Disertační práce

Ekologie *Calamagrostis epigejos* (L.) Roth

Pavla Vachová

ČESKÁ
ZEMĚDĚLSKÁ
UNIVERZITA V PRAZE

2017

Fakulta životního
prostředí

Česká zemědělská univerzita v Praze

Fakulta životního prostředí

Katedra ekologie

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Praha 2017

Třtino, co tě trápí sestřičko? Proč se tak pozachvíváš?
Neboj se, otevři mi srdce tvé, snad se ti uleví...
„Pozachvívám se arci, snad i radostí.
A též já si přeju něčemu to sděliti, tuším... V duši mé probouzí se vědomí, po lidsku řekněme myšlenka. Chcete ji věděti, bratříčku, chcete znát moji myšlenku, duši třtiny?“

Jakub Deml – Moji přátelé

Prohlášení

Prohlašuji, že jsem předloženou dizertační práci vypracovala samostatně a použila pouze prameny uvedené v seznamu literatury.

V Praze dne:............... Pavla Vachová

Poděkování

Děkuji školiteli Michalu Hejcmanovi za odborné vedení. Ráda bych také poděkovala manželovi a mamince, kteří mi byli velkou oporou. Děkuji Alče Walmsley a Ladě Jakubíkové za výbornou spolupráci a skvělou náladu v terénu i mimo něj. Děkuji také Květě a Václavovi, kteří mě vždy dokázali vytrhnout z trudomyslnosti. Mé díky patří i Vendulce Bronové, Evě Soulkové a Janě Novotné, bez nichž by studenti byli často zcela ztracení.

Děkuji Interní grantové agentuře FŽP za podporu mého výzkumu (IGA 20124283, IGA 20134296, IGA 20164226).

Obsah

1. Úvod

Člověk ovlivňuje krajinu ve střední Evropě už více jak deset tisíc let. Buď přímo tím, že začal obdělávat a využívat půdu, nebo nepřímo prostřednictvím lovu velkých býložravců. Díky těmto činnostem se vytvořila jedinečná mozaika biotopů. Na území dnešní České republiky se nejdříve hospodařilo na těch nejúrodnějších půdách, které mají většinou sprašový podklad. S vývojem lidstva se zvyšovaly i nároky na využívání půdy. Významným krajinotvorným elementem byla po staletí pastva. Páslo se nejen na pastvinách, ale i v lesích, sadech a později i na úhorech, kde byla pastva využívána především k odplevelení polí. Pastevní krajinu postupně nahradila různá luční společenstva, která se zformovala na rozličných geologických podkladech.

Pokud jsme tedy přetvořili divokou přírodu v souladu s našimi potřebami, musíme tedy počítat s tím, že bude potřeba dodávat energii na udržení požadované krajinné struktury. Přičemž není podstatné, zda jde o vstavačovou louku, krátkostébelnatou step nebo lipovou alej. Pokud se tyto staletí budované a opečovávané biotopy nechají svému osudu, lépe řečeno sukcesi, s velkou pravděpodobností zarostou nějakým invazním nebo expanzním druhem. Téměř s jistotou se na opuštěných plochách vždy objeví vysoce expanzivní tráva - třtina křovištní (*Calamagrostis epigejos*).

Krajina byla odedávna pozměňována i těžbou nerostných surovin. Malé opuštěné lůmky jsou jistým biotopovým zpestřením (tedy pokud neslouží jako skládka nepotřebného materiálu všeho druhu). Velkolomy zase mění celé krajiny – vytváří se krajina zcela nová. Záleží na způsobu rekultivace, jak bude finální krajina vypadat. Na místech po těžbě mohou vznikat vodní plochy, lesy, travní porosty. Pokud se přikloníme jen k drobným terénním úpravám a následné spontánní sukcesi, vznikne bohatá mozaika biotopů. Ale i tyto plochy jsou poměrně náchylné k zarůstání *C. epigejos*. Tato tráva má mnoho předpokladů pro strategické vítězství nad ostatními druhy. Problematika *C. epigejos* byla již řešena v mnoha vědeckých pracech. Přesto se stále neví, jaké bude její chování při určitých podmínkách spjatých především s přísunem živin, vodním režimem, defoliací, disturbancí a řadou dalších faktorů. Tyto faktory lze souhrnně nazvat managamentem. Na management jsou dále navázány růstové fáze, produkce biomasy, produkce a přežívání semen, či případná dormance. Pokud budeme znát ekologii tohoto druhu v celé jeho šíři (byť se jedná o velmi obsáhlé téma), je možné se následně zaměřit na jeho potenciální využití i v jiných než biologických disciplínách.

Hlavním cílem mojí disertační práce je rozšíření poznatků o ekologii *C. epigejos*. V předložených vědeckých pracích se snažím na problematiku nahlížet v širším kontextu, kdy do experimentů zahrnuji reakci na management (článek I), či nastiňuji, jak by mohlo vypadat společenstvo, které by bylo stabilní z hlediska abundance třtiny, ale ve kterém by zároveň neubýval počet ostatních druhů, jak tomu je doposud (článek II). Studovala jsem, jak je vegetace a půdní fauna ovlivněna topografickou strukturou výsypek (článek III). Zajímala mě také interakce zájmového druhu s potenciálními predátory (článek IV). V poslední studii byl využit velmi široký areál *C. epigejos*. Díky svému rozšíření může být využita pro mapování znečištění těžkými kovy z atmosférické depozice (článek V).

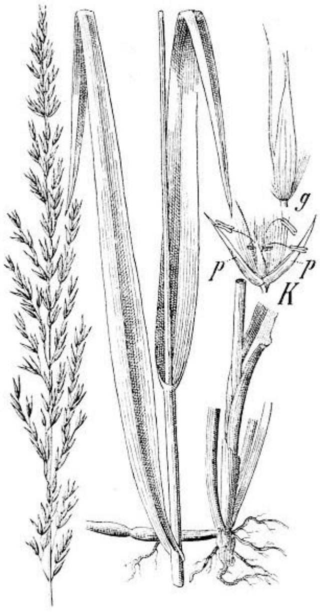
2. Rešeršní část

2.1. Taxonomie

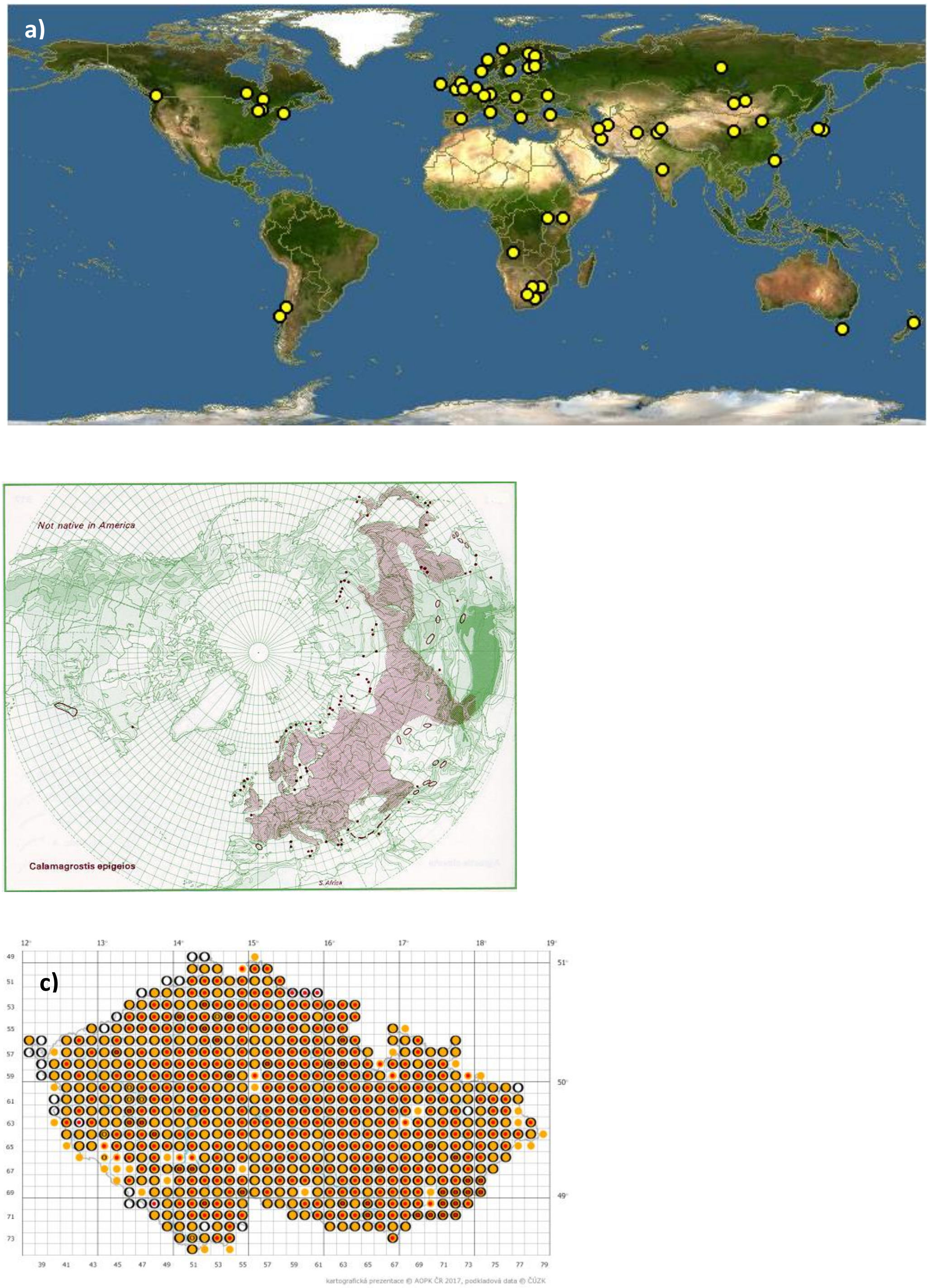
Rod *Calamagrostis* z čeledi lipnicovité (*Poaceae*) je poměrně široký taxon čítající více jak 300 druhů a jejich kříženců. Společnými znaky tohoto rodu jsou jednokvěté, postranně smáčknuté klásky o dvou plevách na delších stopkách a v latě více méně rozkladité, pérovité blizny vyčnívají z klásku obyčejně blíže spodiny. Pod pluchami jsou dvě zřetelné štětičky chloupků, které se později prodlužují. Plucha bývá zpravidla osinatá (Polívka, 1902). Latinský název pochází z řeckých slov κάλαμos (rákos) a αγρωστις. V České republice v současné době roste osm druhů z rodu *Calamagrostis* (Kubát, 2002).

2.2. Rozšíření

C. epigejos (obr. 1) je původní v Evropě, Asii a Africe. Druhotně je rozšířena v Severní a Jižní Americe, na jihu Afriky, ale také v Austrálii (obr. 2a, b). Na mnoha lokalitách bylo zaznamenáno její expanzivní chování, a to zejména na místech, která jsou odlesněná nebo jinak antropogenně zasažená. Roste od nížin do horských oblastí v suchých až velmi vlhkých porostech (Kubát, 2002). Postupné rozšiřování *C. epigejos* v České republice je znázorněno na obr. 2c, kde je vyznačen výskyt druhu podle nálezové databáze ochrany přírody AOPK ČR.



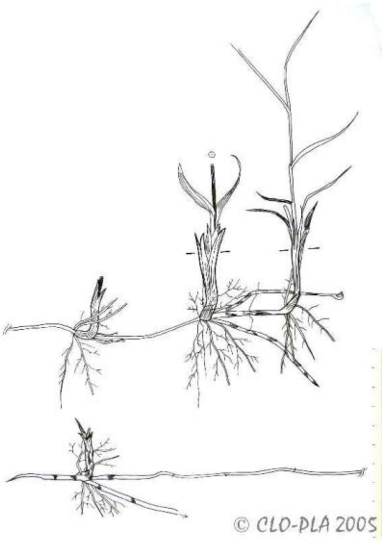
Obr. 1. *Calamagrostis epigejos*. Kresba A. Kašpar, převzato z Polívka (1902).



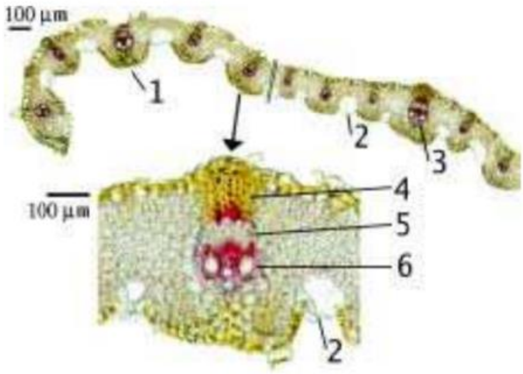
Obr. 2. Rozšíření *Calamagrostis epigejos*: obr. a) ●nálezy převzaté z Discover Life (2017); b) nálezy převzaté z Linneaus Server (1998); obr. c) převzat z AOPK ČR (2017): ◦ - nálezy do roku 1949, ● - nálezy v letech 1950-1989, ● - nálezy v letech 1990-2009, ○ - nálezy od roku 2010.

2.3. Morfologie anatomie a růst *C. epigejos*

C. epigejos (obr. 1) je statná, vytrvalá, vysokostébelnatá, výběžkatá tráva (obr. 3). V závislosti na autorství je uváděno poměrně široké rozpětí jejího vzrůstu, a to od 0.6 m až do 2 m (Kubát, 2002; Rebele et Lehmann, 2001; Regal et Šindelářová, 1970). Stéblo (*culnus*) má většinou 2-4 kolénka (*nodi*) a je pod latou silně drsné (Kubát, 2002). Listy jsou střídavé a jejich pochvy jsou drsné, v dolní části chlupaté, jazýček 2-4 mm, špičatý. Listové čepele jsou 5-15 mm široké, ploché nebo slabě svinuté, na líci drsné. Anatomická stavba listu je zobrazena na obr. 4. *C. epigejos* patří do skupiny tzv. C3 trav (Giraldo-Canas, 2010). Jedná se o evolučně starší způsob fixace CO_2, který využívá většina rostlin mírného pásma (Ehleringer et al., 1991).



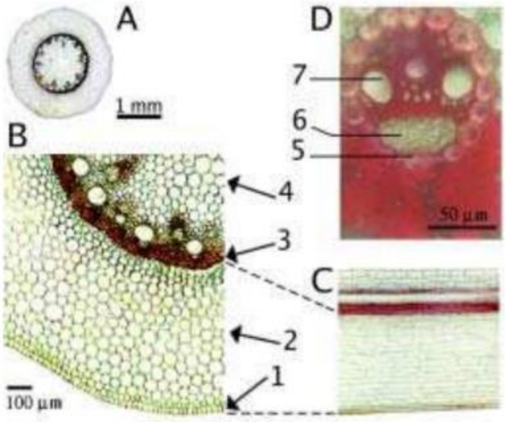
Obr.3. Klonální růst *C. epigejos*. Převzato z Klimešová et Klimeš (2013).



Obr. 4 Anatomická stavba listu. Příčný řez listovou čepelí. Mesofyl s neradiálním chlorenchymem. 1 – adaxiální žebro, 2 – stoma, 3 – cévní svazky, 4 – sklerenchym, 5 – floém (lýko), 6 – xylém. Převzato z práce Duška (2002).

Kořeny sahají do velké hloubky (Rebele et Lehmann, 2001) a jsou často velmi mohutné (obr. 5). Tyto vlastnosti mohou být určující v úspěšném využívání živin (Fiala, 2001). Jak ukazuje obr. 2, klonální šíření této trávy probíhá tak, že oddenek roste nejprve horizontálně, poté přechází ve vertikální růst a vytvoření nového výhonu. Anatomická stavba oddenku je detailněji popsána na obr. 6.

Obr. 5. Kořenový systém *C. epigejos* (foto: P. Vachová).



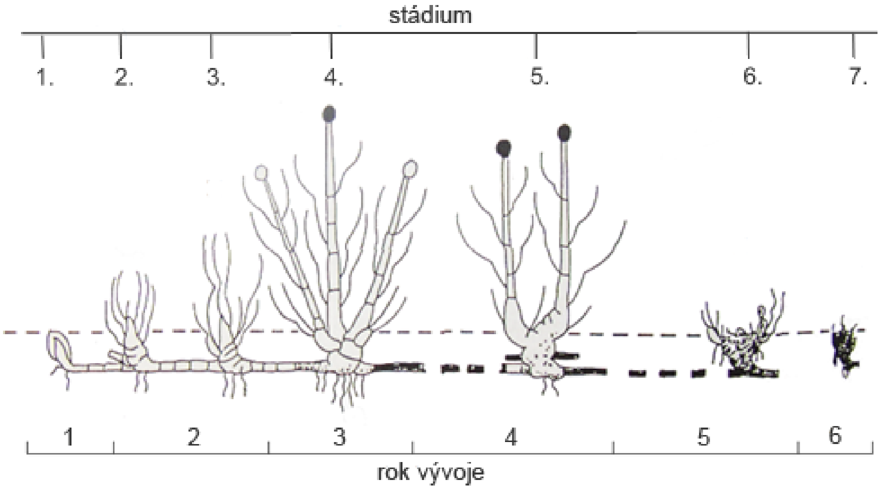
Obr. 6. Anatomická stavba oddenku - řez. A- celkový pohled na oddenek; B – oddenek detail C – podélná část oddenku; D – detail svazků. 1 – epidermis, 2 – kůra, 3 – sklerenchymatický kruh kolem centrálního válce, 4 – centrální válec, 5 – pochva, 6 – floém, 7 – xylém. Převzato z práce Duška (2002).

C. epigejos je rostlina s klonálním růstem, jejíž populační dynamiku lze rozdělit do dvou různých úrovní - na dynamiku ramet a dynamiku genet (Eriksson, 1989). Rameta je část genety, jedná se o nejmenší část klonální rostliny, která je po oddělení od genety schopna samostatného života. Geneta je definována jako soubor ramet, vzniklý z jedné zygoty klonálním růstem (Van der Maarel, 2005). Jednotlivé ramety jsou fyziologicky propojené. Dochází mezi nimi k distribuci sacharidů, živin a vody.

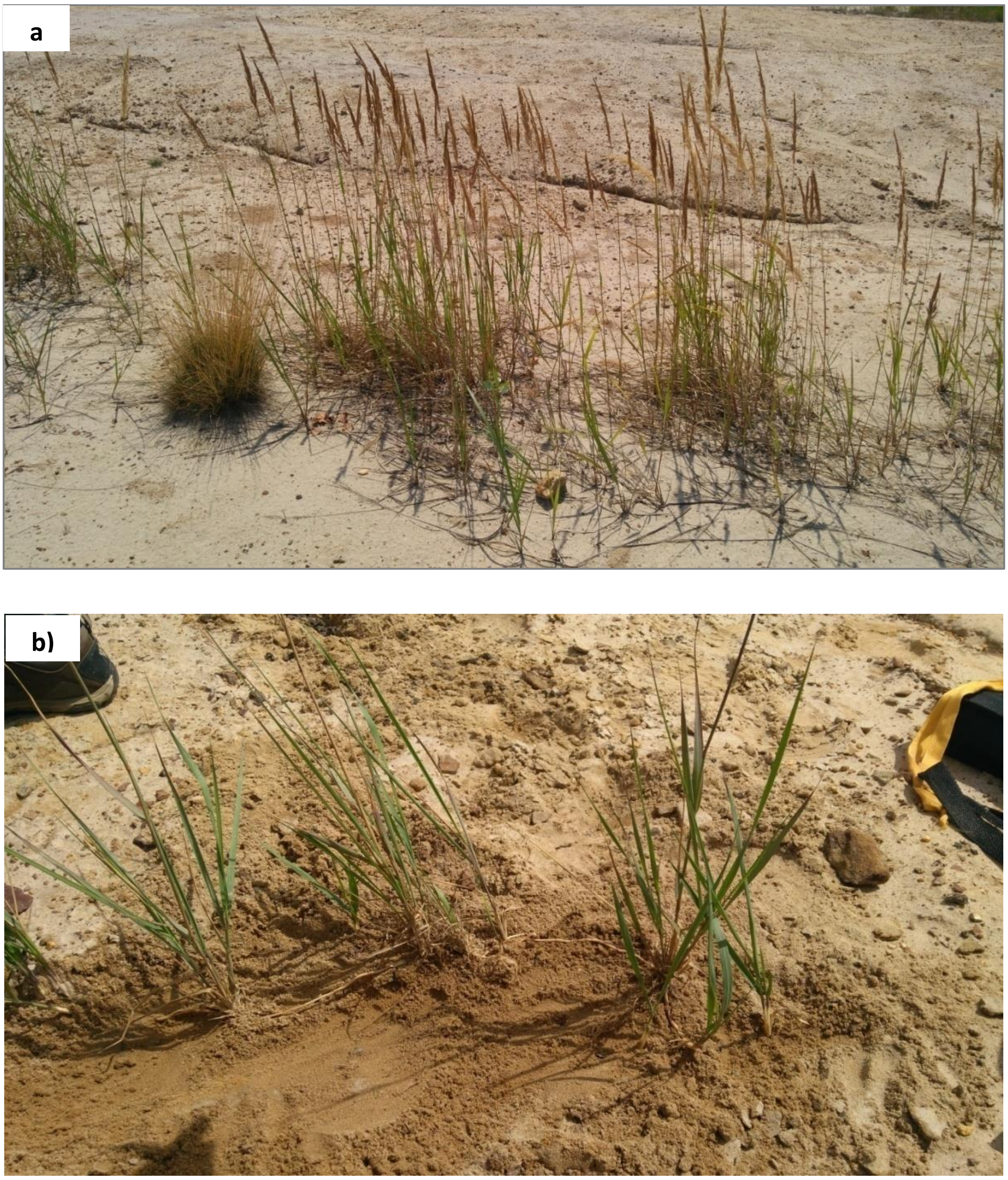
Nicméně toto propojení zřejmě nezvyšuje konkurenceschopnost (Březina et al., 2006).

