – G, Hyd, 0.25 – H, 0.5 – Ch, 0.75 – N, 1 – M; T = therophytes, G = geophytes, Hyd = hydrophytes, H = hemicryptophytes, Ch = chamaephytes, N = nanofanerophytes, M = megaphanerophytes). **Beginning of flowering** and **End of flowering:** these total traits describe the plant's flowering period. **Pollination by animals, Self-pollination,** and **Wind pollination** are binary traits that describe different plant pollination methods. **Medium seed weight:** this is a continuous trait that describes the average seed weight of a plant analysed. **Propagation by seed and vegetative propagation:** these are binary traits that describe different methods of plant propagation. **Presence of mycorrhiza:** this binary trait describes whether a plant has mycorrhiza. **Anemochory, Zoochory, Barochory:** these are binary traits that describe different plant-spreading methods. **Competitiveness, Stress tolerance,** and **Ruderality:** these are ordinal traits that describe different ecological aspects of the plant. **Ellenberg values:** the Ellenberg ecological indices are indicators used to assess plant species' ecological characteristics and habitat requirements (Cornelissen *et al.*, 2003).

SUBSTRATE PHYSICOCHEMICAL ANALYSES

Soil samples weighing approximately 1 kg were taken at five points at 0–15 cm depth at each sampling site. In addition, soil bulk density, total soil porosity, and maximum water-holding capacity were measured. These data will help the understanding of the soil samples physicochemical properties during observation. The detailed measurement procedure is described in research undertaken by Bierza *et al.* (2023). The substrate samples for physicochemical analyses underwent several stages of preparation. First, they were air-dried, ground, and sieved to a fraction smaller than 2 mm.

The substrate samples were then analysed for several physicochemical parameters including: pH – determines the acidity or alkalinity of the soil; electrical conductivity (EC) – measures the ability of the soil to conduct electricity; soil organic carbon content (SOC) – indicates the amount of organic matter in the soil; C loss on ignition – indicates the loss of organic carbon during the combustion process; total nitrogen (TN) is the sum of nitrate (NO₃), nitrite (NO₂), organic nitrogen and ammonia; content of available forms of phosphorus (P₂O₅) – indicates the amount of available phosphorus; available magnesium concentra-

tion (MgO) – measures the amount of magnesium in the soil; exchangeable cations (K⁺, Na⁺, Ca²⁺): measures the concentration of exchangeable cations in the soil; moisture – a percentage that determines the water content of the soil.

SUBSTRATE RESPIRATION (RS) MEASUREMENTS

Soil respiration was measured using a portable infrared gas analyzer (IRGA) connected to the soil respiration chamber. The soil respiration chamber had an area of 78 cm² and a volume of 1171 cm³. The edge of the soil respiration chamber was inserted into the soil to a depth of 1–2 cm. The detailed measurement procedure is described by Woźniak *et al.* (2022).

DATA ANALYSIS

In order to determine the relationships between environmental factors and functional attributes of the species (Tab. 1), the RLQ ordination followed by the improved fourth-corner method (Dray *et al.*, 2014) was used as it links three data matrix tables: table L with abundance values noted for species growing on a series of plots, table R with variables describing the plots, and table Q containing the chosen traits of particular species. Table L contained the 192 vascular plant species with their abundance in 324 studied plots. Table R contained the environmental variables, mainly soil traits (physical-chemical properties, granulometric composition, pH, presence of soil enzymes, SRL).

The table Q had 23 plant traits for 192 vascular plant species found in this study. Prior to RLQ, a default option, i.e., standardised principal component analysis (PCA), was computed on the matrix of environmental variables by sites (table R) as well as for the traits-by-species matrix (table Q). For the RLQ analysis, statistical analyses were undertaken using the fourth-corner statistics in the form that (Dray *et al.*, 2014) improved. The analysis was conducted to assess the relationship between each environmental variable and the first two axes of the RLQ and also between the traits and the axes of the RLQ. This statistical tool allowed both the quantitative and qualitative variables to be used. Finally, we analysed the bivariate associations between the variables of two matrices, R and Q. The significance was tested using a permutation procedure. For this analysis, the fourth corner was used. To examine the overall functional diversity of vascular plant composition noted in the plots, four components of functional diversity were computed: richness (*FRic*), evenness (*FEve*), divergence (*FDiv*), and dispersion (*FDis*). The default function dbFD in the FD package was used. It was assumed that *FRic* can be interpreted as a measure of low habitat filtering. Functional evenness (*FEve*), a measure of niche overlap and functional divergence (*FDiv*) as a degree of functional heterogeneity, can be treated as signs of competition in a community. Apart from functional factors, taxonomical diversity was calculated: species richness (*S*), Shannon–Wiener index (*H*), evenness (*E*), and Simpson dominance index using “vegan” and “abdiv” packages. To assess the relationship between FD components and SRL against the species diversity, detrended correspondence analysis (DCA) with passive projection (999 permutations of Monte Carlo test) was applied. The SRL Spearman rank correlation test was used to check whether there are relationships among the functional diversity parameters.

Table 1. The list of functional traits of the recorded plant species that were analysed

Name of trait	Code	Type
Height	height	continuous
Leaf area	le_area	continuous
Bud height	BudHeight	ordinal (0 – T, 0.12 – G, Hyd, 0.25 – H, 0.5 – Ch, 0.75 – N, 1 – M)
Beginning of flowering	flw_early	integer
End of flowering	flw_late	integer
Pollination by animals	poll_zoo	binary
Self-pollination	poll_self	binary
Wind pollination	poll_wind	binary
Medium seed weight	medium seed_wght	continuous
Propagation by seed	seed	binary
Vegetative propagation	weg	binary
Presence of mycorrhiza	Myc	binary
Anemochory	Anem	binary
Zoochory	Zoochory	binary
Barochory	Barochory	binary
Competitiveness	Comp	ordinal (1-C, 0.5-CR, CS, 0.33-CSR)
Stress	Stress	ordinal (1-S, 0.5-CS, SR, 0.33-CSR)
Ruderality	Rude	ordinal (1-R, 0.5-CR, 0.33-CSR)
Ellenberg value for light	<i>L</i>	integer (0–9)
Ellenberg value for temperature	<i>T</i>	integer (0–9)
Ellenberg value for moisture	<i>F</i>	integer (0–12)
Ellenberg value for soil reaction	<i>R</i>	integer (0–9)
Ellenberg value for nitrogen	<i>N</i>	integer (0–9)

Explanations: contribution of Raunkiaer's forms: T = therophytes, G = geophytes, Hyd = hydrophytes, H = hemicryptophytes, Ch = chamaephytes, N = nanofanerophytes, M = megaphanerophytes. The basic classification of CSR plant functional types: C axis of competition (ability of plants to displace the other species under favourable environmental conditions); S axis of stress tolerance (ability to withstand long-term adverse environmental conditions); R axis of ruderality (ability to grow faster in disturbed sites).

Source: own elaboration.

RESULTS

In order to estimate the impact of the various aspects of diversity of the spontaneous vegetation species composition apart from the taxonomic diversity, some functional traits have been assessed. The list of the traits is presented in Table 1.

In both axes, RLQ explained 68% of total inertia. The proportion of the variance that was accounted for by the RLQ analysis was compared with the results from separate analyses of the data for the species (L), species traits (Q), and environmental data (R).

The first axis of the RLQ analysis accounted for 86.25% of the variability that was explained by the first axis of the separate environmental analysis (i.e., the ratio between the variance of the environmental characteristics in RLQ and the variance of the environmental characteristics in the separate analysis) – Table 2. The variability explained by the first axis of the separate plant trait analysis was 84.5% (Tab. 2). The eight environmental variables and plant traits are positively correlated with the first axis of RLQ, while 11 and 15 of the environmental and trait variables are correlated negatively (Fig. 1).

We considered the RLQ (R = environmental data, L = species, and Q species traits) analysis as the most suitable for examining relationships between among environment, species composition, plant traits, and four functional diversity compo-

Table 2. Decomposition of RLQ analysis

Parameter	RLQ axis 1	RLQ axis 2
Eigenvalue	1.52	0.48
Covariance	1.23	0.69
Correlation	0.39	0.39
Total variance	52.87	16.87
R/RLQ (%)	86.25	83.66
L/RLQ (%)	44.16	53.36
Q/RLQ (%)	84.50	71.92

Explanations: R = environmental data, L = species, and Q = species traits. Source: own study.

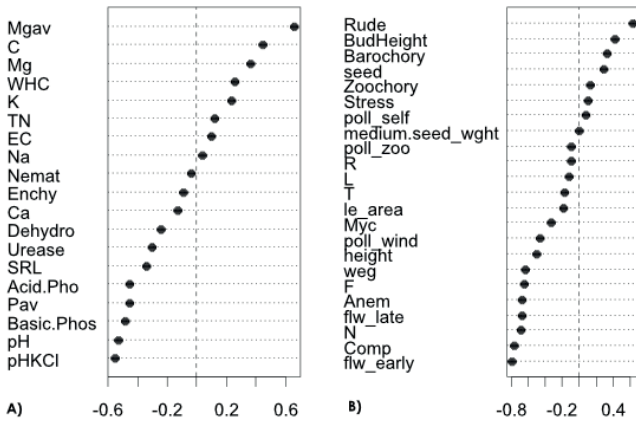


Fig. 1. The weighted correlations of environmental variables and plant traits with the first RLQ axis: A) environmental variables, B) plant traits; the weighted correlations of environmental variables and plant traits with the first RLQ axis; Mg av. = magnesium available, P av. = available phosphorus, Mg = available Mg (MgO) concentration, WHC – water holding capacity, K, Na, Ca = exchangeable cations, TN = total nitrogen, EC = electrical conductivity, Nemat = Nematoda, Enchy = Enchytraeidae, Dehydro = dehydrogenase activity, Urease = urease activity; SRL = soil respiration level, Acid.Pho = acid phosphatase, Basic.Phos = alkaline phosphatase, Rude = ruderality, BudHeight = bud height, Barochory = barochory, seed = propagation by seed, Zoochory = zoochory, Stress = stress; poll_self = self-pollination, medium seed_wght = medium seed weight, poll_zoo = pollination by animals, R = Ellenberg value for soil reaction, L = Ellenberg value for light, T = Ellenberg value for temperature, le_area = leaf area, Myc = presence of mycorrhiza, poll_wind = wind pollination, height = height, weg = vegetative propagation, F = Ellenberg value for moisture, Anem = anemochory, flw_late = end of flowering, N = Ellenberg value for nitrogen, Comp = competitiveness, flw_early = beginning of flowering; source: own study

nents (richness, evenness, dispersion, and divergence). The literature suggests that high soil richness is typical for species-rich ecosystems, so we assume that this parameter scores a high value in sites that represent a high functional evenness (*FEve*) and divergence (*FDiv*). The high values of these functional components indicate high competition, which usually occurs in communities with many species. High functional richness (*FRic*) and dispersion (*FDIs*) values represent low habitat filtering.