Propojení ramet *C. epigejos* trvá déle než dva roky. Samotný životní cyklus od juvenilní ramety po odumírající dílčí shluk ukazuje obr. 7 (Janczyk-Weglarska, 1997). Střelec (2004) sestavil životní cyklus *C. epigejos* dle prací Dolečkové (1989) a Rebela et Lehmann (2001). Z juvenilní ramety po jednom až dvou letech vzniká dílčí shluk vněpochevním odnožováním. Tento shluk žije pět až sedm let a poté postupně odumírá. Od druhého či třetího roku postupně vykvétají jednotlivé prýty. Střelec (2004) dále u třtiny rozlišuje čtyři fáze růstu: (1) obsazování prostoru, (2) zahušťování porostu, (3) bohaté kvetení při dosažení maxima hustoty odnoží, (4) ředění polykormonu. Obr. 8 ukazuje reálné propojení jednotlivých ramet v genetu. Geneta samotná může zahrnovat mnoho ramet, jejichž počet se odvíjí od podmínek prostředí. Pokud jsou podmínky příznivé, *C. epigejos* vytvoří během jedné sezony i několik ramet.

C. epigejos je často považována za ryze klonální trávu, nicméně je dokázáno, že například disturbované plochy jsou osidlovány především ze semen vzniklých pohlavně (MacDonald et Lieffers, 1991). Pohlavní rozmnožování může být omezeno biotickými i abiotickými faktory prostředí, především na okrajích populací a často vede k jeho potlačení ve prospěch klonálního růstu (Eckert, 2001).



Obr. 7. Životní cyklus *C. epigejos* **1** – juvenilní rameta; **2, 3** – vegetativní dílčí shluk; **4** – generativní dílčí shluk; **5** – generativní dílčí shluk s kvetoucími bočními prýty; **6, 7** – odumírající dílčí shluk (převzato z Janczyk-Weglarska, 1997).



Obr. 8.. *C. epigejos*: a) geneta *C. epigejos* v písčitém substrátu; b) po odhrabání svrchní vrstvy substrátu je vidět propojení jednotlivých ramet (foto: P. Vachová).

Spektrum biotopů, ve kterých se *C. epigejos* vyskytuje, je velmi rozsáhlé. Prakticky se jedná o všechny antropogenně vytvořené či ovlivněné plochy, které jakýmkoli způsobem pozbyly managementu. Příkladem mohou být bývalá vojenská cvičiště, brownfieldy, výsypky, opuštěné travní porosty nebo pole (Mudrák et al., 2010; Prach et Wade, 1992; Rebele, 1996) Na těchto místech často působí jako sukcesní blokant. Třtina má mnoho vlastností, které jí přinášejí vysokou konkurenceschopnost. Mezi ně patří hlavně vysoká produkce biomasy a následná tvorba pomalu se rozkládající hmoty. Díky těmto vlastnostem dokáže třtina vytvářet specifické mikroklimatické podmínky omezující výskyt ostatních druhů a půdních mikroorganismů. Dalšími vlastnostmi jsou velká tolerance k abiotickému stresu a efektivnější využití vody a dusíku, než je tomu u jiných druhů rostlin (Fiala et al., 2003; Pruchniewicz et Żołnierz, 2016). V posledních letech vzniklo mnoho studií zkoumajících vliv dusíku na metabolismus a růst *C. epigejos* (Gloser, 2005; Gloser et al., 2004; Kavanová et Gloser, 2005). Z experimentů vyplývá, že rychlejší růst a reprodukce tohoto druhu jsou způsobovány příznivými vlhkostními a živinovými poměry (především dusíkem) a že při absenci managementu se stává konkurenčně silnou travou (Hejcman et al., 2009; Rebele et Lehmann, 2001; Tůma et al., 2005). V závislosti na zdrojích využívají mladé rostliny propojení ramet (Březina et al., 2006). *C. epigejos* je navíc vysoce tolerantní k různým druhům zátěže jako je spad popílku (Mitrović et al., 2008) a brání obnově původních druhů (Somodi et al., 2008). Existuje tedy mnoho důvodů, proč je tato rostlina významná, a to nejen v evropském měřítku (Chytrý et al. 2001).

2.4. Problematika expanzí

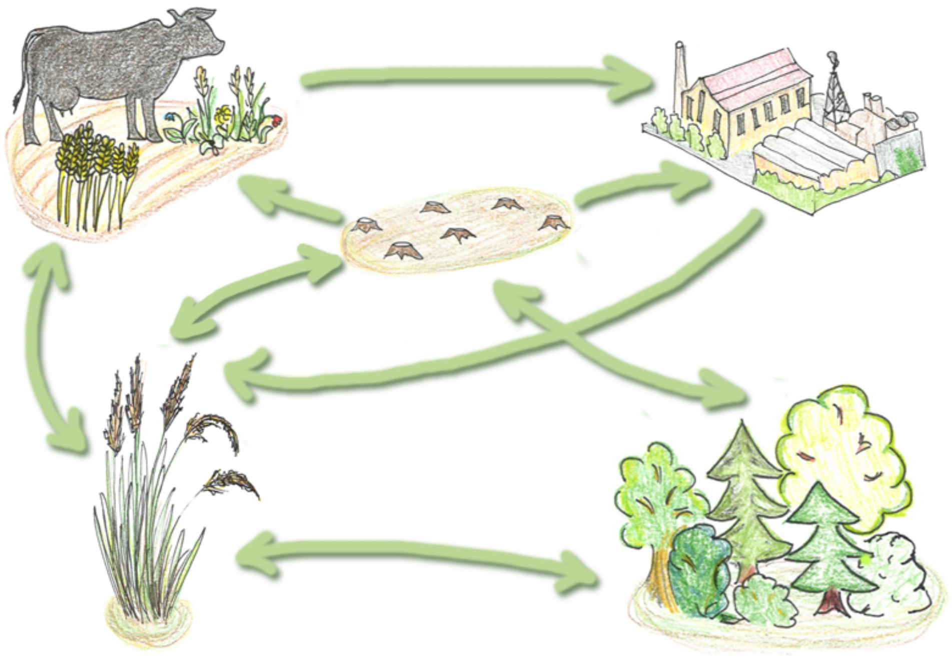
Expanze a invaze jsou často zkoumaným fenoménem napříč biologickými disciplínami. Expanzní a invazní druhy mají mnoho společného. Důležitá je i historie druhu, která bezpochyby ovlivňuje schopnost organismu invadovat nebo expandovat (Connell, 1980). Termín expanze poprvé použili Prach et Wade (1992) u původních druhů (apofytů), které se rychle šíří a obsazují nové plochy. Invazní druhy jsou takové nepůvodní druhy, které se nekontrolovatelně šíři na nová stanoviště. Ať už se jedná o expanzi či invazi, tento jev s sebou přináší změnu stanovištních podmínek (Fiala et al., 2003), jež může skončit degradací celého biotopu. Pochopení principu invazního chování je klíčové pro zachování původních biotopů a biodiverzity. Za zakladatele moderní invazní biologie je považován Charles Elton svou knihou Ecology of Invasions by Animals and Plants. Jeho hypotéza biologické odolnosti říká, že druhově bohatá společenstva jsou odolnější vůči invazím, protože jsou schopna využívat zdroje efektivněji než druhově chudá společenstva (Elton, 1958). V závislosti na druhu mohou být invaze a expanze ovlivněny i změnou klimatu. Nově příchozí druhy se mohou stát dominantnější v introdukci než druhy původní, a to ředevším díky odlišné interakci mezi půdou a rostlinou. Půda vždy hraje důležitou roli při rozšiřování druhů, protože ovlivňuje dominanci, vegetační dynamiku, ale také sukcesi (Hickling et al., 2006; Van Grunsven et al., 2010).

Expanze některých druhů je zcela přirozenou součástí vývoje vegetace nejen ve střední Evropě. Například expanze trav v pozdním miocénu byla způsobena nejen změnou poměrů složek atmosféry, ale byla poháněna i dalšími faktory, jakými byla např. tektonická aktivita spojená se suchem v nízkých zeměpisných šířkách nebo změna v globálních srážkových modelech (Pagani et al., 1999). Šíření trav bylo podpořené také předpokládaným přirozeným bezlesím na konci interglaciálu v pozdním holocénu (Magri, 1995). Paleobotanické studie dokazují, že invaze a expanze jsou běžným jevem (Pokorny et al., 2004). Většinou nastávají v důsledku lidské činnosti, ale nesmíme zapomínat, že právě díky ní vznikla mozaikovitá krajina a zvýšila se biodiverzita, jejíž zachování je aktuálním tématem (Pokorný, 2005).

Expanze stepí začala po antropogenním odlesňování na začátku neolitu (Jankovská et Pokorný, 2013). S rozmachem zemědělství expandovali *Plantago lanceolata* a *Calluna vulgaris*, ve středověku *Alopecurus pratensis* nebo *Veronica chamaedrys* a jiné plevele raných sukcesních stadií (Kozáková et Pokorný, 2007; Pokorný, 2005; Sádlo et al., 2008). V současnosti jsou za nejvýznamnější expanzní druhy v rámci České republiky považovány *C. epigejos*, *Gallium aparine*, *Taraxacum* sec. *Ruderalis* nebo *Urtica dioica*. Hnací silou současného expanzního chování rostlin bývá většinou eutrofizace, zvýšení obsahu živin (hlavně dusíku) a degradace biotopů (Haková et al., 2004). Obecně můžeme říci, že typický expanzní druh je autochtonní plevel s R nebo CR životní strategií, snadno se šíří, velmi dobře prospívá v eutrofizovaných půdách, jeho rozšíření souvisí s ruderalizací a apofytizací krajiny (Hájek, 2002; Sádlo et al., 2008). Teprve změnou stanovištních podmínek se rozšiřují druhy nepůvodní nebo expandují druhy, kterým nové podmínky svědčí (Czech et al., 2000).

Invaze jsou známy už od neolitu, kdy lidé začali významněji ovlivňovat své okolí. Nepůvodní druhy z tohoto období nazýváme archeofyty. Další významný okamžik nastal s objevením Ameriky na konci 15. století. Otevřely se nové obchodní cesty a po nich přicházely nové druhy – neofyty. Klasická invazní hypotéza je, že rozmanitost zvyšuje rezistenci společenstva vůči invazím (Tilman et al., 1996). Česká republika je invazím velmi otevřená. Důležitou roli přitom hraje husté osídlení, hustá síť řek a komunikací, četné disturbance, ale také blízkost velkých krajinných celků, pestrá mozaika biotopů a hojná migrace. Neměli bychom zapomínat na fakt, že mnoho druhů má na našem území severní hranici rozšíření.

Hovoříme-li o invazích, je důležitým pojmem invazibilita. Invazibilita je vznikající vlastnost prostředí, která je způsobena několika faktory, včetně regionálního klimatu, narušování režimu prostředí a konkurenčních schopnostech původních druhů (Lonsdale, 1999). Chytrý et Pyšek (2009) uvádí, že nejdůležitějším faktorem pro invadovanost společenstva je jeho typ, přičemž tento vliv je markantnější v případě archeofytů než u neofytů. Z invadovanosti lze do jisté míry usuzovat na invazibilitu. Nejvíce invadovaná společenstva jsou také nejvíce invazibilní. Jako nejvíce zasažená jsou ruderální společenstva a plevelová vegetace. Invaze jsou nebezpečné především pro jejich těžkou predikci a poměrně dlouhou lag-fázi. Hobbs et Humphries (1995) apelují na změnu lidské činnosti v introdukování nepůvodních druhů . Nejvíce invazibilní jsou disturbovaná společenstva s přebytkem zdrojů (živiny, světlo, voda) (Burke et Grime, 1996).



Obr. 9. Využívání krajiny ve vztahu k rozšíření *C. epigejos*. Šipky naznačují směr možného vývoje těchto vybraných stanovišť. (kresba: P. Vachová)

2.5. Expanze *C. epigejos*

Krajinu České republiky tvoří mozaika různorodých biotopů (Chytrý, 2010). Mnoho z nich je postiženo expanzí *C. epigejos*. Jedná se především o úzkolisté a acidofilní suché trávníky (Chytrý et al., 2001). V lesních společenstvech expanduje hlavně do *Luzulo albidae-Quercetum petraeae*, *Abieti-Quercetum, Mastigobryo-Piceetum* a dalších méně zastoupených jednotek (Neuhäuslová, 2001). Jako diagnostický, konstantní i dominantní druh je uváděna z třídy *Epilobietea angustifolii* (Chytrý et Tichý, 2003).

Expanze této trávy byla zmapována na různých lokalitách, např. v aluviích řek (Fiala, 2001; Fiala et al., 2003; Sedlaková et Fiala, 2001), lesních ekosystémech (Halarewicz et Pruchniewicz, 2015) či posttěžebních lokalitách. Biotopy typické pro expanzi *C. epigejos* jsou také rekultivované plochy (Hodačova et Prach, 2003; Mudrák et al., 2010; Šebelíková et al., 2016). Zajímavá, z hlediska expanzního chování *C. epigejos* jsou opuštěná vojenská cvičiště. Tyto plochy zahrnují různé typy vegetace a mohou být útočištěm pro mnoho druhů vzácných živočichů (Čížek et al., 2013; Reif et al., 2011). *C. epigejos* se dokáže šířit velmi rychle i na velké vzdálenosti (Lehmann et Rebele, 1994). Kovářovy (2004) studie odkališť prokázaly rozdílný způsob šíření v závislosti na typu substrátu. Zatímco na těžších půdách z rudní činnosti se tato tráva rozšiřuje pomocí typu falanga (obr. 10a), v lehčích půdách, vznikajících na popílkových odkalištích, se jedná především o typ guerilla (obr. 10b).

C. epigejos dokáže velmi dobře vytlačit jiné druhy (Mudrák et al., 2010) a pokud má dostatek dusíku, stává se dominantním druhem (Holub et al., 2012; van den Berg et al., 2005). K úspěšnému šíření této trávy přispívají i další faktory jako je zastínění (Somodi et al., 2008) nebo velké množství pomalu se rozkládající hmoty (Fiala et al., 2011). Stařina totiž pohlcuje světlo, zastiňuje semena a semenáčky a ovlivňuje teplotu půdy. Mnoho semen se díky silné vrstvě biomasy ani nedostane na půdu a nemá tedy šanci vyklíčit. To samozřejmě ovlivňuje utváření společenstva (Facelli et Pickett, 1991). Ve společenstvu, které je zasaženo expanzí, je obvyklé, že druhy ubývají úměrně s přibývající početností *C. epigejos* a některé zcela mizí (Fiala et al., 2003; Sedlaková et Fiala, 2001).

Obr. 10. Formy klonálního růstu u *C. epigejos*. a) typ Falanga; b) typ Guerilla. (Foto: P. Vachová)

2.6. Faktory ovlivňující růst a expanzi *C. epigejos*

2.6.1. Živiny

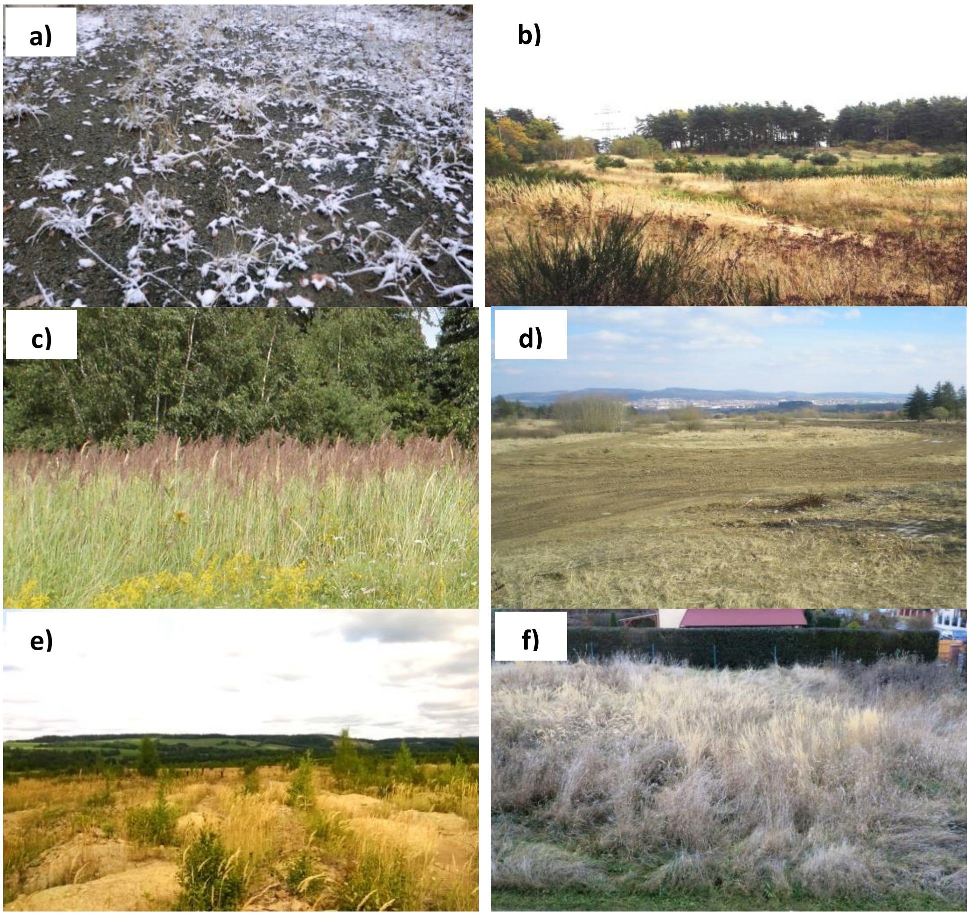
Nejen bez vody není života (Rada Evropy, 1968), ale kromě vodíku a kyslíku potřebují organismy i další prvky jako je uhlík, dusík, hořčík, fosfor, síru, draslík, vápník, mangan, železo, měď nebo zinek. Ovšem každý druh má jiné potřeby. Dostupnost živin, které jsou pro určitý druh důležité, může ovlivnit disperzi onoho druhu v prostředí (Begon et al., 1997). Reakce společenstva na dodané živiny bývá velmi rychlá a zpravidla vede na místní úrovni ke snížení druhové bohatosti (Grime, 2006; Huston, 1999). Dusík, forfor a draslík (tzv. NPK živiny) mohou totiž rychle měnit druhovou skladbu (Hejcman et al., 2012). Uvádí se, že za snižováním počtu rostlinných druhů stojí především vysoká dostupnost dusíku v kombinaci s fosforem, ale také absence kosení (Bakelaar et Odum, 1978; Elisseou et al., 1995; Kull et Zobel, 1991).

Klonální rostliny jsou obecně nenáročné na dostupné živiny (Klimeš et al., 1997). Nicméně studie, které se v minulosti zabývaly vlivem živin na růst a prospívání *C. epigejos* (např. Holub et al., 2012; Holub et Záhora, 2008; Tůma et al., 2009), jasně ukazují pozitivní vliv živin na její růst. Ovšem reakce tohoto druhu na živiny, zvláště v konkurenčně silném prostředí, se může projevit až po několika letech jejich pravidelné aplikace (např. Rebele, 2000). V expanzním chování *C. epigejos* hraje důležitou roli dusík. Spolu s fosforem možná roli hlavní. Pokud má tato rostlina dostatek dusíku, stává se dominantním druhem (Holub et al., 2012;

van den Berg et al., 2005) podobně jako při dostatečném množství dostupného fosforu (Lammerts et Grootjans, 1997 Hejcman et al., 2010; Suss et al., 2004). *C. epigejos* je konkurenčně silnější při dotaci dusíkem a absenci odstraňování biomasy (Tůma et al., 2005). Jako další vlivné vlastnosti byly určeny velká tolerance k abiotickému stresu a efektivnější využití vody a dusíku, než je tomu u jiných druhů rostlin (Fiala et al., 2003).

2.6.2. Antropogenní vlivy

C. epigejos expanduje především na antropogenně zasažených lokalitách (obr. 11), které pozbyly managementu (např. Prach et Wade, 1992). Bylo prokázáno, že tato tráva velmi dobře prosperuje i na znečištěných stanovištích (Mitrović et al., 2008). Ovšem její využití při remediaci se ukázalo jako nevhodné (Lehmann et Rebele, 2004).



Obr. 11. Příklady antropogenně zasažených lokalit s dominantní *C. epigejos*. a) opuštěný lom (Vráž u Písku), b) bývalé vojenské cvičiště (Oldřichov), c) neobhospodařovaná louka (Kostelec nad Černými lesy) d) disturbovaná plocha (Krašovice), e) hnědouhelná výsypka (Hornojiřetínská výsypka), f) neudržovaná zahrada v intravilánu (Kostelec nad Černými lesy) (foto: P. Vachová).

2.6.3. Arbuskulární mykorhiza

Výzkum zaměřený na vztah arbuskulárních hub (AMF) a rostlin je velmi zajímavý a jeho šíře je nad rámec této práce. Nicméně, několik projektů z této oblasti bylo zaměřeno právě na *C. epigejos*, která (ostatně jako většina druhů trav) není nejspíše na AMF významně závislá (Hetrick et al., 1992; Malcová et al., 2001; Rydlová et Vosátka, 2001). Příčina samostatnosti trav je v jejich jemném, rozvětveném kořenovém systému. To ovšem neznamená, že AMF v travních porostech chybí. Zdá se, že množství arbuskulárních hub je ovlivněno přítomností invazních, resp. expanzních rostlin. Důsledkem může být nižší diverzita arbuskulárních hub u původních rostlin a jejich horší prospívání (Endresz et al., 2013; Vogelsang et Bever, 2009). Pokud je arbuskulární mykorhiza zjištěna, zpravidla zvyšuje u *C. epigejos* biomasu, záleží ovšem na druhu inokulované houby (Püschel et al., 2007). *C. epigejos* využívá arbuskulární mykorhizu pro příjem fosforu, u dusíku to však prokázáno nebylo (Püschel et al., 2007; Rydlová et al., 2016).

2.6.4. Klasický management

Čtyřleté nebo i kratší období je pro potlačení expanzních druhů jako je *C. epigejos* velmi krátké (Křivánek et al., 2004). Po návratu managementu totiž začínají tyto druhy ustupovat až po šesti letech (Dostálek et Frantík, 2012). Šíření *C. epigejos* může být způsobeno nedostatečným managementem a dobrou dostupností dusíku (Hejcman et al., 2009). Nejen zastínění (Somodi et al., 2008), ale i velké množství pomalu se rozkládající hmoty (Fiala et al., 2011) brání znovuobjevení druhů typických pro pastviny. Zdá se ovšem, že na suchých písčitých a živinově chudých půdách může *C. epigejos* koexistovat s větším počtem druhů (Rebele, 2014).

Na druhově chudých trávnících bylo zaznamenáno, že pravidelné kosení jednou až dvakrát za sezónu může být pro tento druh stresující a třtina se nedokáže v takovýchto podmínkách šířit a postupně se zlepšuje i druhová skladba (Hakrová et Wotavová, 2004). Některé studie uvádějí, že po druhém (Házi et al., 2011) nebo po třetím (Rebele et Lehmann, 2002) roce seče se snižuje pokryvnost tohoto druhu. Házi et al. (2011) uvádí, že po čtyřletém sečení se začíná zvyšovat druhová bohatost. Důležité je obnovení managementu. Tím se zastaví degradace luk (Pruchniewicz, 2017) Nástrojem na zabránění rozrůstání by mohla být pastva (např. Stroh et al., 2002). *C. epigejos* se daří eliminovat i pomocí herbicidů (Janauer, 1995; Regal et Šindelářová, 1970). Na heterogenních plochách s dostatkem živin dokáže *Arrhenatherum elatius* vykompetovat *C. epigejos* (Tůma et al., 2009, 2005). U vřesovišť s výskytem *C. epigejos* je vhodné strhnout drn i se svrchní vrstvou půdy, přičemž vypalování při expanzi tohoto druhu není vhodné, protože jen napomáhá vegetativnímu rozšiřování (Prausová et Sádlo, 2004; Sedláková et Prausová, 2004).

2.6.5. Alternativní možnosti ovlivnění expanze *C. epigejos*

Klasické způsoby managementu travních porostů často nenaplňuje očekávání v podobě potlačení expanzních druhů jako je *C. epigejos*. V posledních letech proto přichází v úvahu alternativní možnosti eliminace nežádoucích druhů, kdy jedním z těchto způsobů je použití dosevu rostlin druhu kokrhel (*Rhinanthus* sp.). *Rhinanthus* je poloparazitická rostlina, jejímiž hostiteli jsou právě rostliny z čeledi *Poaceae* (Kocián, 2016). Kokrhel se svými haustorii napojí na kořenový systém *C. epigejos* (obr. 12) a tímto způsobem čerpá vodu, živiny a ve výsledku vede ke snížení biomasy této trávy (Těšitel et al., 2017). Nejlepších výsledků při snižování pokryvnosti *C. epigejos* bylo dosaženo, když byla před výsevem kokrhele odstraněna stařina (Mudrák et al., 2014). Tento způsob zásahu našel uplatnění i v plánech péče o zvláště chráněné území (Jongipierová et al., 2015).

Obr. 11. Napojení haustorií druhu *Rhinantus* na kořen *C. epigejos* (foto: J. Těšitel).

Další perspektivně se jevící možností, jak eliminovat *C. epigejos*, je využití larev kovaříků (drátovců), jež predují její výhonky (Roubíčková et al., 2012). Tomuto tématu se věnují studie předkládané jako součást této práce **(článek III a IV)**.

3. Cíle disertační práce

Disertační práce je zaměřena na ekologii *C. epigejos*. Hlavním cílem je přispět k rozšíření poznatků o tomto druhu a nalezení jednoduchých řešení, která mohou omezit růst a další rozšiřování *C. epigejos*. Tyto poznatky by mělo být možné využít jak v ochranářské, tak v lesnické a zemědělské praxi. Pozornost byla soustředěna především na tyto cíle:

Hlavní cíle práce:

1. Zjistit, jak probíhá expanze ve společenstvu s *C. epigejos*. (**článek I**)
2. Zjistit reakci *C. epigejos* na dodané živiny. (**článek I**)
3. Zkoumat teoretickou míru stability fytocenóz s dominantní *C. epigejos*. (**článek II**)
4. Otestovat možnosti eliminace *C. epigejos*. (**článek III** a **IV**)
5. Navrhnout praktické využití *C. epigejos*. (**článek V**)

4. Výsledky disertační práce

Článek I

The effect of management on expansion of *Calamagrostis epigejos* in grasslands

podíl autora: sběr, zpracování a analýza dat, návrh metodiky, tvorba manuskriptu

Článek II

Stochastic identification of stability of competitive interaction in ecosystems

podíl autora: sběr dat, návrh základního rámce metodiky, tvorba manuskriptu

Článek III

Topography of spoil heaps and its role in plant succession and soil fauna presence

podíl autora: sběr, zpracování a analýza dat, návrh metodiky, tvorba manuskriptu

Článek IV

Wireworms suppress spreading of the expansive weed *Calamagrostis epigejos* (L) Roth by feeding on its rhizomes

podíl autora: sběr, zpracování a analýza dat, návrh metodiky, tvorba manuskriptu

Článek V

Using expansive grasses for monitoring heavy metal pollution in the vicinity of roads

podíl autora: sběr, zpracování a analýza dat, návrh metodiky, tvorba manuskriptu

I.

The effect of management on expansion of *Calamagrostis epigejos* in grasslands

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Abstract

Spreading of *Calamagrostis epigejos* constitutes a serious problem for a wide spectrum of stands. We were interested in the height of vegetation, species diversity, abundance of *C. epigejos* and type of plant community created by management. We created an experimental area of fully randomized blocks. Each block consisted of five types of treatments differing in the quantities of phosphorus, nitrogen and potassium applied in different combinations of kg per hectare (N100, N100P30, N100P30K100, P30). We determined the composition and representation of individual species. We also collected soil samples, in which the amounts of P, Ca, Mg, K, N, C were analysed.

Presence of *C. epigejos* is positively correlated with the total height and coverage/biomass of vegetation. Application of phosphorus itself affects adversely both the height of vegetation and the total abundance and abundace of *C. epigejos*. The number of species is negatively affected by the supply of nutrients, nitrogen in particular, and by high abundance of *C. epigejos*. Good availability of nitrogen and the ability to eliminate smaller heliophytic species through shading their seedlings are main factors promoting expansion of *C. epigejos*. The one-year mowing doesn‘t affect the ability of the grass to spread.

Keywords Disturbed areas; Diversity; Nutrients; Mowing, Vegetation, Abundance

Nomenclature of vascular plants by Kubát et al. (2002)

1. Introduction

The landscape of Czech Republic comprises a mosaic of various biotopes (Chytrý, 2010). Many of these biotopes are affected by expansion of *Calamagrostis epigejos*. These are mainly nearrow-leaved dry grasslands and acidophilous dry grasslands (Chytrý et al., 2001). In forest biotopes, *C. epigejos* expands to the *Luzulo albidae-Quercetum petraeae, Abieti-Quercetum, Mastigobryo-Piceetum* and other underrepresented units (Neuhäuslová, 2001). It is reported from Class *Epilobietea angustifolia* as a diagnostic, constant and dominant species (Chytrý, Tichý, 2003). Abandoned military training grounds form an interesting phenomenon in the Czech landscape. These areas include different types of vegetation and can be refugees for many rare animal species (Čížek et al., 2013; Reif et al., 2011). Unfortunately, these areas are also frequently affected by the expansion of *C. epigejos*. This plant is a persistent, long-stemmed, rhizomatous grass, which has a spreading behaviour especially in locations that have been deforested or otherwise anthropogenically affected (F Rebele, Lehmann, 2001). It thrives from lowlands to mountain areas and in dry to very moist stands (Kubát, 2002). In these locations, *C. epigejos* frequently acts as a succession blocker. It has numerous traits that predispose it to be highly competitive - especially high biomass production and subsequent creation of slowly decomposing plant material, which enables this reed grass to transform microclimatic conditions so that they are not suitable and compatible with the existence of other plants and soil microorganisms. High tolerance to abiotic stress and more efficient use of water and nitrogen in comparison to other plant species have been established as further influential properties (K Fiala et al., 2003). In recent years, there has been a number of studies examining the influence of nitrogen on the metabolism and growth of *C. epigejos* (V Gloser et al., 2004; Vít Gloser, 2005; Kavanová, Gloser, 2005). Hejcman et al. (2009) examined the influence of mowing, not mowing and fertilization on the expansion of *Calamagrostis villosa* into subalpine Nardus grass stands. After an extended time period, it was determined that *C. villosa* spreads more to areas that have undergone fertilization and thus the discontinuation of agricultural management and increasing nitrogen availability contributed to the expansion of this grass. Due to a similar strategy of growth and resource utilization, we can apply this finding also to *C. epigejos*. It was also confirmed by Tuma et al. (2005) in a growing-pot experiment which demonstrated that *C. epigejos* is competitively stronger when nitrogen is added and trimming is not performed. It is also interesting how integrating ramets are used mainly by young plants and depending upon the resources available to them (Březina et al., 2006).

The objective of our experiments was to identify low-cost and simple solution for limiting the growth and further spread of *C. epigejos* without endangering species diversity and community stability. Although a certain reduction of *C. epigejos* biomass under the influence of UVB light has been reported (Oudejans et al., 2001), we created an experimental design that would enable us to observe this in conditions that can easily be simulated by means of fertilization and mowing. Response of community to the applied nutrients is very fast and usually leads to a reduction in local species richness (Grime, 2006; Huston, 1999). It is noted that primarily nitrogen in combination with phosphorus but also in the absence of mowing has a potential to reduce the number of species. (Bakelaar, Odum, 1978; Elisseou et al., 1995; Kull, Zobel, 1991). The basic questions are the following: 1) What kind of plant community is created under the influence of fertilization and mowing? (2) How do management practices affect stand height? (3) How does management affect species diversity? (4) How does management affect the abundance and number of *C. epigejos* shoots in individual squares?

2. Material and Methods

2.1. Experimental sites and design of experiment

The study site is located near Oldřichov, in the vicinity of Písek (49°19′46.274″N, 14°4′36.340″E) in southern Bohemia. This site is part of the Central Bohemian Pluton within the Moldanubian Zone. The geological bedrock of most of the area is the Central Bohemian Pluton of amphibole-biotitegranodiorites, Červensko type (Fišera, 1982). The experimental location is in an area with annual average air temperature of 7.1°C – 8.0°C, which range falls right into the middle of average temperatures within the Czech Republic. Mean annual precipitation is 501–600 mm. As true for all of central South Bohemia, this is an area with relatively sparse precipitation. This is caused mainly by its position relative to the Šumava mountains (CHMI, 2012). Phytogeographically, this area is in the Březnické Podbrdsko subregion of the Bohemian–Moravian mezophyte (Hejný, Slavík, 1988). The experimental design was proposed by the long-running experiments in Central Europe (Hejcman et al., 2007; Lepš, 1999). The experiment is done in full randomized blocks with three repetitions. Each block consists of five types of treatments, differentiated according to the amount of applied nutrients - phosphorus, nitrogen and potassium - in various combinations in kilograms per hectare (N100, N100P30, N100P30K100 P30) (Table 1). We provided the nutrients using commercial fertilizers at the beginning of the growing season. Nitrogen was applied in the form of ammonium nitrate with limestone, phosphorus as superphosphate, and potassium intake was simulated using potassium chloride. The squares were also differentiated according to whether or not they would be mowed. The area of a single square is defined as 9 m^2. To prevent edge effects, we evaluated only the central part of each square (Mládek et al., 2011).

The species composition including percentage was investigated in 2010 according to Moravec (1994). Nutrients were applied in April 2011, 2012 and 2013. In year 2010, the nutrients weren’t applied. The percentage of found species was assessed in the individual squares in July 2011, 2012 and 2013. The compressed sward height was measured with a rising-plate-meter (Castle, 1976; Correll et al., 2003).

2.2. Soil analyses

Soil samples were taken by sampler lawns at five randomly selected points within each square simultaneously with the species inventory. These samples were taken each years. The sampler has a diameter of 23 mm and a length of 10 cm. Samples were dried at room temperature and sieved through fine-sieve with a mesh size of 2mm. Soils were analysed for essential nutrients. P, Ca, Mg and K were analysed as available nutrients in the extraction solution by Mehlich III (Tran et al., 1990).

The plant accessible phosphorus was determined spectrometrically as phosphomolybdic blue at the wavelength of 750 nm light. Potassium was investigated by the atomic absorption spectrometer using air-acetylene flame. Calcium and magnesium were analysed with the same method, but the solution of lanthanum was added to the sample to suppress ionization.

Total Carbon (TC) and Total Nitrogen (TN) were determined using high temperature catalytic combustion with the the Primacs SNC equipment. The samples were introduced into a high temperature combustion oven, where at 1050°C carbon is completely oxidized to CO_2 at the presence of a catalyzer. CO_2 was measured by Non Dispersive Infra-Red Detection (NDIR) for Total Carbon. Total nitrogen was converted to N_xO_y which was then reduced at 600°C to N_2. The N_2 gas was measured by Thermal Conductivity Detection (TCD).

2.3. Statistical analyses

One way ANOVA and ANOVAR were used to evaluate the effect of treatment on all collected data. After obtaining significant results, multiple comparisons using Tukey's HSD test were applied to identify significant differences between treatments. Correlation coefficient was used for interspecies interaction. All analyses were performed using STATISTICA 9.0 software (Statsoft, Tulsa). CANOCO 5 program (Šmilauer, Lepš, 2014) was used to RDA analysis. It was applied for interaction treatment, individual plots and plant species in time.

3. Result

3.1. Height of vegetation

Vegetation height is positively affected by increasing abundance of *C. epigejos* (r=0.620). *Gallium verum* and *Knautia arvensis* can reach higher height, so shading by a tall grass doesn't affect them. The heliophilous species as *Plantago lanceolata*, *Dianthus deltoides*, *Festuca pratensis*, *Potentilla argentea* or *Viola tricolor* receded when vegetation height increased (fig.1). According to Tab 2, no differences between the individual treatments were observed in 2010 (F = 1.025, p = 0.42).

The addition of nutrients had an effect on vegetation height in the first year after application. A significant difference in height of vegetation was found between treatments P and NP, NPK in 2011 (F = 4.746; p <0.01). In 2011 the height of the vegetation was significantly lower in the P treatment in 2011 than in other treatment., This difference persisted even in following season of 2012 (F = 4.547; p = 0.01). A difference in the vegetation height wasn't observed for individual treatments in 2013 (F = 1.788; p = 0.18). According to the results for repeated measurement ANOVA indicated in Table. 3, the height of vegetation is unchanged in each treatment (F = 2.937; p = 0.873). We note demonstrable increase in height of vegetation during the monitored years (P = 2.5434; p <0.01) (tab2).

3.2. Number of species

The number of species in monitored squares was negatively affected by increasing amounts of *C. epigejos* and also over time (Fig 1)..

The negative correlation between number of species and the total abundance is shown in Fig. 4, in a RDA graph. The most affected species are *Veronica officinalis*, *Plantago lanceolata*, *Epilobium sp*., *Lotus corniculatus*, *Potentilla argentea*, *Festuca pratensis*, *Hypericum perforatum*, *Thymus chamaedrys*, *Gnaphalium sylvaticum*, *Trifolium arvense*, *Trifolium pratense*. These species disappear soon due to expansion of *C. epigejos* (Fig. 1). The results of repeated measurements ANOVA in Table 3 show that in variants, N, NP and NPK proven to reduce the number of species over the years (F = 18.26; p = 0.02). However, the number of species is not different in the monitored years, (F = 0.963; p = 0.51).

3.3. Abundance of *C. epigejos*

The most striking change in the abundance of *C. epigejos* was observed for the control treatment. There was a sharp increase in its abundance 2013 (F = 3.556; p = 0.03) (Tab. 3). The abundance of *C. epigejos* increased to similar levels in our experiment during the reporting period (Fig. 5). *Plantago lanceolata*, *Festuca pratensis*, *Centaurea jacea*, *Lotus corniculatus*, *Potentilla argentea*, *Dianthus deltoides*, *Taraxacum sect*. *Ruderalia* or *Trifolium arvense* receded immediately after expansion of *C. epigejos*. There is no a species that would have been positively impacted by the presence of *C. epigejos* in the plant community - as shown in Fig. 1 and Fig 4. Increasing abundance of *C. epigejos* negatively affects a number of species (r = -0.420).