The analyses that present the main gradients of the collected data show that the negative impact of some environmental parameters, such as acidity of the soil substrate, available phosphorus, phosphorus-dependent soil enzyme activity, calcium, and sodium, and determines the gradients. The environmental parameters responsible for the positive part of the gradient are sodium, electrical conductivity, total nitrogen, potassium, water holding capacity, available Mg (MgO) concentration, C loss of ignition, and magnesium availability.

While among the analysed plant traits, presenting the gradients and influencing it negatively, the following traits should be listed: early flowering, traits responsible for competitiveness, Ellenberg value for nitrogen, beginning of flowering, anemochory, Ellenberg value for moisture, vegetative propagation, height, wind pollination, presence of mycorrhiza, leaf area, Ellenberg value for soil reaction, Ellenberg value for light and Ellenberg value for temperature. Lesser plant traits are correlated with the RLQ axis shaping the gradient, including traits responsible for stress resistance and adaptivity to conditions of the ruderal part of the continuum, zoochory, barochory, and bud height.

Permutation tests of the fourth-corner analysis showed associations between the first two axes of the RLQ analysis and the environmental variables and plant traits, respectively (Fig. 2). The positively significant environmental parameter shaping the AxQ1 is the available Mg (MgO) concentration. In contrast, the negatively significant environmental parameters shaping the AxQ1 are the pH, available phosphorus, dehydrogenase activity, and alkaline phosphatase.

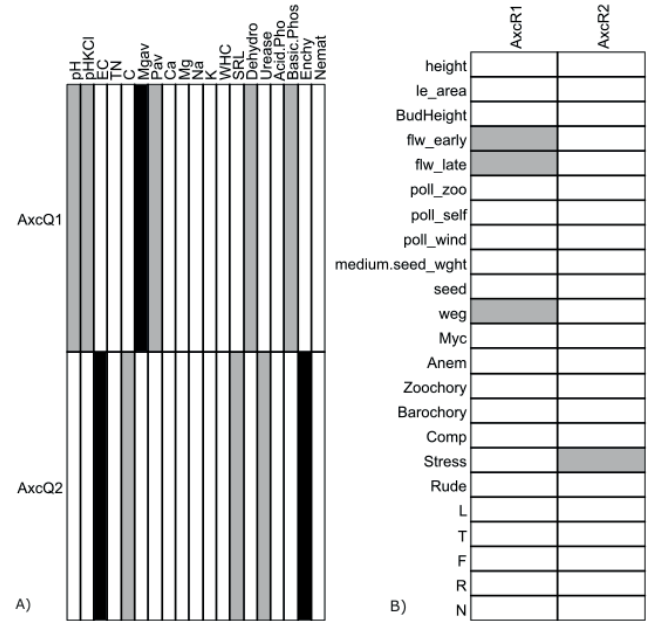


Fig. 2. The fourth-corner tests between the first two RLQ axes for the vascular plant traits (AxQ1/AxQ2) and environmental variables (A) and fourth-corner tests between the first two RLQ axes for the environmental variables (AxR1/AxR2) and vascular plant traits (B); positive and significant ($p < 0.05$) associations are represented by black cells and negative significant ones by grey cells, while white cells indicate non-significant associations; other explanations as in Fig. 1; source: own study

The positively significant environmental parameters shaping the AxQ2 are electrical conductivity and Enchytraeidae. In contrast, the negatively significant environmental parameters shaping the AxQ1 are the C loss of ignition, water holding capacity, and urease activity.

The ordination analysis of the plant traits that are represented by species composition of the spontaneous vegetation of post-mining sites reveals that the manner of seed dispersion, the height of bud location, and ruderal traits are divided by the first axis from the species that are competitors, anemochorous and spreading vegetatively. Concerning the ordination of the environmental factors, electrical conductivity, available magnesium, and water holding capacity, together with nitrogen, carbon, and potassium content in the soil substratum, are one part of the ordination. The biotic functional traits parameters of competitiveness, zoochory, end of flowering, wind pollination, beginning of flowering, bud height, mycorrhiza, seed weight, height, Ellenberg value for temperature, self-pollination, ruderality, leaf area, and vegetative propagation is shown at the top of the chart. The second axis separates the above plant species traits from the parameters represented by plant species that possess the traits of adaptation to Stress on the far down of the functional trait distribution (Fig. 3).

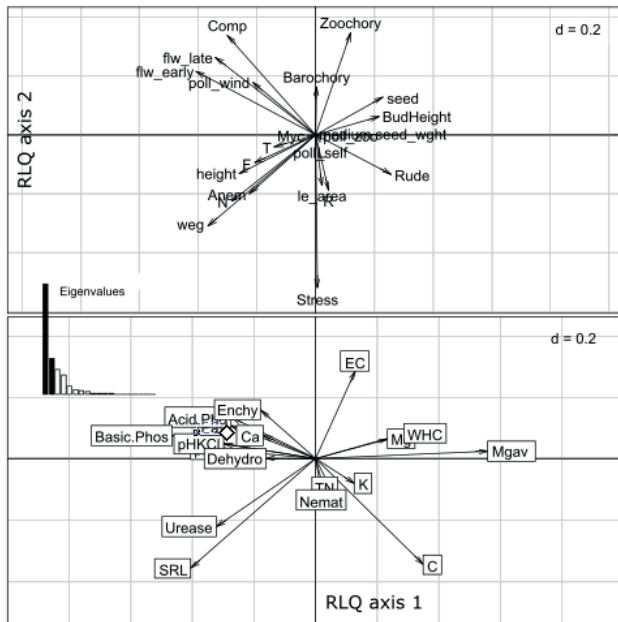


Fig. 3. The RLQ ordination, showing relationships between plant traits (upper) and environmental factors (down) along the two first axes; explanations as in Fig. 1; source: own study

The RLQ ordination was performed for environmental factors. The first axis is determined by the two extremes shaped by the available magnesium (Mg av.) and the activity of basic phosphorus and pH (in KCl). The EC and SRL determine the second axis. The other parameters distributed along the first axis are the water-holding capacity, calcium, dehydrogenase activity, and magnesium. Those parameters which lie, along the second axis are, C loss on ignition, urease activity, Enchytraeidae, Nematoda, and total nitrogen.

The vegetation type with the lowest number of species, *Phragmites australis*, the value of soil respiration is the highest. This result reveals that the vegetation with the higher diversity in the coal mine heaps novel ecosystem does not present a higher soil respiration value (Tab. 3).

The fourth-corner correlation analysis has shown that the soil respiration value is positively correlated with vegetation

patches in which plant species composition is represented by species characterised by vegetative spreading together with plant species that have traits responsible for adaptation to stressed habitat conditions (Fig. 4).

The significance of functional and taxonomic diversity is apparent in the gradient for species composition (Fig. 5B). The higher functional richness, divergence, and dispersion values positively correlate with the Shannon–Wiener index and species richness. Both taxonomical and functional evenness were non-significant factors. Among functional components only and in the case of functional dispersion, it is weakly negative and significantly correlated with SRL (Fig. 5A).

The relationship between temperature and SRL is weak and positive but significant (Fig. 6). An increase in temperature is associated with a slight increase in SRL. The value of the correlation coefficient ($r = 0.17$) confirms this weak relationship. In addition, the p -value = 0.0018 indicates the statistical significance of this relationship, meaning that the probability that the observed correlation is due to chance is very low.

DISCUSSION

THE ROLE OF TAXONOMIC AND FUNCTIONAL DIVERSITY IN ECOSYSTEM FUNCTIONING

The ecosystem development on disturbed habitats such as post-mining mineral sites, follows the sequence of processes and functionality established during the natural, spontaneous vegetation process. Identifying the critical factor in a disturbed environmental situation of the structure and function affecting the biodiversity of the vegetation of the non-analogous species composition in general and the microbial communities is frequently impossible due to the complexity of the various biotic and abiotic factors (Berg and Smalla, 2009). In the presented study, we attempt to identify the environmental gradients that influence the diversity expressed as taxonomical and functional vegetation community composition and, based on the identified gradients, analyse the relationships between different measures of vegetation diversity and soil substratum respiration parameters.

Table 3. The list of spontaneous vegetation types with the biodiversity indices (maximum, minimum, and mean) along with the value soil respiration level (SRL) rate in the vegetation patches of the particular vegetation types

Vegetation type groups	<i>Poa compressa</i>	<i>Tussilago farfara</i>	<i>Daucus carota</i>	<i>Chamaenerion palustre</i>	<i>Phragmites australis</i>
Maximum number of species	19	18	20	16	10
Minimum number of species	8	1	7	8	3
Mean number of species ±SD	13.8 ±2.90	8.11 ±3.62	12.6 ± 3.00	11.5 ±2.02	10 ±5.06
The mean Shannon–Wiener diversity index ±SD	1.92 ±0.24	1.17 ±0.473	1.84 ±0.277	1.66 ±0.262	1.06 ±0.594
The mean species evenness ±SD	0.73 ±0.08	0.56 ±0.15	0.732 ±0.0605	0.685 ±0.0883	0.454 ±0.187
Maximum SRL rate ((g CO ₂)-m ⁻² -h ⁻¹)	1.21462	0.82552	0.15826	0.161759091	0.97638
Minimum SRL rate ((g CO ₂)-m ⁻² -h ⁻¹)	0.00752	0.00506	0.00158	0.0086	0.271
Mean SRL rate ((g CO ₂)-m ⁻² -h ⁻¹)	0.227	0.20173	0.0526	0.1617	0.5842

Explanation: SD = standard deviation.
Source: own study.