Coverage of *C. epigejos* decreased in the first year after application of nutrients in all treatments (Tab. 3). A large reduction in abundance of *C. epigejos* was observed in P variation in 2012 and also the lowest total abundance was found in this treatment during the year (Tab. 2). The development of control treatments is also interesting. The first three seasons we observed very similar abundances of *C. epigejos*. However, a sharp increase in abundance of this species was observed in 2013 (F = 3.555; p = 0.034). The other treatments had similar outcomes, but this development did not have a statistically significant character (to see Fig. 5).

3.4. The community response to fertilization and mowing

We found that the content of soil nutrients in individual treatments was unchanged during the year for phosphorus (F = 0.879; p = 0.62), potassium (F = 1.2; p = 0.52) or for nitrogen (F = 1.783; p = 0.52). However, the nutrient enrichment resulted in changes in vegetation (Fig. 4). *Arrhenatherum elatius*, *Gallium pomeranicum* and *Carex hirta* are the species preferring a regime of high nitrogen and phosphorus input. The species of *G. album* and *G. verum* were largely replaced by their hybrid *G. x pomeranicum* during the reporting seasons Increasing abundance of *C. epigejos* negatively correlated with the abundances of *A. elatius* (r = -371). Figure 2a shows a representation of the percentage of each species in individual treatments. Control – with non-intervention regime - is uniquely suited to *Lotus corniculatus*, *Centaurea jacea*, *Plantago lanceolata*, *Festuca pratensis* but also to *Arrhenatherum elatius*. We also cannot ignore the fact that the occurrence of species *Trifolium pratense*, *Lotus corniculatus*, *Trifolium arvense*, *Vicia angustifolia*, *Holcus lanatus* or *Centaurea jacea* are positively correlated with the presence of *A. elatius*. (Fig. 1) We recorded an increase in abundance of *A. elatius*, *L. corniculatus*, *P. lanceolata* in the first year after application of nutrients. However, these species except *P. lanceolata* retreated again in subsequent years (Fig. 2b). Figure 4 illustrates well how the mown plots with sufficient nitrogen are more prone to expansion of *C. epigejos* than others. Other species prefer uncut treatments with NP, P and C addition.

The community has been changing continuously in time (Fig. 3), with increasing abundance of *C. epigejos* playing the major role. *H. perforatum*, *P. pratensis*, *P. argentea*, *V. officinalis*, *T. chamaedris*, *G. sylvaticum*, *B. sterilis* and *Epilobium sp*. have been declining in coverage during the observed years (Fig.1).

4. Discusion

The result investigate about height vegetation corresponds with the results of other studies about the impact of the available nitrogen on the rapid formation of biomass. However, the generally accepted positive effect of phosphorus on the amount of biomass (Hejcman et al., 2010; Suss et al., 2004) hasn't been confirmed.

The number of species in monitored squares was negatively affected by increasing amounts of *C. epigejos* and also over time. Although Házi et al. (2011) reported that an increase in species richness begins after four years of mowing.

The species disappear soon due to expansion of *C. epigejos*. This grass competes other species by shading (Somodi et al., 2008), but also a large amount of slowly decomposing materials (Karel Fiala et al., 2011). These factors and absent traditional farming prevents the reappearance of species typical for pastures. It appears that *C. epigejos* may coexist with a plurality of species only on dry sandy and nutrient poor soils (Franz Rebele, 2014). Some studies indicate that mowing reduces abundance of this species after two years (Házi et al., 2011) or after three years (Franz Rebele, Lehmann, 2002). However abundance of *C. epigejos* increased to similar levels in our experiment during the reporting period. This species can suppress other species very well indeed (Mudrák et al., 2010). When the nitrogen supplied, *C. epigejos* becomes the dominant species (Holub et al., 2012; van den Berg et al., 2005).

P. lanceolata was negatively correlated with the abundance of *C. epigejos,* but this doesn't mean that it would be completely expelled from the monitored squares - its presence can be supported by poor management and the availability of nitrogen (Hejcman et al., 2009).

The low cover of total abundance in squares could be due to a greater amount of old grass. Litter creates a physical barrier (intercepts light, shades seeds and seedlings, reduces soil temperature) for seedling and sprout emergence and also prevents seeds from reaching the soil. This affects the formation of plant communities (Facelli, Pickett, 1991).

The broad ecological amplitude allows *C. epigejos* adapt to different environmental conditions. Grazing donkeys could be a tool to prevent sprawl (Stroh et al., 2002), the presence of wireworms which predate its shoots (Roubíčková et al., 2012). Reaction of the grass to a new method of management is not immediate but can be fast. *G. x pomeranicum* . is very adaptable to conditions which are prevalent in habitats dominated by *C. epigejos* and good nutrient availability. It is therefore possible that these more resistant hybrids begin to dominate under pressure of the environment and the original parent plants disappear into more favorable biotopes. *A. elatius* grows frequently at species-poor stands (Scotton et al., 2014), in the next successional stages (Ondřej Mudrák et al., 2012), therefore it thrives in heterogeneous unmanaged areas with plenty of nutrients (Hejcman et al., 2007). It may be the strongest competitor of *C. epigejos* in this case (I Tůma et al., 2005; Ivan Tůma et al., 2009), as our results also suggest. *A. elatius* loses more nitrogen than *C. epigejos* when being cut (Holub et al., 2012), because it accumulates it in aboveground biomass (Berendse et al., 1992), therefore *A. elatius* needs a regular intake of nitrogen to maintain its fitness (Buckland et al., 2001). *L. corniculatus* is the species that does not occur at shaded places but it has a relatively broad ecological and phytocenological amplitude (Jones, Turkington, 1986) and responds positively to sufficient phosphorus (Kelman, 2006). *Centaurea jacea* occurs most often in community *Molinio-Arrhenatheretea* class but it is a common species of ruderal meadows, pastures and forest margins where it grows on nutrient rich soil. This species doesn't prefer shaded biotopes as *F. pratensis* and *P. lanceolata* (Slavík, B, 2004). The community, which it was part of here, if there wasn’t an expansion of *C. epigejos*, probably fell into the category T1.1., *Mesic Arrhenatherum* meadows, according to the Catalogue of biotopes (Chytrý et al., 2001). This idea is based on information from old maps. The most important factor causing changes in the community over time is probably spontaneous succession, however, it tends to lead to monospecific stands of this grass.

5. Conclusions

The experiment based on management practices represented by fertilization and mowing in different combinations didn't lead to finding an effective solution on eliminating expansion of *C. epigejos* in the anthropogenically influenced grassland. However, this study has contributed to deepening our knowledge about the ecology of this species; sowe can now better predict the direction of development of biotopes with *C. epigejos*.

C. epigejos positively affects the vegetation height and coverage. The species richness is closely related and negatively correlated with the abundance of *C. epigejos* – if the abundance of this grass increases, the other species disappear. The number of species is also negatively affected by nutrient addition, especially of nitrogen, concurrently supplied nutrients greatly accelerated the expansion of *C. epigejos*.

A complete change in communities over time is often driven by the expansion of *C. epigejos*. This grass expands irrespectively to most environmental parameters, because it is able to push out smaller heliophilous species through shading their seedlings (and creating an impenetrable layer of litter on the soil surface, which prevents seed burial and seedling), however, its expansion can be accelerated by high availability of nitrogen. Therefore there are very few anthropogenically influenced biotopes that *C. epigejos* fails to colonize and mowing throughout one year doesn't affect spreading of the grass. In absence of any further interventions, *Mesic Arrhenatherum* meadows are changing to species-poor communities with the dominant *C. epigejos* and they lose their ecological value and function.

Acknowledgments

Finalization of this paper was supported by the projects IGA 20124283 and IGA 20134296 funded by the Internal Grant Agency of the Czech University of Life Sciences.

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Table 1 Fertilizer treatments and amounts of nutrients applied in individual squares

Treatment	N(kg*ha^{-1})	P(kg*ha^{-1})	K(kg*ha^{-1})
N	100	-	-
P	-	30	-
NP	100	30	-
NPK	100	30	100
C	-	-	-

Table 2 Result of ANOVA and Tukey post hoc test for years in treatment, treatments with the same letter did not differ significantly at *p* <0.05.

	year	C	N	P	NP	NPK	F	p	F	p
Vegetation height	2010	26^{a}	33^{a}	30.5^{a}	28^{a}	26^{a}	1.025	0.423	2.5434	0.00751
	2011	30.2^{ab}	37^{ab}	23^{b}	39.2^{a}	38.8^{a}	4.746	< 0.01		
	2012	33.4^{ab}	32.2^{ab}	21.5^{a}	31.4^{ab}	41.2^{b}	4.547	0.011		
	2013	38.2^{a}	42.2^{a}	29^{a}	28.6^{a}	41.6^{a}	1.788	0.178		
Number of species	2010	14.6^{a}	13.8^{a}	17.5a	14.2^{a}	13.4^{a}	1.387	0.28	0.96255	0.51084
	2011	13.6a	10.2^{a}	13.5^{a}	11.4^{a}	9.8^{a}	1.932	0.151		
	2012	13.6^{a}	8.2a	14.5^{a}	10.0^{a}	9.6^{a}	2.708	0.065		
	2013	10.4^{a}	7.4a	12.0^{a}	8.6^{a}	9.4^{a}	2.058	0.132		
Total abundance	2010	105^{a}	101^{a}	104^{a}	107^{a}	108^{a}	0.186	0.943	1.2631	0.26335
	2011	106^{a}	101^{a}	104^{a}	107^{a}	108^{a}	0.795	0.545		
	2012	112^{a}	107^{a}	84^{c}	119^{ab}	131^{b}	5.558	0.005		
	2013	123^{a}	130^{a}	129^{a}	129^{a}	130.6^{a}	0.085	0.986		
Abundance of *C. epigejos*	2010	59^{a}	60^{a}	35^{a}	58^{a}	72^{a}	0.871	0.502	0.94515	0.52813
	2011	37^{a}	59^{a}	25^{a}	58^{a}	74^{a}	1.516	0.242		
	2012	47^{ab}	56^{ab}	16^{a}	51^{ab}	80^{b}	2.803	0.059		
	2013	84^{a}	93^{a}	80^{a}	83^{a}	90^{a}	0.767	0.562		

Table 3 Result of ANOVA and Tukey post hoc test for tratments in year, treatments with the same letter did not differ significantly at $p < 0.05$.

		2010	**2011**	**2012**	**2013**	**F**	**p**	**F**	**p**
Vegetation height	**C**	21.5	25.5	30.5	33.5	1.1956	0.3381	0.43	0.873
	N	24.5	21.5	20.5	36.5	0.319	0.8115		
	P	30.5	28	21.5	23	0.631	0.633		
	NP	33.5	36	36.5	39	3.496	0.0402		
	NPK	40	40	45	46.5	2.427	0.097		
Number of species	**C**	15.5^{a}	17.5^{a}	14a	5.5b	2.242	0.116	18.26	0.019
	N	14.5^{b}	10.0^{ab}	12^{ab}	7.5^{a}	6.876	0.003*		
	P	17.5^{a}	13.5^{a}	14.5^{a}	12.0^{a}	0.778	0.565		
	NP	14.5^{b}	11.5^{ab}	11.5^{ab}	8.0^{a}	16.81	< 0.001		
	NPK	12^{a}	9.0^{a}	10.5^{a}	10.5^{a}	7.222	0.002*		
Total abundance	**C**	74	99	107	128	1.39	0.276	6.972	0.071
	N	54	84	92	133	2.959	0.058		
	P	104	113	84	129	3.94	0.109		
	NP	100	66	131	124	1.515	0.243		
	NPK	112	109	131	146	3.619	0.032		
Abundance of C. epigejos	**C**	27.5^{a}	17.5^{a}	17.5^{a}	90^{b}	3.555	0.034*	3.768	0.155
	N	10.0^{a}	17.5^{a}	20.0^{a}	90.0^{a}	2.863	0.64		
	P	35.0^{a}	25.0^{a}	16.0^{a}	80.0^{a}	2.089	0.244		
	NP	55.0^{a}	50.0^{a}	80.0^{a}	90.0^{a}	1.549	0.241		
	NPK	75.0^{a}	70.0^{a}	67.5^{a}	85.0^{a}	2.305	0.109		

Figure caption

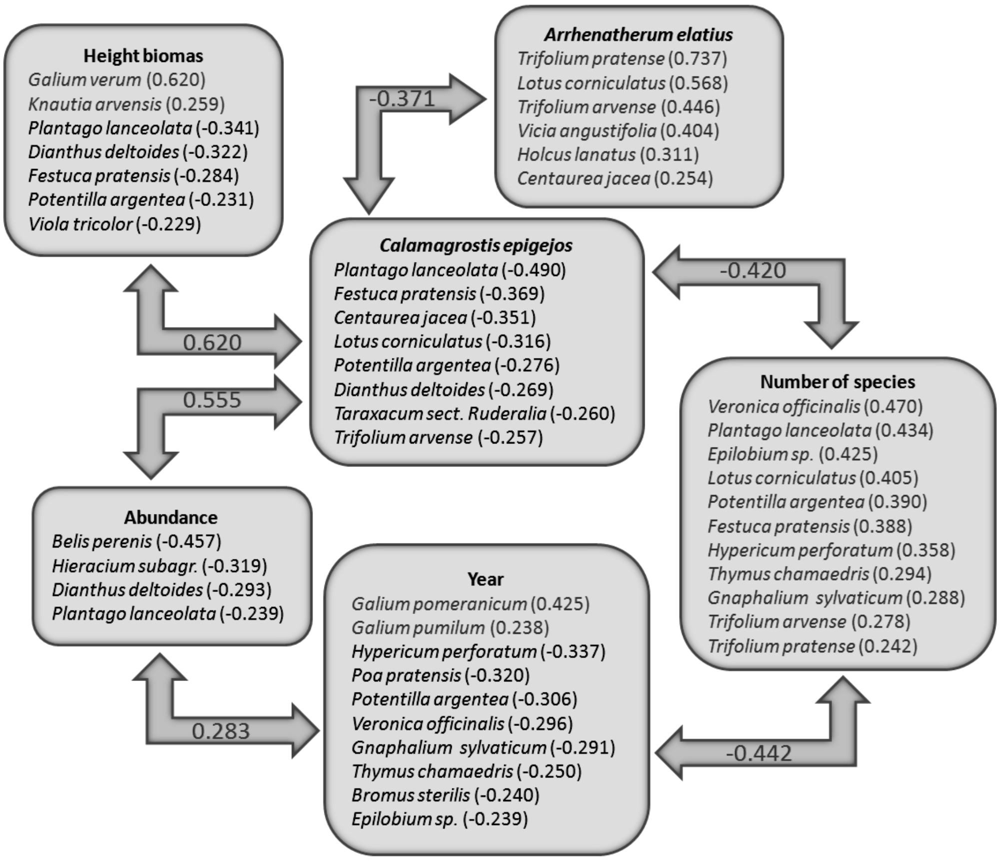

Fig. 1 Correlation diagram. The numbers in parentheses are the correlation coefficients between variable in the header box and abundance of plant species. Numbers in the darts are correlation coefficients between the variables in the header boxes. light gray are marked positive, correlation dark gray are negative.

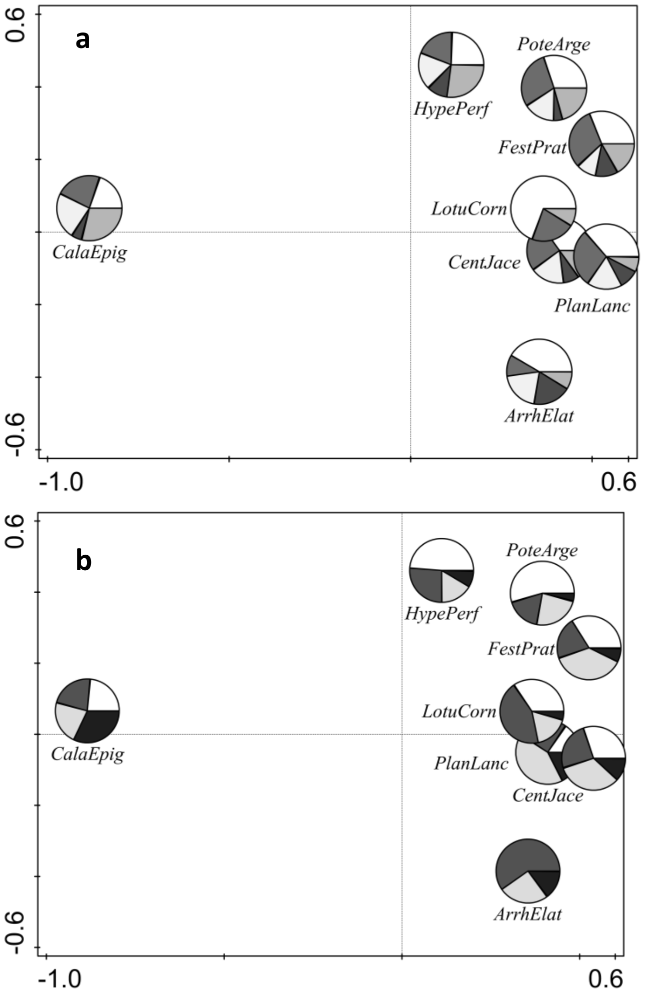

Fig. 2 Ordination diagram showing the results of RDA (CANOCO 5) of plant species in experimental sites. Pies represent 8 the best fitting species of a community. The first axis explains 9,45% of cumulative variance. **a)** The size of sector in pie represents percentage species abundance in treatment. The colours in pies represent treatment. Colour of treatment: light gray-N, black–P, dark gray-NP, medium gray-NPK, white-control. **b)** The size of sector in pie represents percentage species abundance in different years. The colours in pies represent year. Colour of treatment: white-2010, dark gray-2011,.light gray-2012, black–2013. ArrhElat – Arrhenatherum elatius, *CalaEpig – Calamagrostis epigejos, CentJace – Centaurea jacea, FestPrat – Festuca pratensis, HypePerf – Hypericum perforatum, LotuCorn-Lotus corniculatus, PoteArge– Potentilla argentea, , , PlanLanc – Plantago lanceolata,.*

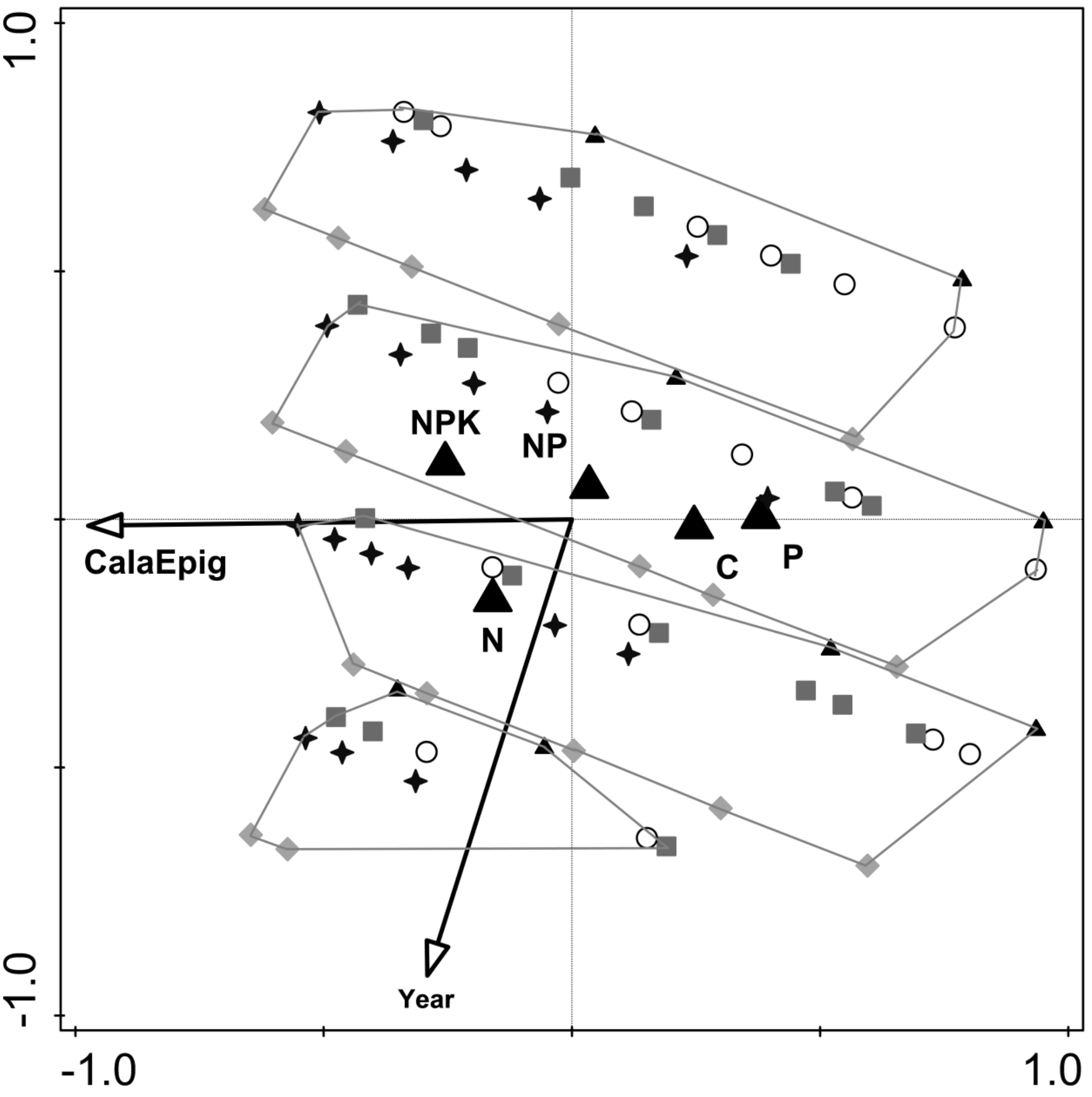

Fig. 3 RDA ordination diagram (CANOCO 5). Displayed the samples and three environmental variables (abundance C. epigejos, year, treatment). The first axis explains 9,45% of cumulative variance. The Polygons express observed years. Treatment: diamont – N, triangele – P, squere – NP, star – NPK, circle - C

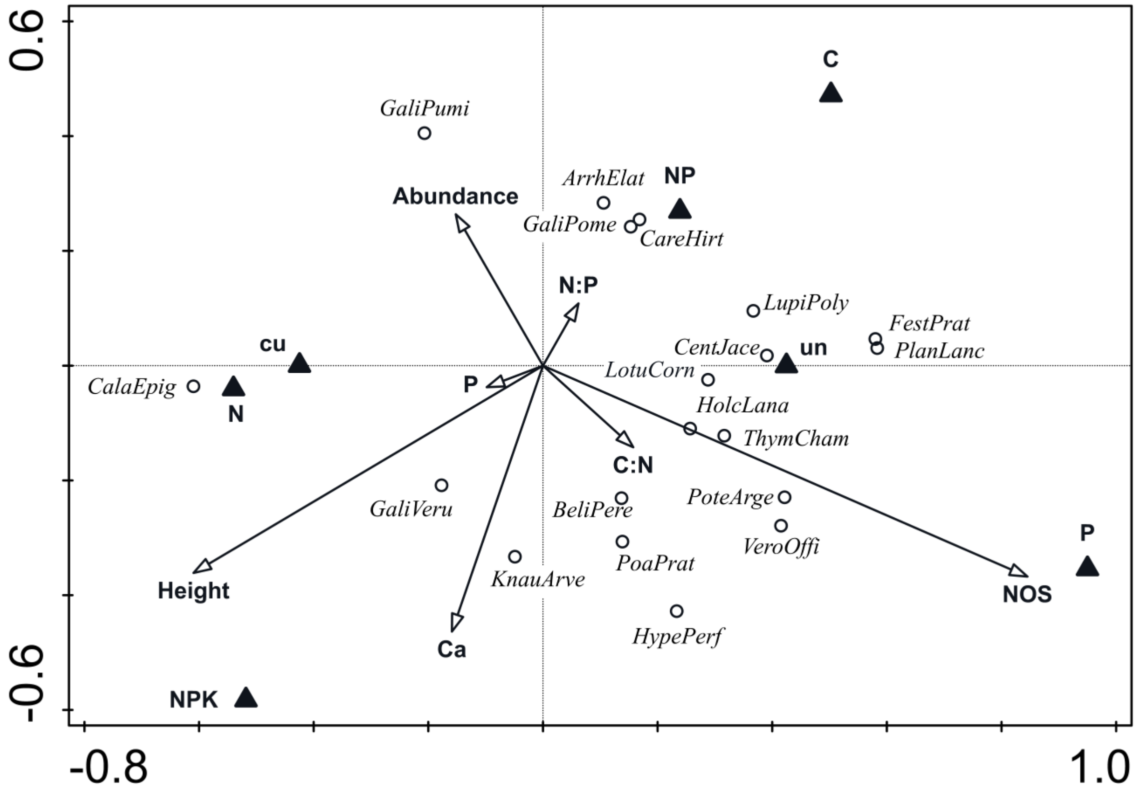

Fig. 4 Redundancy analysis (RDA, CANOCO 5) based on interactive forward selected environmental variables. The first axis explains 8.54% of cumulative variance. Environmental variables: Height – biomas height in sample. Abundance – total abundance in sample. NOS – number of species. Arrows Ca, P, N:P, C:N - nutrients content in the soil. Triangle N, P, NP, NPK – treatment. Triangle cu – mowed, triangle un – unmowed. *ArrhElat–Arrhenatherum elatius, BeliPere - Belis perenis, CalaEpig – Calamagrostis epigejos, CareHirt – Carex hirta, CentJace - Centaurea jacea, FestPrat – Festuca pratensis, GaliPumi – Galium pumilum, GaliPome – Galium pomeranicum, GaliVeru – Galium verum, HolcLana – Holcus lanatus, HypePerf – Hypericum perforatum, KnauArve – Knautia arvensis, LotuCorn – Lotus corniculatus, LupiPoly – Lupinus polyphyllus, PlanLanc – Plantago lanceolata, PoaPrat–Poa pratensis , PoteArge–Potentilla argentea, ThymCham – Thymus chamaedrys, VeroOffi – Veronica officinalis*.

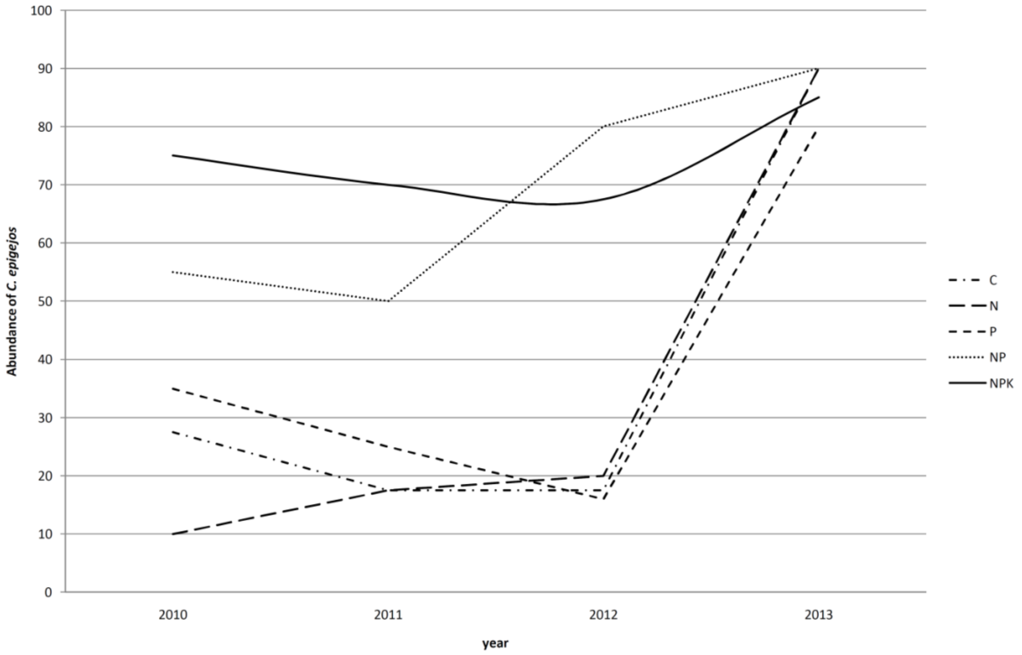

Fig. 5. Graph abundance of *C. epigejos* in the monitored years. The lines represent particular treatments.

II.

Stochastic identification of stability of competitive interaction in ecosystems

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Plos One

https://doi.org/10.1371/journal.pone.0155023

PLOS | ONE

RESEARCH ARTICLE

Stochastic Identification of Stability of Competitive Interactions in Ecosystems

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Abstract

The problem of finding an optimum within a set of possibilities that represent the varying successfulness of numerous subjects competing with one another is highly relevant in the field of ecosystem interactions. We propose a method for solving this problem by the application of the Nash equilibrium concept, which is frequently used in ecology. The proposed model is based on the transformation of the initial payoff vectors of subjects that interact in different situations into a statistical set of symmetrical game matrices that consist of permutations of payoff values. The equilibrium solution is expressed as values of the probability of Nash equilibrium occurrence with uniform distribution over all possible permutations based on uncertainty of positions of payoff values in the matrix. We assume that this equilibrium solution provides information on the distribution of the degree of stability among individual situations and interacting subjects. In this paper, we validate this assumption and demonstrate its application to a dataset that represents interspecies interactions in plant ecology. We propose that the use of the Nash equilibrium in the analysis of datasets formalized according to the Pareto optimality scheme is applicable in numerous other contexts.

Citation: Vach M, Vachová P (2016) Stochastic Identification of Stability of Competitive Interactions in Ecosystems. PLoS ONE 11(5): e0155023. doi:10.1371/journal.pone.0155023

Editor: Chris T. Bauch, University of Waterloo, CANADA

Received: November 4, 2015

Accepted: April 22, 2016

Published: May 12, 2016

Data Availability Statement: All relevant data are within the paper.

Funding: This work was supported by Internal Grant Agency of the Czech University of Life Sciences IGA 20134296 521/08/1131 (http://www.ga.czu.cz/, PV).

Competing Interests: The authors have declared that no competing interests exist.

Introduction

The problem of finding an optimum within a set of possibilities that represent the varying successfulness of numerous subjects competing with one another is not only a highly relevant basic topic in the field of economics but also in other disciplines. The basic approach for solving this problem in economics is to search for the Pareto optimality (PO) that corresponds to the state in which no subject can achieve a better result without worsening the result of another subject. The PO problem is often encountered in economics research. Of recently published research, the paper of Luc [1] is notable. The paper provides a detailed, exact analysis of a PO problem. The PO concept is practical, and it is frequently used in many contexts including environmental sciences [2–5]. Nevertheless, an evaluation of the equilibrium optimum within the set of possibilities that represent various successful competitive environments, e.g., the species represented in an ecosystem, may be realized using other approaches. In the first place, the application of the Nash equilibrium (NE), which is a fundamental principle of game theory [6], is offered as an option. Additionally, both principles—PO and NE—may be compared within the framework of the matrix game scheme. PO can be found in states that are not necessarily identical with NE [7].

In this paper, we propose a method for solving the problem of the equilibrium optimum in a set of situations with various payoffs for interacting subjects by applying the NE concept, which is frequently used in ecology. Most cases that involve the application of advanced game theory are referred to as evolutionary game theory (EGT). In the theory of evolutionary processes, game models are viewed as the basic unit of frequency-dependent interspecies interactions. Basic interaction types (e.g., plant–plant competition, plant–herbivore interactions, and plant–mutualism interaction [8]) are modeled based on defined strategies that species acquire during evolutionary development.

When simulating adaptive dynamics in phenotype space, a game model of interactions is applied in the replicator equation, the solution to which provides a picture of short- and long-term evolution [9]. The simulated population's per-capita growth rate can be determined using the G-function [10]. This function, which includes a specific game model, is dependent on the sizes and strategies of the interacting populations as well as the particular strategy, i.e., a trait of the species whose evolutionary development is being simulated [11, 12].

In EGT, the attainment of an evolutionarily stable strategy (ESS) is viewed as the optimal solution [13]. Every ESS is a strategy in a Nash equilibrium in the stage of linear game, although not all NE are made up of ESS. Matrix games as models of evolutionary interaction have been applied in many studies [14–20]. EGT has been applied to a number of specific cases, such as adaptations by organisms to recent climate changes [21], competitive interactions between root systems of plants [22], and many others. An example of an EGT-based study is the application of advanced game theory to the coordination of ecosystem management strategies [23]. In this example, the replicator equation follows from a bioeconomic game model, and the stable state is a NE.

In sum, the practical application of game theory in environmental science results in interactive models in the form of game types of various degrees of complexity. However, in contrast to the game concept, the problem analyzed in this research, i.e., the search for the equilibrium optimum within a set of variant situations that represent various payoffs for interacting subjects, does not include a deterministic selection of strategies. The problem that we solve may be regarded as a purely stochastic one. We propose a model for the evaluation of the equilibrium optimum in a set of observed data. In principle, this evaluation may be considered analogous to statistical data processing.

The proposed approach is based on transformation of the initial payoff vectors of subjects who interact in different situations in a statistical set of symmetrical game matrices that consist of permutations of payoff values. Additionally, the concept of variable payoff vectors in game models is introduced within a framework of the inclusion of more types of possible behavior of individual players. However, this proposed approach does not exclusively concern a stochastic problem. The result of the game is determined by the selection of strategies even though these strategies are linked with a specific type of player behavior defined by a probability value. Thus, extended game models were established by Harsanyi [24, 25] and have been developed particularly in the field of cooperative game theory [26, 27] and related research.

The solution proposed in this paper also transcends the category of stochastic games formed by a sequence of random states. The actual payoff depends on previous states of the game. Stochastic games were introduced by Shapley [28].

Our proposal for solution of the equilibrium optimum in a set of various situations is based on permutation of N-plets of payoff values of N interacting subjects in a symmetrical game matrix of a corresponding dimension. The individual variant configurations of this matrix are fully independent. That is, they do not exhibit a relative structural connection. Each incurred matrix represents a linear game in normal form with at least one NE. Therefore, the substance of the proposed model consists of the equilibrium solution of a symmetrical game matrix derived from payoff values of subjects that interact in a set of various situations. This equilibrium solution is expressed as values of the probability of NE occurrence with uniform distribution over all possible permutations given by the uncertainty of the positions of *N*-plets of payoff values in the matrix.

We assume that this equilibrium solution provides information on the distribution of the degree of stability among the individual situations and the interacting subjects. The problem of ecosystem stability is the topic of many works, where the relationship between stability and diversity is often evaluated [29–34]. We propose a stochastic model for calculation of the degree of stability that can be used to evaluate e.g. the selected data sets of ecosystem descriptors. Our approach can be applied in the simplest case to the numerical example of two species observed at four sampling sites mentioned in Chapter 4 of Legendre and Legendre Numerical Ecology [35]. A similar scheme is of course addressed in many research works, not only in ecology.

The model

Let a set of *K* alternative situations be defined by different external conditions. In each *K* situation, *N* subjects interact with one another. The results of this interaction are expressed by payoff values for each subject in all *K* situations. That is, the individual situations are advantageous to different degrees for the interacting subjects. The aim is to find the continuous distribution of individual situations along an axis that represents the equilibrium determined by a total degree of advantageousness for all interacting subjects. The maximum degree of equilibrium will be displayed by the situation that provides the best possible compromise of payoff values for all subjects. However, the evaluation of equilibrium is based on the NE concept, which cannot be found in a set of individual vectors of payoff values. Therefore, for the proposed method of evaluation, it is necessary to transform the initial payoff vectors into a normal form game matrix of dimension *N*. However, no information is available on the possible strategies of the interacting subjects that could determine the particular configuration of the game matrix. Nevertheless, it is possible to define two basic, apparent conditions for this transformation of payoff vectors into the game matrix. The first starting principle is that the derived game matrix should be symmetrical with respect to the number of interacting possibilities—therefore, formal strategies of interacting subjects in the sense of defining the game in the basic form. The second condition is connected with the stochastic substance of the proposed approach, which can be expressed as follows. Any information that substantiates a particular form of game matrix for NE evaluation is unavailable. Then, the only correct possibility is to include a complete set of all possible forms of game matrices to be derived, which can differ depending on the NE position within the limits of the elements of the game matrices. Of course, the elements in the derived matrices correspond to payoff values of *N* subjects in *K* individual situations $\pi_1^k, \ \ldots\pi_N^k$. Permutations that result in the formation of individual game matrices are identical in relation to interacting subjects (i.e., players). The individual game matrices concern permutations of *K* situations with fixed payoff values for players $\pi_1^k, \ \ldots\pi_N^k$, which are invariable with respect to changes in position in the matrix. The maximum, which is represented by a complete set of all possible permutations that determine the occurrence of NE in derived games, is achieved in the symmetrical payoff matrix of dimension *N*. If strategies are not made concrete, i.e., if (considering the evaluated NE position of the elements in the matrix) the sequence of columns or rows of the matrices to be derived does not matter because the total number of available games V with the possibility of a different NE result for N subjects (players) is given as follows:

$$V = \frac{(\prod_n^N A_n)!}{\prod_n^N A_n!} \tag{1}$$

An is the number of formal strategies of player n. The number of possible permutations V of the payoff matrix is the maximum for $A_1 = A_2 = \ldots = A_N$; therefore, for a symmetrical game matrix of dimension N. Because $(\prod_n^N A_n)! = K!$ is a constant and for the denominator is: $(A + \tau)! \cdot (K/(A + \tau))! > A!^2, A = \sqrt{K}, \tau \neq 0$ for a pair of interacting entities.

The evaluation of equilibrium for payoff vectors π_n^k primarily requires their linear mapping $\varphi : X_K \rightarrow Y_{K'}$ from space of variant situations X_K into space $Y_{K'}$, dimension K', which will enable the formation of symmetrical matrices, i.e., $K' \epsilon A^N$, where $A > 1$ is the selected number of columns and rows of the game payoff matrix. For example, the outcome for two interacting subjects may be $K' = 9$, i.e., $A = 3$, and the total number of permutations with the possibility of various results with respect to NE in this matrix is $9!/(3!^2) = 10{,}080$.

Each permutation of the derived payoff matrix is a game in the basic form with at least one NE. The condition for the best mutual response of two opponents (i.e., player n and player m) with respect to a Nash equilibrium is as follows [6]: Let x_n and x_m be mixed strategies of players n and m. Then, x_n is the best response to x_m if for $i \epsilon A_n \; x_{n,i} > 0 \Rightarrow (\pi_n \, x_m)_i = \max(\pi_n \, x_m)_k$ applies, and x_m is the best response to x_n if for $j \epsilon A_m \; x_{m,j} > 0 \Rightarrow (\pi_m \, x_n)_j = \max(\pi_m \, x_n)_k$ applies.

NE can be calculated as linear programming tasks for each matrix permutation. Specific calculations can be performed via labeled polytopes [36, 37]. For example, a 3 × 3 bimatrix game can be geometrically interpreted as two polyhedra in space that represents each player's individual strategies: x_1, x_2, x_3 for Player 1 and y_4, y_5, y_6 for Player 2. NE can be evaluated as the completely labeled vertices of these polytopes, which ensures that by definition the vertices are found at local maxima that correspond to identical rows and columns within each payoff matrix. The evaluated options represent games with NE on the generated polytope axes in pure strategies, on faces in mixes of two strategies, and for a proportion of payoff bimatrix permutations at the intersection of three faces in space in mixes of three strategies.