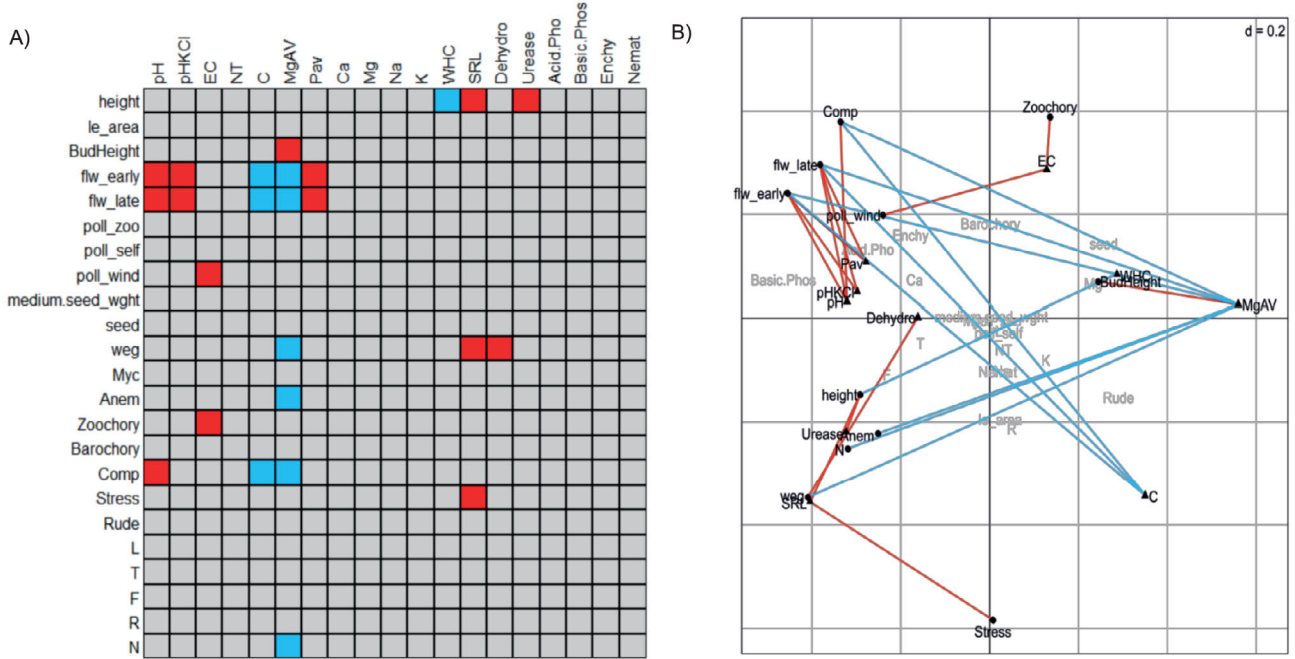


Fig. 4. The results of fourth-corner analysis between environmental variables and plant traits; red and blue cells – positive and negative significant ($p < 0.05$) associations, respectively, grey cells – non-significant associations; explanations as in Fig. 1; source: own study

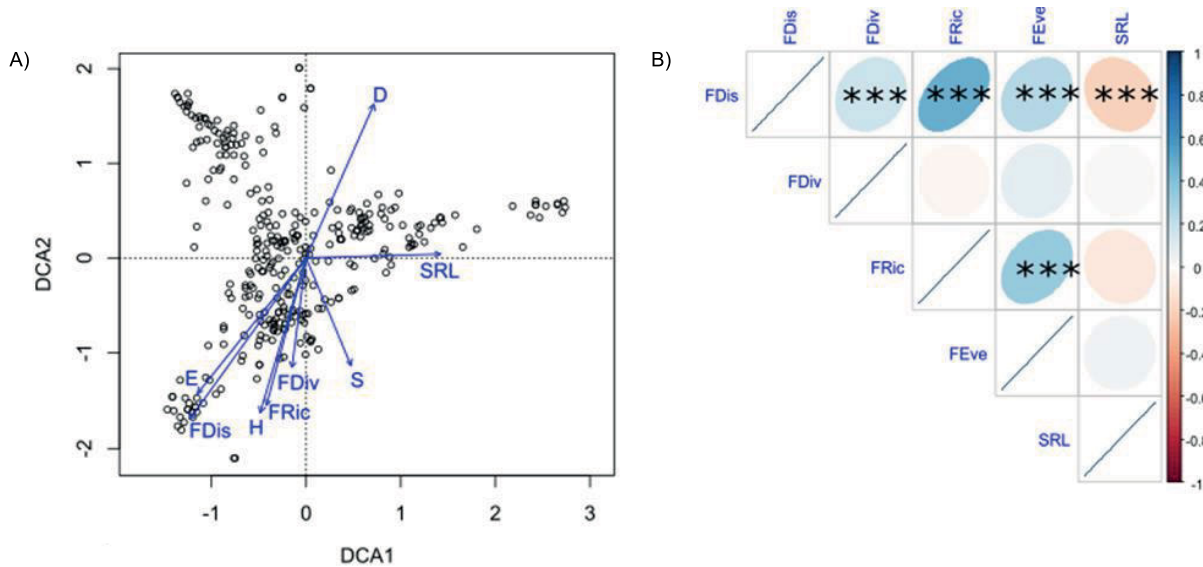


Fig. 5. Passive projection of functional and taxonomical diversity and their relations with soil respiration level (SRL): A) the ordination of DCA, B) matrix correlation; *FDis* = high functional dispersion, *FDiv* = high functional divergence, *FRic* = high functional richness, *FEve* = high functional evenness; source: own study

The taxonomic diversity analysis focuses on plant species composition and indices that measure preliminary species richness and abundance. These indices are primarily used in Europe and are based on the principle that certain plant species have specific tolerance ranges for environmental factors such as light, temperature, moisture, soil pH, and nutrient availability. In these studies, the well-known Ellenberg ecological indices are the Ellenberg indicator values (*EIVs*), which are used to evaluate the ecological preferences of plants along environmental gradients. These values are assigned to plant species based on their observed ecological behaviour in the field. Our study revealed that plant species representing different functional traits

are filtered along the gradient of biotic and abiotic habitat factors. The results showed that only some traits are significant in the spontaneous vegetation on coal mine heap novel ecosystems concerning soil respiration. The low habitat filtering expressed by functional richness and dispersion was associated with relatively high functional evenness and divergence being a proxy of competition, which is not common. Soil respiration was negatively correlated with low habitat filtering and competition (not significantly, but the trend is similar). It means that under novel ecosystem conditions, such as coal mine heaps, soil respiration seems to be affected by many environmental and biotic factors but the effects are of low magnitude.

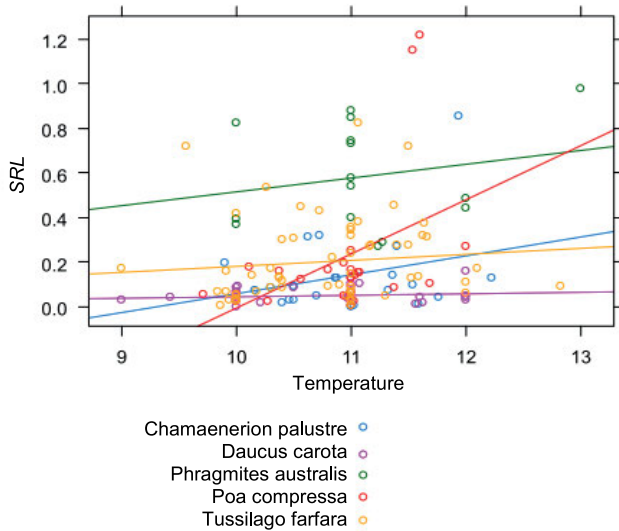


Fig. 6. The relationship between temperature and soil respiration level (SRL); $r_s = 0.17$, $p = 0.0018$; source: own study

FACTORS AFFECTING THE TAXONOMIC DIVERSITY OF THE VEGETATION WITH REGARD TO SOIL RESPIRATION

Plant taxonomic diversity can affect soil respiration by changing the quantity and quality of organic matter supplied to the soil. Plant species can provide different amounts of leaves and other organic materials that are broken down by soil microorganisms, leading to soil respiration. Soil physicochemical properties, such as pH, water content, and nutrient content (e.g., nitrogen and phosphorus), can affect plant taxonomic diversity and soil respiration. For example, soils with low pH can reduce plant diversity and soil microbial activity, affecting soil respiration. Changes in the taxonomic diversity of plants can affect these interactions and, thus, soil respiration (Bierza *et al.*, 2023). In our study, the factors that drive the habitat gradients along which the plant species composition and vegetation types are distributed are identified. The main gradients of the collected data show that the impact of environmental parameters, such as acidity of the soil substrate, available phosphorus, phosphorus-dependent soil enzyme activity, calcium, and sodium, determine the gradients. The environmental parameters responsible for the positive aspect of the gradient are sodium, electrical conductivity, total nitrogen, potassium, water holding capacity, available Mg (MgO) concentration, C content, and available magnesium. Among the analysed plants' traits that reflect the gradients, the following traits should be listed: early flowering, traits responsible for competitiveness, Ellenberg value for nitrogen habitats parameters, beginning of flowering, anemochory, Ellenberg value for moisture, vegetative propagation, plant height, wind pollination, the ability to symbiosis mycorrhiza, the value of leaf area, Ellenberg value for soil reaction acidity of the habitat conditions, Ellenberg value for light and Ellenberg value for temperature.