NE is found for every game from the complete set of evaluated permutations V of a symmetrical game matrix. Every found NE represents a vector of selection of formal strategies. That is, for player n, $x_{n,1} \ldots x_{n,A}$, $\sum_{i=1}^{A} x_{n,i} = 1$. The resulting values $x_{n,i}$ are counted as contributions to the total degree of equilibrium E_{nk} of player n in a situation k that corresponds to the determined NE. In the case of NE in pure formal strategies, the contribution $x_{n,k} = 1$ is the same for all players. For NE in mixed strategies, the contributions may be $x_{n,i}$ in individual situations (representing NE) for each player.

Each complete set of permutations of the symmetrical matrix (therefore, the game) has weight 1. If there is more than one NE in one game, the values of the counted contributions are divided by the number of determined equilibriums l_E. The resulting values of total $E_{n,k}$ are obtained as summary contributions of all V of solved games:

$$E_{n,k} = \sum_V \left(\frac{x_{n,k}}{l_E} \right)_v \tag{2}$$

Degrees of equilibrium for individual situations and players (therefore, interacting subjects) can be additionally relativized by dividing by a total number of evaluated permutations:

$$p_{n,k} = \frac{E_{n,k} A!^N}{A^N!}, \quad \sum_{k=1}^{K} p_{n,k} = 1 \tag{3}$$

This step results in the expression of the equilibrium solution of the derived game in the form of sets of values of probability $p_{n,k}$, thereby determining NE in the arbitrary permutation of a symmetrical game matrix for N interacting subjects in K situations.

The values $p_{n,k}$ characterize Nash equilibrium probabilities which could be interpreted as relative degrees of stability for interacting subjects in individual situations. Higher values indicate a preferable compromise of payoff values of interacting subjects in relation to equilibrium and therefore also a higher stability of evaluated situations.

In general contexts, the approach employed can also be validated (for case of two players) using an analogy with a corresponding elementary card game: Each player has K cards with various values. In each round of the game, one of the players arranges these K cards into a square with A cards per side ($A^2 = K$). The other player matches his or her cards to the cards the first player arranged to make prescribed pairs. K identical pairs are created each round and no other pairs are permitted. The order of the players alternates between individual rounds. The card pairs' square distribution is evaluated in each round such that each player is assigned the points of his or her cards or shares of cards which represent NE. The players' primary effort is to be assigned cards with the highest possible value. However, the players do not know the specific card distribution methods (the payoff bimatrix variations that lead to NE) with the greatest number of these highest cards. The distribution of card pairs in each round is therefore random. If all possible distributions are taken into account, it is possible to determine which cards of each player will win more frequently in the sense of their proportions of the NE found. The game's total points are therefore determined by the card values distributed to each player and how these cards are prescribed within the K defined pairs.

Example

As an example, an application of the proposed model in the field of plant ecology is described. The example concerns the evaluation of the stability of interactions between the expansive bushgrass *Calamagrostis epigejos* and other plant species in nine environments under various types of management.

The evaluated data were acquired over two seasons in an experiment performed in complete, randomized blocks with three repetitions. The experimental design was based on long-term experiments performed in Central Europe [38, 39]. Each block consisted of nine treatments represented by squares with an area of 9 m^2 within which various fertilization rates and mowing / no mowing managements were applied: C-cut, C-uncut, N-cut, N-uncut, K-uncut, NP-cut, NP-uncut, NPK-cut, and NPK-uncut. C designates variants with no fertilization, and C-uncut corresponds to squares without any intervention. Nutrients were supplied by applying commercial fertilizers. A community's response to added nutrients is rapid and typically results in a decrease in species diversity at the local level [40, 41]. During the second season, the relative spatial proportion of *C. epigejos* and the number of other plant species that occurred were evaluated for each square. To eliminate any possible distortion due to the edge effect, data were collected only in the central area of each square.

For the proposed evaluation method, data were available on the determined abundance of *C. epigejos* and the numbers of other species within the nine management treatments (individual abundances of the otrher species have not taken into account). Subject 1 is bushgrass with a

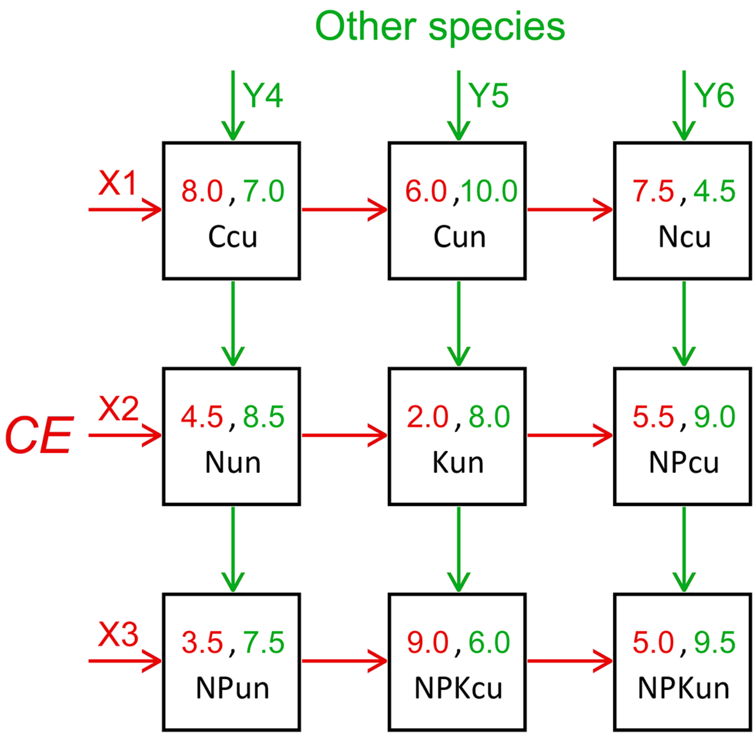

Fig 1. The derived payoff bimatrix: One of the 10,080 permutations. The bimatrix includes abundance values of the bushgrass Calamagrostis epigejos (CE) in multiples of 10% and numbers of the other species that were found (individual abundances of the other species have not taken into account).

doi:10.1371/journal.pone.0155023.g001

payoff table that expresses its abundance within individual squares in multiples of 10%. Subject 2 consists of other found species, and the payoff table states their determined numbers. All values represent the rounded means (with a minimum increment of 0.5) of each treatment's three repetitions. The particular payoff values for both subjects are provided in Fig 1. The vector of payoff values is dimension AN, and its additional transformation is unnecessary. Nine pairs of payoff values may be directly distributed into a 3 × 3 symmetrical matrix (Fig 1).

An overview of the total number of NE calculated for all 10,080 payoff bimatrix permutations (i.e., games) is provided in Table 1. NE was most frequently found in pure strategies, whereas in mixes of three formal strategies, NE occurred in fewer than 10% of the evaluated permutations.

The resulting NE probabilities $p_{n,k}$ for the individual management variants are shown in Fig 2 For each of the nine variants, we can define an expected value of 1/9 corresponding to a uniform distribution of NE probability. Within the context of the approach that was used, this value can be viewed as an indifferent state.

The results show that the application of the proposed concept offers the possibility of solving the problem of equilibrium optimum, i.e., the best compromise among payoff values, which are optimal for each interacting subject. The setting is not trivial, and the maximum payoff is achieved in both subjects in different situations. For *C. epigejos*, NPK-cut is an optimal situation. The other species achieve their highest numbers in the C-uncut situation. The evaluation of the initial payoff values according to the proposed concept unambiguously results in a preferred maximum number of other species, and thus a significantly higher probability of NE

Table 1. Total Nash equilibrium (NE) found.

Variant	Summary NE in pure strategies		Summary NE in mixes of two strategies		Summary NE in mixes of three strategies		Total summary NE	
	Subject 1 CE	Subject 2 Species	Subject 1 CE	Subject 2 Species	Subject 1 CE	Subject 2 Species	Subject 1 CE	Subject 2 Species
C-cut	360.0	360.0	799.0	654.9	101.0	75.0	1260.0	1089.9
C-uncut	3403.3	3403.3	621.9	548.9	64.8	85.9	4090.0	4038.2
N-cut	0.0	0.0	198.5	390.1	48.1	86.1	246.7	476.2
N-uncut	72.0	72.0	353.8	446.2	81.9	76.5	507.7	594.7
K-uncut	0.0	0.0	254.6	198.0	82.1	58.3	336.7	256.3
NP-cut	973.3	973.3	602.5	586.8	76.7	84.2	1652.5	1644.4
NP-uncut	0.0	0.0	265.5	259.6	84.1	68.5	349.6	328.1
NPK-cut	0.0	0.0	352.0	372.9	68.2	61.7	420.2	434.6
NPK-uncut	612.3	612.3	534.9	525.3	69.5	80.0	1216.7	1217.6
Total	5421.0	5421.0	3982.7	3982.7	676.3	676.3	10080.0	10080.0

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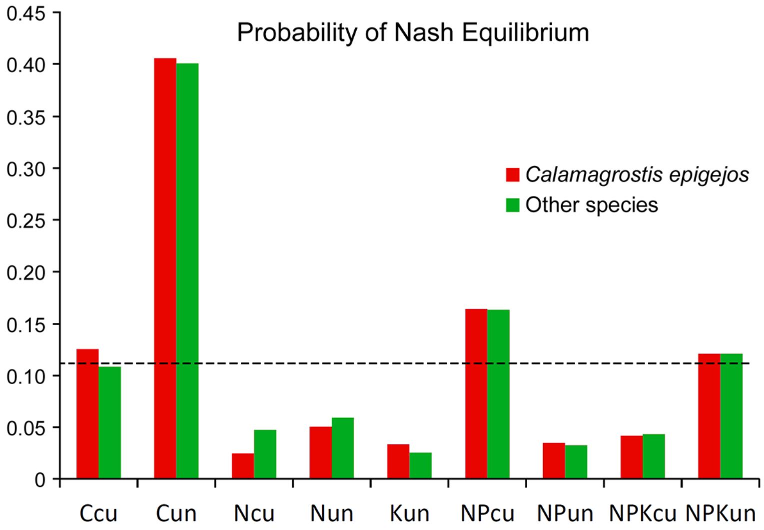

Fig 2. Obtained Nash equilibrium probabilities for individual management treatments. The dotted line is the expected value of 1/9 that corresponds to a uniform distribution of NE probability.

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occurrence $p_{n,k}$ results, which can be interpreted as a degree of stability (Fig 2). This result approximately matches common assumptions. That is, the highest degree of stability is achieved in a situation without interference, and this stability corresponds to the number of occurring species; therefore, corresponds to biodiversity and not to the abundance of expansive bushgrass. This fact is not only demonstrable with respect to the maximum obtained value $p_{n,k}$. The result of the proposed concept is a continuous distribution of the degree of stability of the evaluated situations. The correlation of this dependence with the set of initial payoff values unambiguously results in the beneficial occurrence of other species—$r = 0.617$ against $r = 0.114$ for *C. epigejos* abundance. That is, stability corresponds to biodiversity (Fig 3).

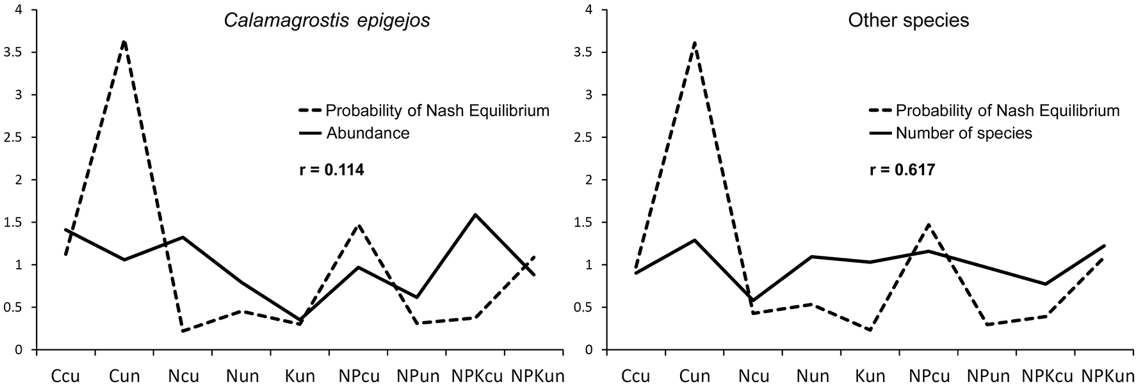

Fig 3. Comparison of the trend of NE probabilities for bushgrass abundance and number of other species. Presented trends are normalized to the mean value of one.

doi:10.1371/journal.pone.0155023.g003

The determinant factor with respect to the result is the occurrence of NE in pure formal strategies such that the calculated distribution of the degree of stability is not significantly different for both subjects. Therefore, it is impossible to deduce a recommendation regarding the type of management that might be more beneficial for the stability of occurrence of other species compared with the abundance of *C. epigejos*. However, the results of the evaluation may represent a contribution to research on the spread of this expansive grass. Based on the evaluation, a summary is possible: there are types of management that result in a more remarkable elimination of the occurrence of *C. epigejos* in plant communities, e.g., K-uncut. However, the assumed degree of stability of these management types is low compared with the non-interference situation, and these states may not be sustainable in the long term.

Discussion

The proposed model of evaluation of the occurrence of NE probability for a set of variant situations and subjects interacting in these situations can be considered to be a solution of a game with vector payoffs. The equilibrium concept in zero-sum matrix games with vector payoffs was developed by Shapley [42]. Variable payoffs may be considered as randomly distributed in commonness, therefore as values with random deviation. Harsányi [24] designed a model of purification of mixed equilibrium that enables a deterministic solution of equilibrium in a game with randomly distributed payoffs. In this paper, we have addressed a situation in which the quantitative results of interactions of the involved subjects (i.e., players) are well known, as are the payoffs in individual situations. However, there is no information on player strategy, which results in a particular limitation of the position of individual situations (linked with player payoffs) in the game matrix. We assume that this uncertainty can be revolved using a statistical set of all permutations of an evaluated situation in a derived symmetrical game matrix. NE is explicitly searched for in every permutation, and its occurrence (in pure or mixed strategies) is gradually counted for individual players in evaluated situations. Thus, our model is simple: the calculation of the probability distribution of NE occurrence does not require complicated mathematical generalization. Considering the established concept of a game with vector payoffs, in our model, all of the vectors in the game matrix are identical. In every solved step (i.e., permutation), the selection of individual payoff values is unambiguously determined by the permutation rules of the evaluated situations (linked with payoffs) in the game matrix.

The presented example of the application of the proposed concept of stability evaluation in datasets of a given type is a simple one with an unambiguous, easily substantiated result. It is obvious why the highest probability of NE occurrence results in the maximum occurrence of other species (in the situation without interference C-uncut) compared with the maximum abundance of *C. epigejos* (in NPK-cut) with the degree of equilibrium being low. The factor that determines the resulting distribution of the degree of equilibrium is NE occurrence in pure formal strategies for the given set of payoff values (Table 1). It is understandable from the initial payoff values that the abundance of *C. epigejos* achieves relatively higher values in a situation that includes the maximum occurrence of other species (i.e., C-uncut). Among the eight other situations, five display a lower abundance value of *C. epigejos* than occurs in situation C-uncut. Thus, the probability of finding NE in purely formal strategies for the situation of the maximum occurrence of other species (i.e., C-uncut) in any permutation of symmetrical game matrix 3 × 3 is 20:56 (0.36), which agrees with the obtained result (Fig 2) (this outcome also includes the lesser NE frequency that results from mixed formal strategies). In contrast, the situation with the maximum abundance of *C. epigejos* (i.e., NPK-cut) includes the smallest number of other species of all nine situations. Thus, the probability of NE occurrence in pure formal strategies is zero for this situation, which is documented by the results presented in Table 1.

Of course, the proposed procedure of stability evaluation in the system represented by the determined payoff values *N* of interacting subjects in *K* situations can be used to evaluate more complex interactions than those included in the presented example. For example, the procedure may be applied to interactions of more subjects with an ambiguous probability preference of equilibrium maxima. In such cases, the obtained stability distribution can be predominantly determined by NE found in mixed formal strategies, and thus, the values of NE probability will be more clearly different for the interacting subjects of the individual situations. Naturally, stability distribution is not required to correlate in individual subjects with their initial payoff values.

The significant feature of the proposed concept is that the result—the evaluation of the degree of stability based on the NE principle—is guaranteed for an arbitrarily complex interactive system. Every permutation of the derived matrix of any dimension *N* can be considered to be a payoff table of a game that has at least one NE. The full statistical set of NE occurrence in the permutations of the game matrix is always available for the evaluation of the distribution of the degree of stability.

The proposed approach is only based on the comparison of measurable or observable values of quantitative parameters without the inclusion of specific internal factors that determine the complex behavior of the ecosystem. The interpretation of a larger probability of NE occurrence as a higher stability of ecological interaction is an assumption that often is not applicable. In addition, the degree of stability defined in this way is only relative. It is larger or smaller only compared with the results obtained for other evaluated situations.

However, approaches based on game theory and NE application are often used in ecology, particularly in evolutionary ecology, as noted in the introduction. The proposed concept, which is exclusively derived from the stochastic game model, can have its foundation in the context of frequent exploitation of game theory principles for solving ecological problems. Out of all of the supposed applications of varying complexity, the use of proposed approach can be demonstrated on general numerical example of two species observed at four sampling sites, cited in the introduction [35]. It is a very simple example of a multidimensional quantitative data set. The statistical evaluation primarily leads to the covariance matrix and the result is an information on the degree of similarity or differences of descriptors of behavior in the set of selected sites. Our proposed model assesses the situation in a completely different way. The result shows the

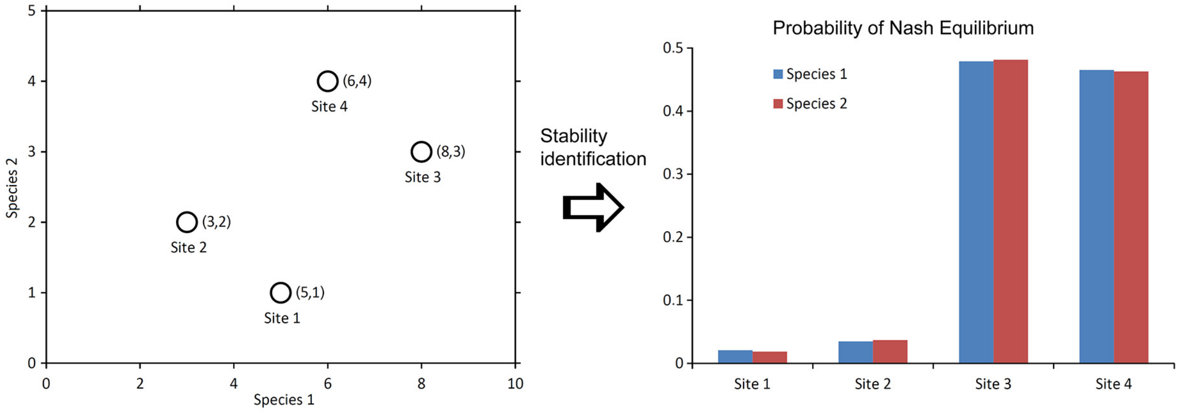

Fig 4. Evaluation of degree of stability expressed as the Nash Equilibrium probability for simple numerical example of two species observed at four sampling sites (mentioned in Chapter 4 of Legendre and Legendre Numerical Ecology [35]).

doi:10.1371/journal.pone.0155023.g004

distribution of degrees of stability of the descriptors in the set of individual sites. We assume, therefore, that we are able to determine more stable and less stable sites (Fig 4).

Relative ecological stability could be evaluated in this way, particularly through the application of our approach to more extensive datasets that reflect, e.g., the ecological parameters of a larger number of landscape elements. In addition, the evaluation of NE probability of occurrence could suitably complement the standard statistical analysis of data of this type. The proposed concept of using NE to solve settings formalized in the Pareto optimality scheme is surely applicable in many contexts including multicriteria analysis of complex ecological and other systems.

Author Contributions

Conceived and designed the experiments: MV PV. Performed the experiments: PV. Analyzed the data: MV. Contributed reagents/materials/analysis tools: MV PV. Wrote the paper: MV PV.

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III.