Based on the primary producers' species composition, the vegetation communities and ecosystems are shaped by intense habitat conditions selection and adaptation processes dependent on the evolutionary, historical, and environmental processes (Purschke *et al.*, 2013; Stevens and Tello, 2014). Much research on the impact of plant species diversity on total soil respiration

(R_s) has been focused, most frequently on taxonomic diversity (Craine, Wedin and Reich, 2001). The fact that species richness can regulate R_s through changes in productivity showed a positive relationship between species richness and R_s (Craine, Wedin and Reich, 2001). In our study, vegetation patches were divided into five groups based on species composition: 1 – *Poa compressa*, 2 – *Tussilago farfara*, 3 – *Daucus carota*, 4 – *Chamaenerion palustre*, and 5 – *Phragmites australis*. The collected data indicate that the most significant group in terms of the number of species is group number 5. Group number 3 showed the highest maximum respiration value, which may indicate its high metabolic activity. Group number 2 showed the minimum number of species (1) and the minimum value for respiration, which may indicate its limited diversity and metabolic activity.

In grassland, R_s was related to species-specific changes (species composition). The changes in species richness were less important in influencing the R_s (Johnson *et al.*, 2008). These differences in results might be connected with the fact that the influence of different species on R_s is the same regardless of the traits of the calculated taxonomic diversity, without linking the different set of traits that particular species composition represents and might influence the value of R_s (Díaz and Cabido, 2001).

The uncertainty between the soil respiration and diversity parameters in varied vegetation types, grasslands, forests, and others might be connected with the dual origin of the total soil respiration. The components of total respiration R_s are plants' autotrophic respiration (R_a), which generates energy for water and nutrient acquisition, survival, and growth, and in contrast, heterotrophic respiration (R_h) from the activity of soil microorganisms that regulates nutrient cycling (Ryan and Law, 2005). Recently, there have been some significant advances in our understanding of the negative impacts of plant diversity loss on ecosystem functions, including net primary production (Liang *et al.*, 2016; Duffy, Godwin and Cardinale, 2017), carbon sequestration (Tilman, Hill and Lehman, 2006) and nutrient cycling (Handa *et al.*, 2014). However, how plant diversity loss affects R_s and its components remains uncertain (Chen and Chen, 2019). Our study focused on the total amount of respiration in the plots that represent the wide spectrum of the identified vegetation types. The range of CO_2 emissions at the analysed sites ranged from 0.00158 to 1.21462 (g CO_2)- $\text{m}^{-2}\cdot\text{h}^{-1}$.

Studies suggest that the biomass created due to primary production increases with plant species diversity and trait heterogeneity due to complementary resource utilisation among constituent species in species-rich ecosystems (Hooper *et al.*, 2005; Chen and Chen, 2018). Increased litter inputs in species-rich ecosystems may lead to the accumulation of soil carbon and nitrogen pools (Fornara, Tilman and Hobbie, 2009; Lange *et al.*, 2015), which can increase microbial respiration (Hector *et al.*, 2000; Hooper *et al.*, 2005; Eisenhauer *et al.*, 2013).

FACTORS AFFECTING THE VEGETATION COMMUNITIES' FUNCTIONAL DIVERSITY REGARDING SOIL RESPIRATION

The relationship between vegetation functional diversity and soil respiration is multifaceted, involving intricate interactions between plant traits, nutrient cycling, microbial communities, and environmental conditions. Understanding these relationships is crucial for predicting how ecosystems may respond to changes in biodiversity and environmental factors, including those

associated with climate change and land-use practices. In natural and seminatural habitats, the vegetation and ecosystem diversity factors are explained by two concepts. The mass ratio hypothesis and niche complementarity hypothesis are used to consider the changes in vegetation plant species composition and ecosystem diversity, mostly about plant functional trait composition (Liu *et al.*, 2022). The mass ratio hypothesis suggests that the characteristics of dominant species have a greater impact on ecosystem processes. In contrast, the niche complementarity hypothesis suggests that diverse communities can use resources more efficiently. In studying the spontaneous development of vegetation of novel ecosystems, the applicability of the above concepts and hypothesis needs to be tested.

Research suggests that the soil water content in the habitat and species richness influence R_s . As indicated by Ren *et al.* (2022), changes in soil microbial community structure and diversity, as well as soil carbon properties, respond to different vegetation types at different soil water levels. Soil water content and species richness also influenced R_s . In our study, the water holding capacity has been assessed in all study plots. The analysis showed that in the mineral habitats of post-mining areas, these parameters are responsible for the positive part of the gradient.

One of the possible aspects of the plant species' functional diversity is identified using the Ellenberg ecological indices. By using Ellenberg ecological indices, a better understanding of the ecological requirements and the environmental conditions of a particular habitat of the plant species composition is possible. This information can be valuable for conservation efforts, land management, and ecological restoration projects. In recent years, functional diversity has become a common approach in studying plant diversity and ecosystem function (McGill *et al.*, 2006). Only temperature, soil reaction, light, and moisture in our study revealed some correlations in the Ellenberg ecological indices in the studied plots.

Among other functional traits, the leaf functional traits, such as leaf nitrogen content and specific leaf area (SLA), are closely related to ecosystem respiration and soil properties (Srivastava *et al.*, 2012; Long De *et al.*, 2019). The functional diversity based on functional traits may be more helpful in explaining R_s . It can provide information on those difficult-to-measure functional traits that may be closely associated with R_s (Cadotte *et al.*, 2009; Stevens and Tello, 2018). There is a high degree of variability in the relationship between different dimensions of biodiversity and environmental factors. These factors may have ecosystem functions with opposing effects (Devictor *et al.*, 2010; Bagousse-Pinguet Le *et al.*, 2019). However, in studies based on real-world problems, the regression parameters vary across spatial locations (Wang *et al.*, 2021). Our study analysed the nitrogen content in soil substrate as a parameter of soil respiration. Our study revealed that fewer plant traits are shaping the gradient, including traits responsible for stress resistance and adaptivity to conditions of the ruderal part of the continuum, zoochory, barochory, and bud height.

The ordination analysis of the plant traits that are represented by species composition of the spontaneous vegetation of post-mining sites reveals that the manner of seed dispersion, the height of bud location, and ruderal traits are divided by the first axis from the species that are competitors, anemochories and spread vegetatively. Concerning the ordination of the environmental factors, electrical conductivity, available magnesium, water holding capacity, nitrogen carbon, and potassium content in soil

substratum are one part of the ordination. In conclusion, the ordination analysis of plant traits and environmental factors in post-mining areas shows complex relationships, which may have important implications for succession and regeneration processes.

The fourth-corner correlation analysis has shown that the soil respiration value is positively correlated with vegetation patches rich in species characterised by high plants that spread mostly vegetatively and plant species traits responsible for adaptivity to stress.

THE ENVIRONMENTAL BIOTIC AND ABIOTIC FACTORS AFFECTING SOIL RESPIRATION IN POST-MINING NOVEL ECOSYSTEMS

Using the spatial heterogeneity of natural environmental gradients to explore the response of plant diversity and ecosystem function to environmental change has become a fruitful approach in ecosystem ecology (Prager *et al.*, 2021). Desert ecosystem biodiversity monitoring provides insight and comprehensive data on habitat conditions, vegetation, and plant community composition; in the extreme desert ecosystem, the biodiversity recordings enable the analysis of the relationships between plant diversity taxonomic, functional, soil properties, and R_s along a severe environmental gradient. The study of the functioning of ecosystems existing in extreme environments, e.g., post-mining sites, and desert ecosystems can enhance the understanding of the mechanisms by which R_s responds to relevant drivers, and may be helpful to the understanding and management of future climate change (Wang *et al.*, 2022).

The diversity of plant composition determines the quality and quantity of the release of particular chemical substances that can recruit particular groups of microorganisms that are useful against, e.g., diseases and insect herbivory (Rudrappa *et al.*, 2008). Releasing plant-derived metabolites that provide a carbon source attracts various microorganisms (Bais *et al.*, 2006; Borymski *et al.*, 2018). Plant diversity and the diversity of living organisms and their varieties (biodiversity) are the main factors driving many ecosystem functions, including the global ecosystem (Chapman and Newman, 2010; Jarzyna and Jetz, 2018; Chen and Chen, 2019). For crucial ecosystem functions like soil respiration, the relation between the individuals of plant species and the diversity and abundance of microorganisms in particular habitats is fundamental (Eisenhauer *et al.*, 2013; Hooper *et al.*, 2005). The presence or absence of one species in a community composition might have more significant functional implications for ecosystems than for another species, even though the species richness in both cases will be the same. However, the two species affect the assemblage functional diversity (FD) differently (Cadotte, Carscadden and Mirotchnick, 2011). The different species' functional attributes are crucial for understanding the dynamic processes behind the temporal and spatial species occurrence and, consequently, vegetation community assembly (Belmaker and Jetz, 2011).

CONCLUSIONS

The plant species and functional diversity of the spontaneous vegetation developing spontaneously on the mineral material of the coal mine novel ecosystem reflects the habitat's variety of biotic and abiotic conditions.

The gradient in species composition explains the functional and taxonomical diversity. The higher functional richness, divergence, and dispersion values positively correlate with the taxonomical diversity expressed by the Shannon–Wiener index and species richness. Both taxonomical and functional evenness were non-significant factors. A weak negative and significant correlation with soil respiration level (SRL) has been identified among functional components only in the case of functional dispersion.

Contrary to our expectation, soil respiration was the highest in the less diverse vegetation types. Among functional components, only functional dispersion (a proxy of low habitat filtering) is weakly negatively and significantly correlated with SRL. However, all functional diversity indices are correlated, which indicates that conditions driving community assembly on coal mine heaps act differently than on near-natural and natural ecosystems. Thus, SRL seems dependent on many abiotic and biotic factors in novel ecosystems and requires further research.

CONFLICT OF INTERESTS

All authors declare that they have no conflict of interests.

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5. Dyskusja

Określenie, jakie czynniki siedliskowe i środowiskowe, regulują ilość uwalnianego CO₂ w wyniku oddychania gleby lub podłoża jest przedmiotem badań w wielu typach ekosystemów. Ilość uwalnianego CO₂ w wyniku oddychanie gleby stanowi $\frac{3}{4}$ dwutlenku węgla i przewyższa ilością wszystkie inne źródła dwutlenku węgla dostające się do atmosfery (Raich i Schlesinger, 1992). Każdego roku przez glebę przenika prawie 10% atmosferycznego dwutlenku węgla (Raich i Potter, 1995).

5.1. Czynniki abiotyczne wpływające na proces respiracji w mineralnych podłożach płatów spontanicznej roślinności na zwałach skały płonnej

Wiedza na temat czynników wpływających na procesy oddychania w układach typu *Novel Ecosystems* nowopowstałych ekosystemach, w płatach roślinności występujących na podłożu mineralnym hałd węgla kamiennego, jest bardzo ograniczona i obejmuje jedynie sporadyczne, niezwiązane informacje. Konieczne jest poznanie mechanizmów, wyjaśniających rolę parametrów siedliskowych na proces oddychania gleby lub podłoża, które wyjaśniłyby ten kluczowy element funkcjonowania ekosystemu m.in. na siedliskach zaburzonych (Buchmann, 2000).

Powiązania parametrów siedliskowych mogą się stać bardziej skomplikowane, gdy spontaniczne płaty roślinności i ekosystemy rozwijają się na nowych siedliskach (Hobbs i in., 2013) np. w miejscach wydobywania surowców mineralnych. Powstałe *de novo* siedliska mineralne, pozwalają na badanie zależności między kolonizującymi je gatunkami roślin, a czynnikami siedliskowymi. Możliwe jest obserwowanie i badanie najlepiej przystosowanych osobników gatunków roślin, oraz określenie ilości biomasy roślinności rozwijającej się spontanicznie. W nowych ekosystemach relacje biotyczne i abiotyczne mogą być kształtowane przez efekt nieznanych wcześniej relacji wynikających z wpływu człowieka na ekosystemy w epoce Antropocenu (Zalasiewicz i in., 2016).

Pierwszym etapem badań była analiza powiązań między warunkami abiotycznymi a intensywnością oddychania podłoża. Większość badań analizuje oddzielnie rolę abiotycznych czynników środowiskowych w oddychaniu glebowym. Jednakże te czynniki nie działają niezależnie, mogą oddziaływać ze sobą i wzajemnie wpływać na oddychanie gleby (Yu i in., 2015). W prezentowanych badaniach przetestowano, który z parametrów abiotycznych ma

największy wpływ na ilość uwalnianego CO₂ z podłoża mineralnego. Oczekiwano, że zawartość C będzie miała najbardziej istotny wpływ na ilość uwalnianego CO₂ z podłoża mineralnego badanych płatów roślinności. Podczas gdy inne parametry abiotyczne, takie jak granulometria, pojemność wodna (WHC), pH, przewodność (EC), nie były oczekiwane jako istotne czynniki wpływające na ilość uwalnianego CO₂ w badanych typach roślinności. Wbrew naszym oczekiwaniom zawartość węgla w glebie nie wpływała istotnie na intensywność oddychania gleby. Może być kilka powodów, dla których wyniki nie są zgodne z założoną hipotezą. Obieg węgla (C) wraz z azotem jest głównym elementem składników odżywczych w ekosystemach (Cusack i in., 2011; Sinsabaugh i in., 2005). Obieg węgla, fosforu i azotu są często powiązane z sobą w ekosystemach (Zarif i in., 2020; Fahey i in., 2013;). W niektórych ekosystemach, co prawdopodobnie powinno być również brane pod uwagę w podłożu mineralnym jako materiale podłoża nowych ekosystemów zwałów pogórnicych, stosunek C:N w glebie wspiera pobieranie dostępnego N przez rośliny (Zarif i in., 2020; Eberwein i in., 2017). Tak więc różnice w stosunku C:N w badanych podłożach mogą mieć znaczenie.

W prezentowanych badaniach aktywność fosfatazy zasadowej jest wskaźnikiem proxy dla parametru zawartości fosforu. Przedstawione wyniki wykazały dodatnią korelację między aktywnością fosfatazy zasadowej a ilością uwalnianego dwutlenku węgla. Większość wcześniejszych badań analizujących poziom oddychania gleby zwracała uwagę przede wszystkim na rolę azotu. Zawartość fosforu została uznana za parametr oddziałujący wraz z zawartością azotu (Helfenstein i in., 2020; Zhang i in., 2020; Zhang i Zhang, 2016;). Mineralny fosfor nieorganiczny (Pi) zmienia się w fosfor organiczny (Po) i może brać udział w biodostępności fosforu w glebie (Helfenstein i in., 2020, 2018; Rosling i in., 2016). Niektóre badania pokazują, że fosfor zwiększa ilość azotu w ściółce, ograniczając mineralizację azotu (Homeier i in., 2017; Mao i in., 2017). Stałe pobieranie azotu, np. azotu nieorganicznego z atmosfery, zmniejsza kwasowość gleby, co może prowadzić do efektu buforowania spowodowanego zwiększoną zawartością fosforu, który utrzymuje pH gleby na stabilnym poziomie (Zarif i in., 2020 ; Mao i in., 2017; Yang i in., 2015;). Żadna z tych zależności nie była testowana w mineralnych podłożach zwałów skały płonnej.

Jednym z czynników abiotycznych jest zasolenie podłoża mineralnego. Zasolenie gleby jest uważane za istotny czynnik w leśnictwie i rolnictwie, szczególnie przy wysokiej ewapotranspiracji i niskich opadach (Bossio i in., 2007; Pannell i Ewing, 2006; Rengasamy, 2006; Beltrán i Manzur, 2005). Jak wskazano, denitryfikacja/nitryfikacja gleby, a w konsekwencji również oddychanie gleby zależą od zasolenia gleby. Stres związany z zasoleniem i suszą to warunki, na które podatne są rośliny budujące wegetację ekosystemów

naturalnych i półnaturalnych. Niektórzy badacze rozumieją, że osobniki roślin nie są w stanie szybko przystosować się do warunków stresowych (Jarvis i in., 2008; Mittler, 2006). Rzadziej bada się, w jaki sposób procesy adaptacyjne, które mogą prowadzić do przystosowania do zasolenia i suszy, mogą różnić się ze względu przebieg procesów eko-fizjologicznych oraz w biochemii osobników roślin. Zmiany w strukturze biochemii roślin wpływają na charakter materii organicznej gleby, a w konsekwencji na proces rozkładu, który jest bezpośrednio związany z uwalnianiem dwutlenku węgla. Zasolenie może ulec zmianie w związku z występowaniem kationów wymiennych i warunków, które mogą wpływać na ich wymianę. Obecność i ilość kationów wymiennych Ca^{2+} , Mg^{2+} , Na^+ i K^+ wpływa na funkcjonowanie gleby w ekosystemach naturalnych i półnaturalnych (Ramos i in., 2018). W prezentowanych badaniach uzyskane wyniki wykazały, że inaczej niż zakładano, zawartość magnezu Mg, potasu K i wapnia Ca oraz wartość pH w mineralnym materiale badanych płatów roślinności są istotnie związane z ilością uwalnianego z badanego podłoża dwutlenku węgla. Wartość pH, wykazywała najwyższą dodatnią korelację z poziomem respiracji gleby, podczas gdy uwalnianie dwutlenku węgla było ujemnie związane z ilością magnezu i wapnia.

5.2. Uwarunkowania biotyczne procesów respiracji w mineralnych podłożach płatów spontanicznej roślinności na zwałach skały płonnej

Nowe ekosystemy, które rozwijają się z mozaiki płatów spontanicznej roślinności na zwałowiskach skały płonnej, spełniają kryteria stosowane w odniesieniu do definicji nowych ekosystemów (*Novel Ecosystem*) – ich biomasa bazuje na *non-analogous* nieanalogicznym, spontanicznym składzie gatunkowym roślinności, który może powodować znacząco różny przebieg procesów funkcjonowania nowo powstających ekosystemów. Niektóre zależności między parametrami biotycznymi a intensywnością oddychania mineralnego podłoża różnią się od tych występujących w naturalnych i półnaturalnych typach roślinności oraz zależnych od nich nowopowstających ekosystemach. W niezburzonych ekosystemach, skład gatunkowy badanych zbiorowisk a w konsekwencji ilość i jakość biomasy, odgrywa kluczową rolę w kontrolowaniu oddychania gleby (Zhang i in., 2022; Allen i in., 2010; Jiang i in., 2008;). Różnorodność mikroorganizmów glebowych i skład ich zbiorowisk są ściśle związane z różnorodnością składu gatunkowego roślin (Waldrop i Zak, 2006).

Jednym z ważniejszych parametrów biotycznych uważanych za istotny dla ilości uwalnianego dwutlenku węgla jest ilość i jakość powstającej w zbiorowisku roślinnym i ekosystemie biomasy. Cała biomasa, a tym samym ilość organicznego węgla, która gromadzi się w różnorodnych typach roślinności w każdym ekosystemie, powstaje dzięki równowadze

fotosyntezy (cała produkcja pierwotna (P) i oddychania roślin (autotrofów), energia potrzebna do wzrostu roślin i procesów życiowych). Różnica między tymi strumieniami to produkcja pierwotna netto (Pn) (Collalti i in., 2020; Chapin i in., 2006). Opierając się na teorii skalowania metabolicznego, hipoteza sugeruje, że oddychanie powinno skalować się wraz z biomasą (West i in., 1999). Według niektórych badań (Reich i in. 2008; 2006) można skalować ilość uwalnianego CO₂ przy znanych zawartościach węgla (C) lub azotu (N) w całej roślinie. W tym podejściu skalowanie jest podobne w obrębie i między różnymi gatunkami, niezależnie od warunków środowiskowych i klimatycznych. Tradycyjny pogląd na dynamikę lasu zakładał izometryczne skalowanie oddychania biomasą określone np. przez Kirę i in. 1967 oraz Oduma 1969 (Odum, 1969; Kira i Shidei, 1967). W przypadku braku znaczących zakłóceń, jeśli oddychanie wzrasta równoległe z biomasą, produktywność pierwotna (Pn) nieuchronnie spada, ponieważ produktywność pierwotna (Pn) nie może rosnąć w nieskończoność, ale staje się względnie stabilna po zamknięciu koron drzew w ekosystemach leśnych (O'Connor i in., 2007). Nasze wyniki pokazały, że zgodnie z naszymi oczekiwaniami typy roślinności o większej ilości biomasy roślinnej oddychają intensywniej. Badania wykazały, że ta zależność w nowych ekosystemach po wydobyciu węgla jest dodatkowo silna i statystycznie istotna między parametrami oddychania biomasy roślinnej i podłoża mineralnego.