Topography of spoil heaps and its role in plant succession and soil fauna presence

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Scientia Agriculture Bohemica

doi: 10.1515/sab-2017-0005

ENVIRONMENTAL SCIENCES

TOPOGRAPHY OF SPOIL HEAPS AND ITS ROLE IN PLANT SUCCESSION AND SOIL FAUNA PRESENCE

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The spoil heaps from brown coal mining without technical reclamation are interesting specific sites for ecological relationships observation. This research was aimed at investigating whether topographic features, which determine soil nutrient and moisture distribution, in combination with soil fauna (wireworm and earthworm) presence, affect plant community composition at a spontaneously revegetated post mining area with an undulating surface. Two sites of different age with three types of topographic features were selected, soil moisture and nutrient contents were measured, and plant community composition and soil macrofauna community were sampled at each position. Wireworms were present at all positions and were most abundant at the bottoms of waves at the younger site; their presence was correlated with the presence of several plant species with high palatability for wireworms, but the direction of the interaction is not clear. Earthworms were only present at the older site and had the highest abundance at flat sections. Earthworm presence affected the amount of nitrogen in soil – the highest nitrogen content was at the site with the highest earthworm density and was followed by a higher diversity of plant community. The plant community composition was generally correlated with plant available nutrient content – especially P and N. We infer that topographic features affect nutrient and soil fauna distribution, which consequently influences the plant community composition.

plant community composition, spontaneous succession, earthworms, wireworms

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doi: 10.1515/sab-2017-0005

Received for publication on February 2, 2016

Accepted for publication on October 4, 2016

INTRODUCTION

During formation of spoil heaps in the brown coal mining areas, the overburden is heaped in longitudinal rows. Most areas are subject to technical reclamations, where the surface is levelled out and later trees or herbs are planted there. However, some parts have been left in their original state, preserving the unique microtopography – waves 1–1.5 m high and 6–7 m wide. These areas are also left to spontaneous development without human intervention. Adjacent to some of these sites we can find areas that have been levelled out but not revegetated, so there is a possibility of comparison of soil and plant community development on flat and undulating landscape during primary succession (Frouz et al., 2001). Studies on succession at various post mining sites showed that leaving spoil material in heaps has a positive effect on diversity of plant and animal assemblages mostly due to high spatial diversity of the stands, age (the older stands have higher diversity than the same old sites with technical recultivation), and material type of heaps. The spontaneous succession can be used on the non-extreme stands surrounded by natural vegetation (Prach, Pysek, 2001; Topp et al., 2001, 2010; Hodacova, Prach, 2003). Spatial variability causes diversity in nutrient availability, soil moisture, soil texture, which then causes diversity in plant community composition (Frouz et al., 2011).

Spatial variability also affects soil fauna – moisture availability, accumulation of litter, accumulation of loose material, accumulation of plants. There is some evidence in literature that wireworms are highly affected by soil moisture and temperature in their activity and distribution, but also depending on the soil type and structure and plant community composition; they generally tend to migrate into areas with higher soil moisture, although avoid soils that are subject to flooding (Campbell, 1937; Evans, 1944; Finney, 1946; Parker, Howard, 2001). At some stands on the spoil heaps the grass *Calamagrostis epigejos* becomes abundant and eventually dominates the plant community, as is common in other disturbed ecosystems at early successional stages (Prach, 1987; Wiegleb, Felinks, 2001; Somodi et al., 2008). This fast growing plant often out-competes all other plant species and creates monospecific stands on soils rich in nitrogen. It is an important ecosystem engineer which alters condition of soil surface. Dominance of this species is caused by very rapid vegetative reproduction and also by very tough, slowly decomposable litter, which inhibits the growth of other plant seedlings (Rebele, Lehmann, 2001; Massey et al., 2007; Mudrak et al., 2010). Even though the autecology of *C. epigejos* has been intensively studied (Rebele, Lehmann, 2001; Gloser, 2002; Kavanova, Gloser, 2005), little is known about its interaction with belowground herbivores and other soil fauna.

It is well known that soil development is driven by changes in vegetation during spontaneous succession (Frouz et al., 2001, 2008), but at the same time, soil organisms can influence plant performance and the composition of the plant community during succession by changing the soil environment or by direct consumption of plant tissue (Brown, Gange, 1989, 1992; Thompson et al., 1993; Wurst et al., 2004; De Deyn, Van Der Putten, 2005; Roubickova et al., 2009).

In the present study we tested whether the method of levelling the ground (which was originally undulating, as a result of the heaping process) at the spoil heaps, without consequent introduction of new species, affects abiotic conditions (moisture, nutrient content). We investigated the change of plant community composition and soil macrofauna presence caused by landscaping consequently. We tested this at two sites of different age, to assess how this effect changes during succession.

MATERIAL AND METHODS

Study area

The study was carried out at a post-mining area in the Sokolov brown-coal mining district in the Czech Republic (50°14′ 30.711″N, 12°40′44.96″E). The average altitude of the spoil heaps is about 500– 600 m a. s. l., mean annual precipitation 650 mm and temperature 6.8°C. The spoil heaps originated from open-cast brown-coal mining and consist mainly of Tertiary clays of the so-called Cypris Formation, which are well supplied with mineral nutrients (Frouz et al., 2001). The pH of the substrate in initial successional stages is 8–9. The study was conducted in two unreclaimed areas – the younger plot (E) was about 18 years old (i.e. the spoil heaps were deposited 18 years ago), the older plot (I) was about 29 years old. Both are characterized by longitudinal rows of depressions and elevations formed during the heaping process, with an adjacent flat area, that has been levelled out but is of similar age. The tops of waves are about 1.5–2 m above the base of the depressions and individual rows are 6–7 m apart.

The younger site has a plant community formed mainly by herbaceous vegetation with dominant *C. epigejos* and with scattered shrubs and small trees (*Betula pendula, Salix caprea, Populus tremula*), the soil surface is bare or has a very thin layer of litter in depressions.

The older site has plant cover formed mainly by herbaceous vegetation with dominant *C. epigejos* and with scattered trees. The litter layer in depressions can be 3–5 cm thick, with greater thickness underneath trees.

At each site we selected three positions according to topography: tops of waves (top), depressions in between waves (bottom), and ground that has been levelled out (flat). At each position we randomly selected 5 plots – replicates.

Soil fauna and plant sampling

A total of 30 samples were collected (15 on younger site and 15 on older site). Five 20 × 20 × 20 cm soil core samples were taken from each plot and each topographic position (tops of waves, bottoms, and levelled ground) and hand-sorted for soil macrofauna, namely wireworms and earthworms. Wireworms were then classified into three groups by sizes – stage 1 (< 5 mm), stage 2 (5–10 mm), and stage 3 (> 10 mm), which roughly correspond with age of the larvae. The Elaterid larvae were then determined into genus according to Klausnitzer (1978). Earthworms were classified as adults and subadults and determined into species according to Pizl (2001). All of the soil sampling was done in late September 2015.

Plant community composition was assessed based on phytosociological plots 1 × 1 m, lying next to soil core samples.

Soil parameters measurements

Composite soil samples were taken from the depth of 0–20 cm at each sampling point for soil moisture, microbial respiration, C, N, and other major element measurements. The soil samples were then kept at 4°C prior to analyses.

Soil microbial respiration was measured in 100 g of dry soil weight according to Frouz, Novakova (2005). CO_2 produced in the vials was trapped by 3 ml of 1M NaOH. Vials were kept at 20°C, and after 10 days the amount of trapped CO_2 was established by

Table 1.Results of ANOVA and Tukey's*post hoc* test for contents of nutrients at individual plots

Plot	Nutrients content (mg/kg)						Moisture (%)	Microbial respiration (mg CO_2/100 g/day)
	P	Ca	Mg	K	C	N		
EB	8.96^{a}	4085.72^{ab}	1247.74	335.30	4962	358^{abc}	28.21	11.64
EF	13.025^{ab}	4480.78^{ab}	1372.38	291.58	4868	295^{ac}	27.04	10.93
ET	9.06^{a}	3887.90^{a}	1343.80	306.26	4702	286^{c}	26.47	10.34
IB	18.50^{ab}	5011.00^{ab}	1264.35	254.30	3718	460^{ab}	30.02	12.28
IF	23.02^{b}	5103.30^{b}	1241.68	263.70	4150	494^{b}	26.90	10.76
IT	21.12^{b}	4989.02^{ab}	1268.00	270.96	3974	454^{ab}	24.30	10.96
F(5;22)	4.965	3.81	1.248	2.118	1.479	6.201	1.696	1.7
P	0.003	0.012	0.321	0.142	0.237	0.001	0.177	0.177

EB = bottoms of waves on younger plot, EF = flats on younger plot, ET = tops of waves on younger plot, IB = bottoms of waves on older plot, IF = flats on older plot, IT = tops of waves on older plot

$^{a–c}$plots with the same letter did not differ significantly at P<0.05

HCl titration, after the addition of $BaCl_2$. The soil moisture was measured gravimetrically – 50 g of sample was dried for 24 h at 105°C.

The rest of the soil sample was dried at room temperature and homogenized by dry sieving through a 2 mm mesh. Afterwards it was analyzed for main elements. P, Ca, Mg, and K were analyzed in the plant available forms in the extract solution according to Mehlich III (Tran et al., 1990). Plant available phosphorus was assessed spectrophotometrically, as phosphomolybdate blue, at wavelength 750 nm on spectrophotometer Cary 60 UV-Vis, Agient Technologies, Santa Clara, USA, . Potassium was measured on atomic absorption spectrophotometer (55B AA Spectrometer, Agient Technologies, Santa Clara, USA), using acetylene-air mixture flame. Calcium and magnesium contents were assessed by the same method, but lanthanum solution was added to the sample for ionization elimination.

Total carbon and total nitrogen were analyzed using high temperature catalytic combustion on the Primacs SNC, Scalar, Breda, Netherlands. The samples were introduced into the high temperature combustion oven, there at 1050°C the carbon was completely oxidized to CO_2 in the presence of a catalyst. The CO_2 was measured by non-dispersive infrared detection for total carbon. Total nitrogen was converted to NxOy which was reduced at 600°C to N_2. The N_2 gas was measured by thermal conductivity detection.

Statistical analyses

One way ANOVA was used to evaluate the effect of plot age and topographic position on all collected data (plants and fauna). For evaluating differences between individual treatments, Tukey's HSD test was used. These analyses were performed in program STATISTICA Version 9.0 (Statsoft, Tulsa). Program CANOCO 5 (Smilauer, Leps, 2014) was used for Canonical Correspondence Analysis (CCA), for

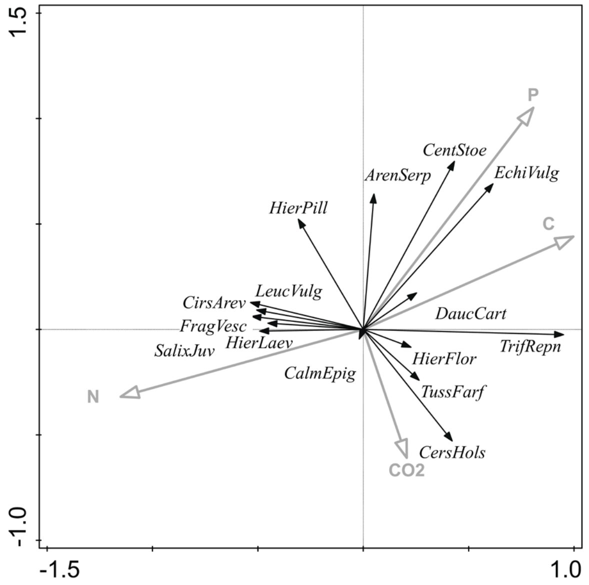

Fig. 1. Regression biplot of environmental variables and species found by Canonical Correspondence Analysis

black arrows indicate species (showing 15 best fitting species), grey arrows indicate the nutrient content of soil (N – nitrogen, P – phosphorus, C – carbon) and soil properties (CO_2 – microbial respiration); environmental variables were selected from a total of 11 using forward selection; the first axis explains 10.0% of cumulative variance; total explanatory variables account 26.3%

ArenSerp = Arenaria serpyllifolia, CalmEpig = Calamagrostis epigejos, CentStoe – Centaurea stoebe, CersHols = Cerastium holosteoides, CirsArve = Cirsium arevense, DaucCart = Daucus carota, EchiVulg = Echium vulgare, FragVesc = Fragaria vesca, HierLaev = Hieracium laevigatum, HierPill = Hieracium pilosella, HierFlor = Hieracium floribundum, LeucVulg = Leucanthenum vulgare, SalixJuv = Salix juvenile, TrifRepn = Trifolium repens, TussFarf = Tussilago farfara establishing the interactions between individual phytosociological plots, plant species, soil macrofauna presence, and environmental conditions. Variation partitioning from CANOCO 5 was used to investigate the contribution of vegetation and soil properties effect on earthworm and wireworm diversity. Vegetation in this analysis was represented by the first axis from RDA (Redundancy analysis) and the soil properties were represented by available phosphorus, soil moisture, and soil microbial respiration.

Table 2. Result of ANOVA and Tukey's*post hoc* test for morphological parameters of *C. epigejos* in plots

	Length of rhizome	Number of ramets	Sheet width
E	8.99^{b}	10.57^{a}	0.57^{b}
I	7.91^{a}	14.21^{b}	0.49^{a}
F(1;26)	4.308	4.393	8.411
P	0.048	0.046	0.008

E = younger plot, I = older plot

^{a,b}plots with the same letter did not differ significantly at *P*< 0.05

RESULTS

Nutrient availability, moisture, and microbial respiration

There were statistically significant differences in the content of total N at different positions ($F = 6.20$; $P = 0.001$). The highest content of total N was at flat areas at site I (494 mg kg^{-1}). The lowest content was at the tops of waves at site E (286 mg kg^{-1}). The other plots cannot be significantly different from each other (Table 1). Statistically significant differences were also recorded for the amounts of plant available P ($F = 4.97$; $P = 0.003$). The highest content of plant available P was at flat areas of site I (23.02 mg kg^{-1}). Similar values were measured for the tops of waves at site I (21.12 mg kg^{-1}). The lowest P content was at the bottom of waves at site E (8.96 mg kg^{-1}). Similar values were measured at the tops of waves at site E (9.06 mg kg^{-1}) (Table 1). There were also statistically significant differences in the amount of calcium ($F = 3.81$; $P = 0.012$) at different positions. The highest content of Ca was at flat areas at site I (5103.3 mg kg^{-1}). The lowest content was at the tops of waves at site E (3887.9 mg kg^{-1}) (Table 1).

There were no statistically significant differences in soil moisture ($F = 1.70$; $P = 0.177$). Soil moisture was the highest in the depressions – at site I 31.7% and at site E 28.2%. Moisture at the tops of waves and flat areas was similar – at site E 26.5% and 26.9% (top and flat respectively) and at site I 24.3% and 26.9%.

There were no statistically significant differences in microbial respiration between the positions or the sites. Respiration was the highest in depressions and similar at the tops of waves and flat parts (Table 1).

Plant community composition

There were thirty plant species in total. Species *Salix* sp., *Cirsium arvense*, *Fragaria vesca*, *Hieracium laevigatum*, and *Leucanthemum vulgare* are generally found together and at locations with high soil N content. *Trifolium repens*, *Daucus carota*, *Arenaria serpyllifolia*, *Centaurea stoebe*, and *Echium vulgare*, on contrary, grow on N poor stands (Fig. 1). *Hieracium pilosella*, *Cirsium arvense*, *Faragaria vesca*, *Salix juv.*, *Hieracium laevigatum*, and variosus hybrids by *Epilobium* sp. are plant species that grow at the older site (I), while *Cerastium holosteum*, *Trifolium repens*, and *Echium vulgare* were only found at the younger site (E) (Fig. 2). *Calamagrostis epigejos* was unaffected by environmental variables, by site or topographic position.

The phytosociological plots show similarities between the tops of waves and the flat sections, and they are most closely correlated with available P (Fig. 3). However, tops of waves had significantly lower number of species than flat sections at both sites ($F(2, 25) = 3.480$; $P = 0.046$). The plots at the bottoms of waves have a uniform plant community composition and are best correlated with microbial respiration.

We also followed the morphological differences in plants of *C. epigejos* represented by the number of ramets, numbers of inflorescences, weight and length of inflorescences, surface area of leaves, and length of underground shoots in one patch.

The statistically significant differences were recorded for the average length of rhizome ($P = 0.048$), number of ramets ($P = 0.046$), and leaf width ($P = 0.008$) among the younger plot (E) and older plot (I) (Table 2).

Soil macrofauna presence

The wireworms were determined as *Agriotes lineatus* (L.) according to Klausnitzer (1978). The highest numbers of wireworms were found at site E in depressions, 3 individuals per sample on average, which equals to 75 individuals per m^{2}. At the tops of waves the numbers were the lowest, at both sites there was only one individual in one out of 5 samples (on average 5 individuals per m^{2} for both sites). In general, there were higher numbers of wireworms at site E – 22 in total. At site I there were only 4 individuals in total (from all soil samples) (Fig. 4). The differences were statistically significant, with the site effect being stronger ($F = 10.75$; $P = 0.003$) than the topographic position ($F = 6.72$; $P = 0.004$). Most of the larvae were in medium size group (68%).

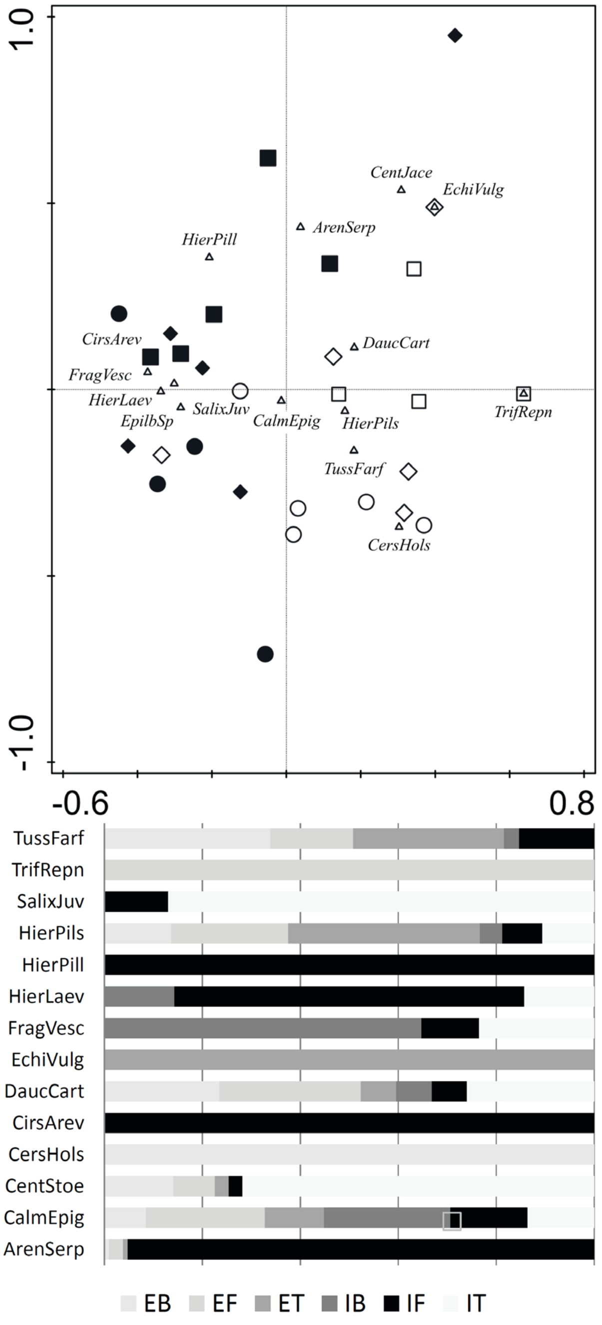

Fig. 2. Canonical Correspondence Analysis species scatter plot

species are displayed by pie charts with individual sections showing their presence at individual stands; the 15 best fitting species are shown as triangle; the first axis explains 10.0% of cumulative variance; total explanatory variables account 26.3%; bar graph shows the ratio of topographic position at 15 best fitting species; species registered in different positions

transparent circle = bottoms of waves on younger plot, transparent square = flats on younger plot, transparent diamond = tops of waves on younger plot, full circle = bottoms of waves on older plot, full square = flats on older plot, full diamond = tops of waves on older plot, *ArenSerp = Arenaria serpyllifolia, CalmEpig = Calamagrostis epigejos, CentStoe = Centaurea stoebe, CersHols = Cerastium holosteoides, CirsArve = Cirsium arvense, DaucCart = Daucus carota, EchiVulg = Echium vulgare, EpilbSp = Epilobium sp., FragVesc = Fragaria vesca, HierLaev = Hieracium laevigatum, HierPill = Hieracium pilosella, HierFLor = Hieracium floribundum, SalixJuv = Salix juvenile, TrifRepn = Trifolium repens, TussFarf = Tussilago farfara*

There were no earthworms found at site E. At site I there were 11 earthworms found in total, 55% were adults and all were determined as species *Aporrectodea caliginosa* according to Pizl (2001). Numbers of earthworms were higher in the flat areas (average 1.4 individuals per sample, 35 individuals per m^2), however the differences, due to overall low numbers of earthworms, were not statistically significant.