Hipoteza badawcza zakładała, że w typach roślinności z większą ilością materii organicznej procesy respiracji podłoża będą przebiegały intensywniej, oraz że typy roślinności o wyższej aktywności enzymatycznej i większej liczebności fauny glebowej w strefie korzeniowej charakteryzują się większą intensywnością oddychania podłoża mineralnego.

Sugeruje się, że aktywność enzymów glebowych jest wskaźnikiem jakości gleby, który może odzwierciedlać zmiany w funkcjonowaniu ekosystemu. Kondycję ekosystemu można scharakteryzować za pomocą enzymów glebowych ściśle związanych z kluczowymi parametrami jakości gleby, takimi jak biomasa, materia organiczna gleby i właściwości fizyczne gleby (Sharma i in., 2022). Aktywność enzymów glebowych, takich jak dehydrogenaza, kwaśna fosfataza i zasadowa fosfataza w naszych badaniach była dodatnio skorelowana z ilością uwalnianego CO₂, jednak nie było korelacji między aktywnością ureazy a ilością uwalnianego CO₂.

Zebrane dane dotyczące, liczebności mezofauny i biomasy pozwoliły nam poszukać dowodów na wzajemne powiązania między elementami biotycznymi badanych płatów roślinności. Stwierdzono relacje pomiędzy ilością biomasy a liczebnością badanych organizmów glebowych, wazonkowców i nicieni. Powstawanie pierwszych relacji w toku

sukcesji pierwotnej na podłożu mineralnym, jest jednym z najciekawszych zagadnień biologii środowiskowej. Badania nad zgrupowaniami fauny glebowej na różnych etapach sukcesji na terenach po górniczych są rzadkie (Tropek i in., 2012).

5.3. Wpływ różnorodności taksonomicznej i funkcjonalnej spontanicznej roślinności rozwijającej się na zwałach skały płonnej na ilość uwalnianego CO₂ z mineralnego podłoża

Zróznicowane warunki chemiczne i fizyczne mineralnych podłoży siedlisk pogórniczych spowodowały powstanie nowych, nieznanych, nieanalogicznych układów gatunkowych roślinności spontanicznej i związanych z nimi organizmów (Helingerová i in., 2010; Woźniak, 2010; Keith i in., 2009). Nieanalogiczny skład gatunkowy roślinności porastającej nowe ekosystemy poeksploatacyjne przedstawia mozaikę płatów roślinności, które są zdominowane przez różne gatunki najlepiej przystosowane do dostępnych mikrosiedlisk (Rawlik i in., 2018). Obserwowana mozaika odzwierciedla różnorodność biotycznych i abiotycznych warunków siedliskowych (Woźniak, 2010). Znamienne jest, że występuje również zależność odwrotna, polegająca na wpływie zbiorowisk roślinnych wczesnych stadiów sukcesyjnych na warunki siedliskowe gleb mineralnych i zależności te są słabo poznane (Orwin i in., 2014; Lamošová i in., 2010).

Wpływ różnorodności gatunkowej roślin na respirację, oddychanie gleby w środowiskach naturalnych i półnaturalnych są nadal ograniczone. Zrozumienie związku między różnorodnością gatunkową roślin a parametrami funkcjonowania ekosystemów, takim jak oddychanie gleby i determinującymi je czynnikami, jest kluczowe (Hillebrand i Matthiessen, 2009; Loreau i Hector, 2001). Różnorodność gatunkowa roślin musi być analizowana w kilku aspektach - zarówno pod kątem różnorodności taksonomicznej, jak i funkcjonalnej roślinności, determinujących je warunki siedliskowe oraz wpływ obu tych czynników na przebieg procesu respiracji gleby lub podłoża.

Różnorodność funkcjonalna wskazuje na różne procesy fizjologiczno-ekologiczne i adaptacyjne. Uwzględnianie tylko różnorodności taksonomicznej nie ujawnia pełnego spektrum zmienności roślin i jej wpływu na respirację gleby (Arnan i in., 2015). Dodatkowym aspektem różnorodności roślin jest różnorodność cech funkcjonalnych. Funkcjonalne cechy roślin są ściśle związane ze wzrostem gatunków, reprodukcją i konkurencją i mogą lepiej odzwierciedlać bezpośredni wpływ gatunków na funkcjonowanie ekosystemu (McGill i in., 2006). W ostatnich latach różnorodność funkcjonalna stała się głównym nurtem w badaniach nad różnorodnością roślin i ich funkcjami w ekosystemie (McGill i in., 2006).

W celu oszacowania wpływu różnych aspektów różnorodności składu gatunkowego roślinności spontanicznej poza różnorodnością taksonomiczną oceniono niektóre cechy funkcjonalne. Wśród analizowanych cech roślin należy wymienić: wczesne kwitnienie, cechy odpowiedzialne za konkurencyjność, wartość wskaźników Ellenberga dla parametrów siedlisk azotowych, anemochorię, wysokość roślin, zapylanie przez wiatr, zdolność do symbiozy mikoryzowej, wielkość powierzchni liści. Związek między różnorodnością funkcjonalną roślinności a oddychaniem gleby jest wieloaspektowy i obejmuje skomplikowane interakcje między cechami roślin, obiegiem składników odżywczych, zbiorowiskami drobnoustrojów i warunkami środowiskowymi. Zrozumienie tych zależności ma kluczowe znaczenie dla przewidywania, w jaki sposób ekosystemy mogą reagować na zmiany w różnorodności biologicznej i czynniki środowiskowe (Liu *i in.*, 2022).

6. Podsumowanie i wnioski

W pracy postawiono kilka hipotezy badawczych, które były weryfikowane w trakcie prowadzonych badań i analiz. Hipoteza badawcza dotycząca określenia zależności między abiotycznymi warunkami podłoża mineralnego w płatach spontanicznej roślinności występującej na zwałach skały a ilością uwalnianego CO₂ zakładała, że ilość węgla w podłożu będzie ma największy wpływ na poziom uwalniania dwutlenku węgla z powierzchni, którą porastały analizowane typy roślinności. Założono, że parametry abiotyczne, takie jak: skład granulometryczny, pojemność wodna, przewodność elektrolityczna oraz pH, nie będą miały znaczącego wpływu na intensywność przebiegu procesu uwalnianiem CO₂ z mineralnego podłoża zwałów skały płonnej. Wbrew oczekiwaniom, intensywność respiracji mineralnego podłoża zwałów skały płonnej nie jest związane wyłącznie z zawartością węgla. Wszystkie analizowane czynniki siedliskowe wpływały istotnie statystycznie na badany proces, poza zawartością azotu ogólnego. Poziomy potasu (K) i oddychania gleby wykazały istotną korelację. Warunki abiotyczne występujące w płatach roślinności zdominowanych przez *Daucus carota*, wpływały istotnie powodując silną ujemną korelację między abiotycznymi parametrami mineralnego podłoża a respiracją podłoża.

Kolejny etap analiz dotyczył określenia zależności między biotycznymi warunkami podłoża mineralnego w płatach spontanicznej roślinności występującej na zwałach skały a ilością uwalnianego CO₂. W tej części skoncentrowano się na analizie aktywności enzymatycznej, obecności przedstawicieli fauny glebowej nicieni i wazonkowców (liczebności) oraz ilości biomasy roślinności w kontekście różnorodności gatunkowej oraz ilości materii organicznej (SOM) w mineralnym substracie glebowym. Hipoteza badawcza zakładała, że w typach roślinności z większą ilością materii organicznej procesy respiracji podłoża będą przebiegały intensywniej oraz że typy roślinności o wyższej aktywności enzymatycznej i liczniejszej obecności fauny glebowej w strefie korzeniowej charakteryzują się większą intensywnością oddychania podłoża mineralnego. Uzyskane wyniki pokazały, że zgodnie z naszymi oczekiwaniami typy roślinności o większej ilości biomasy roślinnej oddychają intensywniej. Aktywność enzymów glebowych, takich jak dehydrogenaza, kwaśna fosfataza i zasadowa fosfataza w prezentowanych badaniach była dodatkowo skorelowana z ilością uwalnianego CO₂, jednak nie było korelacji między aktywnością ureazy a ilością uwalnianego CO₂ z mineralnego podłoża zwałów.

W trzeciej części pracy celem było zidentyfikowanie gradientów środowiskowych wpływających na różnorodność roślinności oraz analiza zależności między różnymi miarami różnorodności roślinności a parametrami oddychania mineralnego podłoża glebowego. Skoncentrowano się na identyfikacji czynników środowiskowych, które kształtują różnorodność roślinności, zarówno pod względem taksonomicznym, jak i funkcjonalnym, oraz ustalenie, jak te różnice wpływają na procesy oddychania gleby. Postawiono hipotezę, że różnorodność składu gatunkowego roślinności spontanicznej występującej na podłożu zwałów skały płonnej, kształtuje intensywność oddychania podłoża w nowych ekosystemach siedlisk mineralnych terenów pogórnicych. Im większa różnorodność taksonomiczna w danym płacie roślinności, tym intensywniejsza respiracja. Oddychanie podłoża różni się znacznie między typami roślinności i jest większe w płatach roślinności o dużym zróżnicowaniu funkcjonalnym. Wbrew oczekiwaniom oddychanie gleby było najwyższe w mniej zróżnicowanych taksonomicznie typach roślinności. Wśród komponentów funkcjonalnych tylko dyspersja funkcjonalna (wskaźnik niskiego filtrowania siedlisk) jest słabo ujemnie i istotnie skorelowana z respiracją podłoża mineralnego zwałów. Jednak wszystkie wskaźniki różnorodności funkcjonalnej są skorelowane, co wskazuje, że warunki sprzyjające zrzeszaniu się zbiorowisk roślinnych na mineralnym podłożu na hałdach skały płonnej w sposób odmienny niż w ekosystemach półnaturalnych i naturalnym.

W związku z tym wykazano, że respiracja jest zależny od wielu czynników abiotycznych i biotycznych w badanych płatach roślinności nowych ekosystemów i wymaga dalszych badań.

Postawione w pracy hipotezy zostały sfalsyfikowane. Wskazuje to, że przebieg badanych procesów: relacji między czynnikami biotycznymi i abiotycznymi, różnorodnością zbiorowisk roślinnych spontanicznie rozwijających się na mineralnym podłożu zwałów skały płonnej a procesami uwalniania dwutlenku węgla, w nowo powstających ekosystemach (*Novel ecosystems*), jest odmienny, niż w ekosystemach naturalnych i półnaturalnych.

Podsumowując, przeprowadzone badania dostarczyły dodatkowej wiedzy o zależnościach między elementami biotycznymi i abiotycznymi, różnorodnością spontanicznej roślinności a ilością uwalnianego CO₂ z podłoża poszczególnych płatów roślinności w nowo powstających ekosystemach na hałdach skały płonnej. Do tej pory nie prowadzono wieloaspektowych analiz z uwzględnieniem czynników abiotycznych oraz biotycznych na terenach hałd pokopalnianych. Informacje zawarte w niniejszej pracy są istotne,

ze względów poznawczych oraz praktycznych. Mogą korzystać z nich praktycy zajmujący się ochroną środowiska i zarządzaniem terenami pogórnymi oraz zaburzonymi. Wnioski płynące z tych badań, mogą być kluczowe dla opracowania strategii zarządzania środowiskiem naturalnym oraz ochrony bioróżnorodności, uwzględniając wpływ różnych czynników na procesy biogeochemiczne w glebie.

7. Streszczenie

Różnorodność biologiczna, w tym przede wszystkim różnorodność taksonomiczna oraz funkcjonalna roślin i jej wpływ na przebieg procesów ekosystemowych i funkcjonowania ekosystemów, pozostaje od dawna kluczowym przedmiotem badań nauk przyrodniczych na całym świecie. Pewne aspekty relacji między różnorodnością taksonomiczną i funkcjonalną a procesami oddychania gleby zostały poznane tylko w niektórych ekosystemach naturalnych, półnaturalnych oraz agrocenozach. Działalność człowieka w coraz większym stopniu wpływa na środowisko naturalne, istnieje pilna potrzeba zrozumienia, w jaki sposób zmiany te wpływają m.in., na oddychanie gleby, a tym samym na globalny obieg węgla.

Wielu badaczy uważa, że nowo powstałe siedliska będące efektem ubocznym eksploatacji surowców mineralnych stwarzają niepowtarzalną okazję do badania procesów sukcesji pierwotnej w szerokim zakresie różnorodnych warunków siedliskowych. Specyficzne biotyczne i abiotyczne warunki siedliskowe prowadzą do zrzeszania się tzw. *non-analogous species composition* zbiorowisk roślinnych o nieznanym dotąd składzie gatunkowym i prowadzą do powstania ekosystemów nazywanych *Novel Ecosystems* (*sensu* Hobbs i in., 2013; Keith i in. 2009). Według wielu badaczy nowe ekosystemy reprezentują odmienne niewystępujące wcześniej jednostki ekologiczne i powinny być identyfikowane i poznawane zgodnie z własnymi, niezależnie opracowanymi zasadami badania procesów biologicznych, i środowiskowych.

Górnictwo, eksploatacja surowców mineralnych znacząco przekształca krajobraz naturalny. Skomplikowane warunki siedliskowe obszarów pogórnich stanowią unikatowy układ eksperymentalny, który pozwala w wielkiej skali prowadzić badania spontanicznych procesów przyrodniczych. Stwarza to podstawę do realizacji studiów nad zrozumieniem funkcjonowania *Novel Ecosystems* (nowych ekosystemów), złożoności relacji między mikroorganizmami, roślinami oraz warunkami abiotycznymi i biotycznymi. Badania prowadzone w układach typu *Novel Ecosystems* pozwolą na poszerzenie rozumienia zależności w ekosystemach naturalnych i półnaturalnych.

Celem pracy jest **przeprowadzenie kompleksowej analizy wpływu czynników biotycznych i abiotycznych na przebieg procesów uwalniania dwutlenku węgla w płatach roślinności spontanicznej występujących na mineralnym podłożu nowych ekosystemów**

rozwijających na hałdach skały płonnej, które powstały w związku z eksploatacją węgla kamiennego.

W ramach niniejszej pracy przeprowadzono kompleksową analizę wpływu warunków abiotycznych i biotycznych, różnorodności taksonomicznej i funkcjonalnej, spontanicznej roślinności na ilość uwalnianego CO₂ z mineralnego podłoża zwałów skały płonnej w nowo powstających ekosystemach, wykorzystując różnorodne metody badawcze. W celu określenia biomasy nadziemnej dominanta i gatunków towarzyszących zastosowano techniki pomiaru masy roślinnej, umożliwiające dokładne wyznaczenie udziału poszczególnych gatunków w strukturze zbiorowisk roślinnych i ekosystemu. Analizę procesów metabolicznych mikroorganizmów glebowych wspierały pomiary respiracji podłoża mineralnego, umożliwiając ocenę aktywności metabolicznej. Dodatkowo określono aktywność enzymatyczną, wyrażoną przez aktywność dehydrogenazy, ureazy, fosfatazy kwaśnej i zasadowej, co pozwoliło na ocenę funkcji biologicznych gleby oraz procesów biogeochemicznych. Analiza fauny glebowej, oparta na ilościowej ocenie obecności wazonkowców i nicieni, stanowiła kluczowy aspekt badawczy, pozwalający na zrozumienie dynamiki ekosystemu glebowego oraz relacji między organizmami. Przeprowadzono szczegółowe analizy fizykochemiczne podłoża, obejmujące m.in. pomiary pH, zawartości składników odżywczych oraz tekstury gleby. Te badania miały na celu zrozumienie fizycznych i chemicznych właściwości mineralnego podłoża gleby, kluczowych dla utrzymania różnorodności biologicznej oraz funkcjonowania procesów ekologicznych. Metody badawcze wykorzystane w ramach niniejszej pracy stanowiły kompleksowy zestaw narzędzi analizy ekosystemu, umożliwiający jak najpełniejsze zrozumienie jego struktury, funkcjonowania oraz interakcji między organizmami. Otrzymane wyniki mogą znacząco przyczynić się do rozwoju wiedzy przyrodniczej oraz mieć praktyczne implikacje dla ochrony i zarządzania środowiskiem naturalnym.

W pierwszej części rozprawy celem było zbadanie zależności między czynnikami abiotycznymi mineralnego podłoża zwałów skały płonnej a intensywnością uwalniania dwutlenku węgla. Skupiono się na analizie poziomów oddychania gleby w kontekście parametrów abiotycznych, takich jak zawartość wody, tekstura gleby, pH, zdolność zatrzymywania wody (WHC), wymienne kationy, zawartość azotu oraz przewodność elektrolityczna (EC). Porównano te parametry z uwalnianiem CO₂ z hałd kopalnianych w ekosystemach typu *Novel Ecosystem* w strefie korzeniowej płatów spontanicznych typów roślinności. Wbrew oczekiwaniom intensywność respiracji mineralnego podłoża zwałów skały

płonnej nie jest związane wyłącznie z zawartością węgla. Wszystkie analizowane czynniki siedliskowe były istotne statystycznie, poza zawartością azotu ogólnego.

W kolejnym etapie badając zależności między czynnikami biotycznymi mineralnego podłoża zwałów skały płonnej a intensywnością uwalniania dwutlenku węgla, skoncentrowano się na analizie aktywności enzymatycznej, obecności nicieni i wazonkowców (liczebności) oraz ilości biomasy roślinności w kontekście różnorodności gatunkowej, oraz ilości materii organicznej (SOM *soil organic matter*) w mineralnym podłożu glebowym. Hipoteza badawcza zakładała, że w typach roślinności z większą ilością materii organicznej procesy respiracji podłoża będą przebiegały intensywniej, oraz że typy roślinności o wyższej aktywności enzymatycznej i liczniejszej obecności fauny glebowej w strefie korzeniowej charakteryzują się większą intensywnością oddychania podłoża mineralnego. Nasze wyniki pokazały, że podłoże płatów roślinności o większej ilości biomasy wykazują wyższe wartości respiracji, oddychają intensywniej. Badania wykazały, że ta zależność w płatach spontanicznej roślinności, tworzących nowo powstające ekosystemy na siedliskach mineralnych zwałów skały płonnej powstających po wydobyciu węgla kamiennego, jest dodatkowo silna i statystycznie istotna między parametrami oddychania podłoża mineralnego i ilością biomasy roślinnej. Aktywność enzymów glebowych, takich jak dehydrogenaza, kwaśna fosfataza i zasadowa fosfataza w naszych badaniach była dodatkowo skorelowana z ilością uwalnianego CO₂, jednak nie było korelacji między aktywnością ureazy a emisją CO₂ z gleby.

W trzeciej części pracy celem było określenie zależności między różnorodnością spontanicznej roślinności a intensywnością procesu respiracji mineralnego podłoża zwałów skały płonnej. Na podstawie wiedzy dostępnej z badań roślinności w ekosystemach naturalnych i półnaturalnych, przyjęto hipotezę, która zakłada, że im większa różnorodność w danym płacie roślinności, tym intensywniejsza respiracja. W pracy w pierwszej kolejności zidentyfikowano gradienty środowiskowe wpływające na różnorodność badanej roślinności zarówno pod względem taksonomicznym, jak i funkcjonalnym. Następnie przeanalizowano zależności między różnymi miarami zróżnicowania roślinności a parametrami oddychania podłoża glebowego. Ustalono, że intensywność oddychania podłoża mineralnego różni się w poszczególnych typach roślinności. Wbrew naszym oczekiwaniom oddychanie, uwalnianie CO₂ z podłoża mineralnego, było najwyższe w mniej zróżnicowanych typach roślinności. Wśród komponentów funkcjonalnych tylko dyspersja funkcjonalna (wskaźnik niskiego filtrowania siedlisk) jest słabo ujemnie i istotnie skorelowana z respiracją (SRL). Jednak wszystkie wskaźniki różnorodności funkcjonalnej są skorelowane, co wskazuje, że warunki

sprzyjające zrzeszaniu się zbiorowisk roślinnych na mineralnym podłożu na hałdach skały płonnej w sposób odmienny niż w ekosystemach półnaturalnych i naturalnym. W związku z tym wykazano, że respiracja jest zależny od wielu czynników abiotycznych i biotycznych w nowych ekosystemach i wymaga dalszych badań.

Poznanie i zrozumienie funkcjonowania układów typu *Novel Ecosystems* nowych ekosystemów oraz procesów prowadzących do współwystępowania najlepiej przystosowanych organizmów, oraz ich wzajemne relacje, umożliwi wykorzystanie tej wiedzy do wspomagania regeneracji funkcjonowania środowiska (usługi ekosystemowe i jakość życia ludzi). Wspomaganie powstawania układów typu nowych ekosystemów można uznać za lepszy sposób zarządzania terenami pogórnymi, niż podejmowanie ryzyka utraty czasu i środków finansowych na przywrócenie np. hałd pokopalnianych do stanu poprzedniego.

Zrozumienie zależności pomiędzy różnymi czynnikami abiotycznymi i biotycznymi oraz procesem oddychania gleby jest kluczowe dla oceny wpływu zmian środowiska na funkcjonowanie ekosystemów glebowych. Ponieważ działalność człowieka w coraz większym stopniu wpływa na środowisko naturalne, istnieje pilna potrzeba zrozumienia, w jaki sposób zmiany te wpływają na oddychanie gleby, a tym samym na globalny obieg węgla.

Podsumowując, przeprowadzone badania przynoszą nową wiedzę dotyczącą uwarunkowań zróżnicowania roślinności oraz oddziaływań między elementami biotycznymi i abiotycznymi na siedliskach mineralnych hałd pogórnymi. Do tej pory nie prowadzono szerokich analiz z uwzględnieniem czynników abiotycznych oraz biotycznych na pogórnymi podłożach mineralnych. Wnioski płynące z tych badań, mogą być kluczowe dla opracowania strategii zarządzania środowiskiem przyrodniczym oraz ochrony bioróżnorodności, uwzględniając wpływ różnych czynników na procesy respiracji przebiegające w podłożach mineralnych.

Postawione w pracy hipotezy zostały sfalsyfikowane. Wskazuje to, że przebieg badanych procesów: relacji między czynnikami biotycznymi i abiotycznymi, różnorodnością zbiorowisk roślinnych spontanicznie rozwijających się na mineralnym podłożu zwałów skały płonnej a procesami uwalniania dwutlenku węgla, w nowo powstających ekosystemach (*Novel ecosystems*), jest odmienny, niż w ekosystemach naturalnych i półnaturalnych.

Słowa kluczowe: respiracja gleby, hałdy pokopalniane, czynniki biotyczne, czynniki abiotyczne, różnorodność taksonomiczna, różnorodność funkcjonalna, nowe ekosystemy.

8. Summary

Biodiversity, especially the taxonomic and functional diversity of plants and its influence on ecosystem processes, has long been a significant research subject in the natural sciences worldwide. Certain aspects of the relationship between taxonomic and functional diversity and soil respiration processes have been understood only in some natural semi-natural ecosystems and agrocenoses. Human activities increasingly affect the environment, and there is an urgent need to understand how these changes affect soil respiration and, thus, the global carbon cycle. Many researchers believe that newly created habitats that are a by-product of mineral exploitation provide a unique opportunity to study primary succession processes in a wide range of specific habitat conditions. Specific biotic and abiotic habitat conditions lead to the association of so-called non-analogous plant communities of previously unknown species composition and the formation of novel ecosystems (Hobbs et al., 2013). According to many researchers, novel ecosystems represent distinct ecological units that have not existed before. They should be identified and studied according to their independently developed principles for studying biological and ecological processes.

Mining, the exploitation of mineral resources, significantly alters the natural landscape. The complex habitat conditions of post-mining areas provide a unique experimental system for large-scale studies of spontaneous natural processes. This provides a basis for conducting studies to understand the functioning of Novel Ecosystems and the complexity of the relationship between microorganisms, plants, and abiotic and biotic conditions. Studies conducted in Novel Ecosystem type systems will advance understanding relationships in natural and semi-natural ecosystems.

This study aims to comprehensively analyze the role of biotic and abiotic factors on the processes of carbon dioxide release in patches of spontaneous vegetation occurring on the mineral substrate of new ecosystems developing on waste rock heaps created in connection with coal mining.

This paper conducted a comprehensive analysis of the influence of abiotic and biotic conditions, taxonomic and functional diversity, and spontaneous vegetation on the amount of CO₂ released from the mineral substrate of waste rock piles in newly formed ecosystems, using various research methods. Plant biomass measurement techniques were used to determine the aboveground biomass of the dominant and associated species, allowing accurate determination of the contribution of individual species to the plant community and ecosystem structure. The

analysis of the metabolic processes of soil microorganisms was supported by measurements of the respiration of the mineral substrate, enabling the assessment of metabolic activity. In addition, enzymatic activity, expressed by dehydrogenase, urease, acid phosphatase, and alkaline phosphatase activities, was determined, allowing the assessment of soil biological functions and biogeochemical processes. Analysis of the soil fauna, based on quantitative assessment of the presence of vascular nematodes and nematodes, was a key research aspect for understanding the dynamics of the soil ecosystem and the relationships between organisms. Detailed physicochemical analyses of the soil were conducted, including pH, nutrient content, and soil texture measurements. These studies aimed to understand the physical and chemical properties of the soil's mineral substrate, which is crucial to maintaining biodiversity and functioning ecological processes. The research methods used within the framework of this work provided a comprehensive set of tools for ecosystem analysis, enabling the most possible understanding of its structure, functioning, and interactions between organisms. The results can significantly contribute to developing natural science knowledge and have practical environmental protection and management implications.

In the first part, the main objective was to study the relationship between the abiotic factors of the mineral bed of waste rock piles and the intensity of carbon dioxide release. The focus was on analyzing soil respiration levels in abiotic parameters such as water content, soil texture, pH, water holding capacity (WHC), exchangeable cations, nitrogen content, and electrolytic conductivity (EC). These parameters were compared with CO₂ release from mine dumps in Novel Ecosystems in the root zone of patches of spontaneous vegetation types. Contrary to expectations, the respiration intensity of the mineral substrate of the waste rock piles is not exclusively related to carbon content. All habitat factors analyzed were statistically significant, except for total nitrogen content. The substrate's potassium (K) content and the intensity of soil respiration showed a significant correlation in the plant community patches studied.

In the next stage, investigating the relationship between the biotic factors of the mineral substrate of the waste rock piles and the intensity of carbon dioxide release, the focus was on the analysis of enzymatic activity, the presence of nematodes and vascular nematodes (abundance) and the amount of vegetation biomass in the context of species diversity and the amount of organic matter (SOM) in the mineral soil substrate. The research hypothesis was that in vegetation types with higher amounts of organic matter, substrate respiration processes would be more intense and that vegetation types with higher enzymatic activity and a more

significant presence of soil fauna in the root zone would be characterized by higher intensity of mineral substrate respiration. Our results showed that the substrate of vegetation patches with more biomass shows higher respiration values, respiring more intensively. The study showed that this relationship in patches of spontaneous vegetation, forming newly formed ecosystems on the mineral habitats of waste rock piles formed after coal mining, is positively strong and statistically significant between the respiration parameters of the mineral substrate and the amount of plant biomass. The activity of soil enzymes such as dehydrogenase, acid phosphatase, and alkaline phosphatase in our study was positively correlated with the amount of CO₂ released. Still, there was no correlation between urease activity and soil CO₂ emissions.

In the third part of the study, the goal was to determine the relationship between the diversity of spontaneous vegetation and the intensity of the respiration process of the mineral bed of the waste rock dump. Based on the knowledge available from studies of vegetation in natural and semi-natural ecosystems, a hypothesis was adopted that the greater the diversity in a given vegetation patch, the more intense the respiration. The paper first identifies the environmental gradients affecting the diversity of the studied vegetation both taxonomically and functionally. Next, the relationships between various measures of vegetation diversity and soil substrate respiration parameters were analyzed. It was found that the intensity of the respiration of the mineral substrate differs between vegetation types. Contrary to our expectations, soil respiration was highest in the less diverse vegetation types. Among the functional components, only functional dispersion (an indicator of low habitat filtering) is weakly negatively and significantly correlated with respiration (SRL). However, all indicators of functional diversity are correlated, indicating that the conditions that promote the association of plant communities on mineral substrate in waste rock dumps act differently than in semi-natural and natural ecosystems. Therefore, respiration depends on several abiotic and biotic factors in new ecosystems and requires further research.

Knowing and understanding the functioning of Novel Ecosystems type systems and the processes leading to the co-evolution of the best-adapted organisms and their interrelationships will enable this knowledge to be used to support the regeneration of environmental functioning (ecosystem services and human quality of life). Supporting the emergence of New Ecosystems type systems can be considered a better way to manage post-mining areas than taking the risk of losing time and financial resources to restore, for example, post-mining heaps to their previous state.

Understanding the relationship between various abiotic and biotic factors and the soil respiration process is crucial to assessing the impact of environmental changes on the functioning of soil ecosystems. As human activities increasingly affect the environment, there is an urgent need to understand how these changes affect soil respiration and, thus, the global carbon cycle.

In conclusion, the research brings new knowledge on the determinants of vegetation diversity and the interactions between biotic and abiotic elements in mineral habitats of post-mining heaps. There has yet to be an extensive analysis considering abiotic and biotic factors on post-mining mineral sites. Conclusions from these studies can be crucial for developing strategies for managing the natural environment and protecting biodiversity, taking into account the influence of various factors on the respiration processes in mineral substrates.

The hypotheses posed in the study were falsified. This indicates that the course of the studied processes: the relationship between biotic and abiotic factors, the diversity of plant communities spontaneously developing on the mineral substrate of waste rock heaps, and the processes of carbon dioxide release in newly emerging ecosystems (Novel ecosystems), is different than in natural and semi-natural ecosystems.

Keywords: soil respiration, coal mine heaps, biotic factors, abiotic factors, taxonomic diversity, functional diversity, new ecosystems.

9. Bibliografia

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