We also tested the effect of the environmental variables (nutrients, moisture, respiration) and vegetation on earthworms and wireworms by CCA analysis. Environment and vegetation together explained 32.4% of the variability of the net effect; vegetation explained 16.4% ($P = 0.004$), environment only 1.9% ($P = 0.286$), 14.1% was explained jointly (Fig. 5).

DISCUSSION

The content of total N, K, Mg, and Ca was rather higher than the average values from grassland areas of the Czech Republic (Kalina, 2005; Mladek et al., 2006). Nitrogen is a very important element that affects growth of plants, their spreading, and is also building material of plant biomass. The contents of total N generally increase with increasing age of soils (Ciarkowska et al., 2016). In our study, the highest total N was measured at the bottoms of waves at the younger site. Tops of waves were poor in N probably due to erosion, greater temperature swings, and smaller retention of water at these positions, which causes faster mineralization and depletion from tops and transport to the depressions, together with water, litter, and finer soil particles (Zhu et al., 2014). The alkaline Tertiary clays that form these spoil heaps are generally rich in total P (Sourkova et al., 2005; Frouz et al., 2008), but situation is different for the content of the plant available P – compared to the amount of available P from permanent grasslands in the Czech Republic (Kalina, 2005), we found a very low content. This is most probably related to the high content of Ca, which forms insoluble calcium phosphate, which generally reduces P availability to plants in soil (Tunesi et al., 1999; Addiscott, Thomas, 2000; Braschi et al., 2003). Higher amounts of available P at the older site are results of the combination of biotic and abiotic processes during succession, which involve P uptake by plants, mineralization of organic matter, extraction of the insoluble forms of P by mycorrhizal fungi and decrease in pH, which causes dissolution of calcium phosphate (Ciarkowska et al., 2016). Higher contents of P, Mg, and Ca in the flat areas could be attributed to higher levels of weathering on the flats – during the process of levelling of ground the layers of cypris clays are disrupted and broken into smaller particles, which are then more susceptible to mineral and chemical weathering (Smeck, 1985; Sourkova et al., 2005).

The plant community composition differed between the two sites, which can be attributed to different successional stages but also to different positions at the spoil heap, causing differences in species pool sources. The species found only at the younger site are *Cerastium holosteum*, *Tussilago farfara*, *Daucus carota*, *Trifolium repens*, and *Echium vulgare*. These species belong to the intermediate strategy type (CSR), except *E. vulgare*, which poorly copes with stress and is rather an R-strategy plant. *Fragaria vesca*, *Cirsium vulgare, Arenaria serpyllifolia* or *Centaurea jacea* were found only at the older site, but even these species are not clear-cut in life strategy. Ellenberg's indicator value did not show different ecological claims. The plant community composition differed between sites E and I, which can be attributed to the different age (difference between sites is 11 years) and associated successional stage of these sites, but the plant communities do not show distinct life-strategies shifts at this stage.

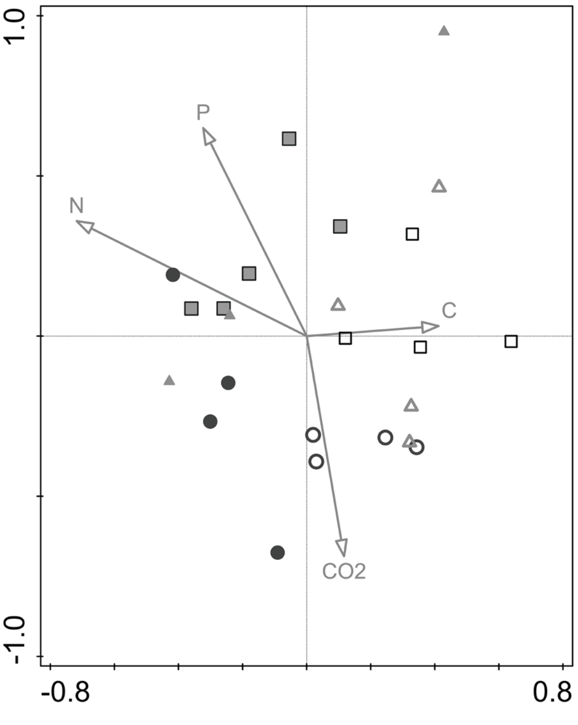

Fig. 3. Canonical Correspondence Analysis shows the samples and four environmental variables by forward selection (P – phosphorus, N – total nitrogen, CO_2 – soil microbial respiration, C – total carbon)

the first axis explains 10.0% of cumulative variance; total explanatory variables account for 26.3%
transparent circle = bottoms of waves on younger plot, transparent square = flats on younger plot, transparent triangle = tops of waves on younger plot, full circle = bottoms of waves on older plot, full square = flats on older plot, full triangle = tops of waves on older plot

The plant communities on leveled-out areas were similar to the ones at the tops of waves, which is related to similar moisture, N content, and microbial respiration values (Fig. 3). This can be caused by the properties of the soils – they are very rich in swelling clay (montmorillonite), which has a high water-holding capacity (Frouz et al., 2001, 2008) so as our measurements were conducted in periods with relatively high precipitation (end of May, September), there was not time for them to dry out. Nevertheless, when the montmorillonite clay does dry out, in tends to shrink and become very hard, so it presents hostile conditions for plant roots as well as for soil fauna and may reduce their migration (Lavelle, Spain, 2001; Dunger, Voigtlander, 2009) or cause absence of some species that cannot cope with these conditions. The fact that *C. epigejos* can grow well under various conditions corresponds with the findings of Rebele, Lehmann (2001) and Lehmann, Rebele (2005). However Lehmann, Rebele (2005) found high phenotypic plasticity in populations growing in diverse conditions, which is not the case at the stands followed by us. This could be caused by smaller initial pool of genotypes, as the spoil heaps

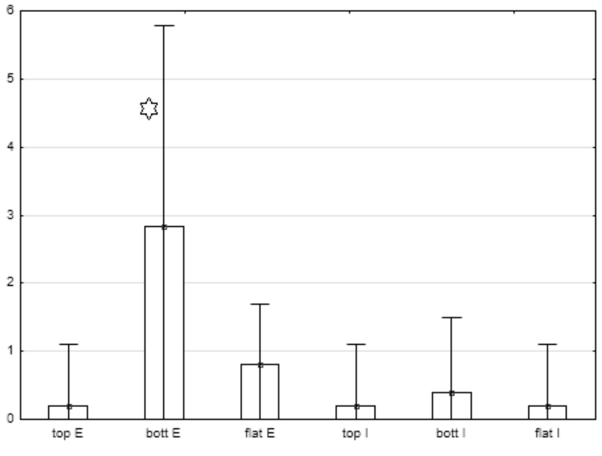

Fig. 4. Average numbers of wireworms at individual topographic positions at both investigated sites top E = tops of waves, site E; bott E = bottoms of waves, site E; flat E = flat section, site E; top I = tops of waves, site I; bott I = bottoms of waves, site I; flat I = flat section, site I. Error bars show standard deviation, asterisk indicates a significantly different result

function as an island in the surrounding landscape, therefore some of the rules of island biogeography imply here (Ash et al., 1994; Prach, 2003).

Numbers of soil fauna were affected both by site age and by topographic position. Wireworms were most numerous in depressions at both sites. This could be attributed to higher moisture content (Campbell, 1937; Evans, 1944; Finney, 1946; Parker, Howard, 2001) or more shade, therefore less extreme temperatures, but according to results of multivariate analysis it seems to be related more to biotic interactions between the soil fauna and vegetation. This is also supported by the fact that numbers of wireworms are lower at site I, which has different plant community composition from site E. Because wireworms are generalist root herbivores, they prefer roots with high palatability and high nutrient content (Brown, Gange, 1989, 1992; Rasmann, Agrawal, 2008), such as are the roots of *Tussilago farfara, Cerastium holosteoides or Daucus carota*, plant species that are most abundant at depressions at site E.

The finding that earthworms occur only at sites 20 years old or older corresponds with results of Frouz et al. (2008) and is related mainly to soil conditions (Roubickova, Frouz, 2014). These soil animals seem to prefer the flat areas, although soil moisture is lower here than in the depressions, which may be due to patchiness of the habitat in the undulating part, as hills present migration constraints for earthworms. Because site I has been colonized only recently (A. Walmsley, personal observation; Frouz et al., 2008), earthworms have not yet reached their potential population densities. Earthworm presence is generally followed by quick increase of N content in soil (Bohlen et al., 2004; Frouz et al., 2006), which could explain why there is the highest N content in the soil at flat areas of site I, while at site E it is by far highest in depressions.

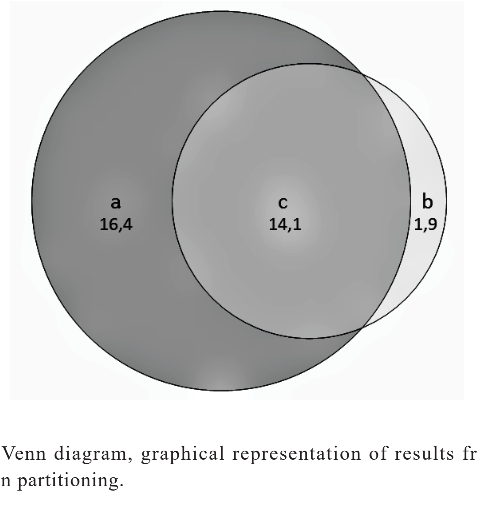

Fig. 5. Venn diagram, graphical representation of results from the variation partitioning.

The effects of environment and vegetation explain 32.4% of variability (a + b + c), net effect of vegetation is 16.4% ($P = 0.004$) (**a**); the ambience effect is 1.9% ($P = 0.286$) (**b**); 14.1% jointly explained (**c**). Explanatory data: vegetation, environmental; response data: worms. The first group (the bigger circle) is represented by CaseR1 from vegetation data, the second group (the smaller circle) is represented by phosphorus amount in soil from environmental dat

It seems that vegetation is a better predictor of wireworm and earthworm presence than the measured environmental factors. Variability, which is explained by the vegetation, reflects the biotic interactions between the soil macrofauna and vegetation. These relationships were noted in other studies as well (Frouz et al., 2008; Roubickova et al., 2009, 2012) and can be explained by direct or indirect trophic interactions. Plants present a food source for both wireworms and earthworms and therefore their life strategy that is related to chemical composition of their body and consequent palatability for herbivores and saprophags, can affect the abundance of soil fauna (Frouz et al., 2001, 2008; Wardle et al., 2005). On the other hand, earthworms affect soil properties and processes, such as aggregate formation (Marashi, Scullion, 2003), water holding capacity (Frouz et al., 2006), activity of microflora by mixing plant litter and mineral soil (Frouz et al., 2006), and nutrient availability (Lavelle, Spain, 2001). Generalist root herbivores can also substantially affect the abundance of some plant species and that way change the plant community composition (Brown, Gange, 1989, 1992; Blossey, Hunt-Joshi, 2003).

Spontaneous succession is a reclamation technique successfully used in areas with anthropogenic disturbances, especially in post-minig landscapes. Our study shows that mechanical terrain reshaping following overburden deposition affects the nutrient distribution in soils, which then influences plant community composition and soil fauna presence. It can also speed up the migration of some soil fauna to the sites. Presence of levelled-out areas together with areas with wave-like structure therefore presents a positive contribution to the habitat diversity at spontaneously revegetated sites.

ACKNOWLEDGEMENT

We would like to thank Jan Frouz for his kind advice, Jarda Kukla for help in getting around the mining sites, Sokolovska Uhelná a.s. for enabling us to perform the research at the spoil heaps, and Tereza Březinová for help with the chemical analyses.

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IV.

Wireworms suppress spreading of the expansive weed *Calamagrostis epigejos* (L) Roth by feeding on its rhizomes

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European Journal of Soil Biology

https://doi.org/10.1016/j.ejsobi.2017.06.001

European Journal of Soil Biology 81 (2017) 19–24

Contents lists available at ScienceDirect

European Journal of Soil Biology

journal homepage: http://www.elsevier.com/locate/ejsobi

Original article

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ARTICLE INFO

Article history:
Received 13 July 2016
Received in revised form
19 April 2017
Accepted 2 June 2017
Available online 9 June 2017

Handling editor: Prof. C.C. Tebbe

Keywords:
Calamagrostis epigejos
Wireworms
Root herbivory
Pot experiment
Rhizome
Spoil heaps

ABSTRACT

We tested whether wireworms (Elaterid beetle larvae) prefer the expansive grass *Calamagrostis epigejos* to other early-successional plant species from the spoil heaps in two microcosm experiments and in a field survey of wireworm abundance and *C. epigejos* coverage on the spoil heaps at different topographic positions at the spontaneously revegetated sites with an undulating surface. In the first experiment, wireworms reduced rhizome biomass of *C. epigejos* the most out of the belowground organs of the 5 plant species, but did not affect its root biomass. In the second greenhouse experiment, wireworms negatively affected the growth of rhizomes of a growing *C. epigejos*, whilst they did not affect the other plant species or the aboveground biomass. In the field survey, *C. epigejos* coverage was lowest at the bottoms of depressions, despite the higher moisture and nutrient levels at this position. Wireworm numbers were the highest in depressions. The results of this study support the hypothesis that wireworms can negatively impact the *C. epigejos* by feeding on its rhizomes and have the potential to slow down the expansion of this weed.

1. Introduction

Calamagrostis epigejos is often a dominant plant species in ecosystems that undergo intense or regular disturbances, such as post-mining sites, urban or agricultural areas [1–3]; at post-mining sites it often forms monocultures at technically reclaimed sites and also on spontaneously revegetated sites at various successional stages [4]. It is typical by quick expansion over long distances [5]. *C. epigejos* creates monospecific stands especially on soils rich in nitrogen and moisture, as it has a high competitive ability in terms of utilizing mineral resources [6,12]. However, due to its high phenotypic plasticity, it is able to grow in a wide range of conditions and expand also in dry grasslands [2].

It is an important ecosystem engineer which alternates conditions at the soil surface. Dominance of this species is caused by very rapid vegetative reproduction and also by very tough leaves, which have low palatability for herbivores [7] and form slowly decomposable litter which inhibits growth of other plant seedlings [8,9]. It has fleshy rhizomes that serve as vegetative reproductive organs and that also play a role in nutrient transport from source organs of mature plants to young tillers [10,11]. At the post mining area near Sokolov, NW Bohemia, at spontaneously revegetated sites, *C. epigejos* starts forming monospecific stands about 15–20 years after deposition of the material. At sites afforested with conifers and trees that enrich soil in nitrogen (alder) it often dominates the herb layer, especially in early phases [8].

Belowground herbivory can have various effects on plants. Root herbivory typically stimulates production of repellent substances for aboveground herbivores [13] and generally decreases plant fitness, although it may enhance root growth and nutrient uptake by roots [14,15]. Brown and Gange [16] have found that generalist root herbivores have the potential to alter plant community composition by selectively feeding on easily palatable roots that mostly belong to ruderal plant species. The increase in plant diversity leads to a reduction of the negative impact of root herbivory on individual species, as the effect is diluted amongst the plants [17].

Wireworms (Elaterid beetle larvae), are polyphagous root herbivores that are common in ecosystems with disturbances, such as agroecosystems and post-mining soils [18–20]. Some species are important pests of commercial crops [15], but there is absence of studies that focus on effects of these root herbivores on important weed species. Wireworms are feeding generalists, however they have been reported to prefer some palatable species and avoid

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http://dx.doi.org/10.1016/j.ejsobi.2017.06.001

species with extremely tough roots [18] or roots that contain some toxic substances, such as lactone in the *Asteraceae* [21].

Agriotes lineatus, which is the species present in our experiment, is one of the most common species of Elaterid beetles in the Czech Republic, most commonly found in open landscapes with prevailing herb cover, such as fields, meadows [22,23] and early-successional phases at post-mining sites; their abundance decreases when trees begin to dominate the sites (Frouz, unpublished results).

In the current study we investigated feeding preferences of *Agriotes lineatus* larvae (shortly called wireworms) to various species of early–successional plants from the spoil heaps in two microcosm experiments and in a field observation, where we were testing the relationship between wireworm presence and growth of individual plant species.

2. Material and methods

2.1. Study area

The study was carried out at a post-mining area in the Sokolov brown-coal mining district in the Czech Republic (50°14′ 30.711″N, 12°40′44.96″E). The spoil heaps originated from open-cast brown-coal mining and consist mainly of tertiary clays of the so-called cypriss series, which are well supplied with mineral nutrients [24]. The pH of the substrate in initial successional stages is 8–9. The study was conducted in plot was about 18 years old. This area is characterized by longitudinal rows of depressions and elevations formed during the heaping process, with an adjacent flat area, that has been levelled out but is of similar age. The tops of waves are about 1,5–2 m above the base of the depressions and individual rows are 6–7 m apart.

2.2. Field observation

2.2.1. Sampling design

At the spontaneously revegetated site at the spoil heaps we selected 3 positions according to topography: tops of waves (top), depressions in between waves (bottom) and ground that have been levelled out (flat). At each position we randomly selected five replicates.

2.2.2. Sampling method

A total of 15 samples was deployed. Five 20 × 20 × 20 cm soil core samples were taken from each plot and each topographic position (tops of waves, bottoms and levelled ground) and hand-sorted for wireworms. The Elaterid larvae were then determined into genus according to Klausnitzer [25]. The geobotanical reléves were registered at each position and replicate. From this reléves we used only coverage of *C. epigejos* for this study.

2.3. Microcosm experiment 1

We selected 5 plant species that occur at sites with spontaneous succession at the spoil heaps – *C. epigejos, Tussilago farfara, Centaurea jacea, Hieracium pilosella, Festuca rubra* and cut a section of belowground organs from each species, in *C. epigejos* and *T. farfara* we used both roots and belowground rhizomes. Then we weighed the fresh weight of these roots, and introduced them into glass chambers, 5 cm in diameter, with soil from the spoil heaps that was previously sterilized by drying-out at 70 °C. We also weighed another section of root from each species and then dried it at room temperature for 7 days to establish the water content. Then we introduced 5 specimen of *Agriotes lineatus* larvae to each column and left to incubate for 1 month. At the end of the month we weighed the root sections, dried them at room temperature for 7 days and weighed them again. In *C. epigejos* we separated root and rhizome biomass and weighted them separately but in *T. farfara* the roots were too fine to separate them from rhizomes, so we weighted them together.

2.4. Microcosm experiment 2

We selected 4 species common at the spoil heaps – *C. epigejos, Taraxacum* sect. *Ruderalia, Tussilago farfara and Centaurea stoebe*, excavated 8 specimen of each species at the spoil heaps and introduced them into round pots (15 cm in diameter, 15 cm high) with soil from an 18-year old site from the spoil heaps, one specimen of each species into each pot. We added 5 larvae of *Agriotes lineatus* to 4 of the 8 plots and incubated them in a greenhouse at a 12/12 h day/night regime at mean temperature 15 °C with 8 °C fluctuations between day and night. After 4 months all the plants were weighed and the length of rhizomes in *C. epigejos* was measured.

2.5. Statistical analyses

One way ANOVA was utilised for evaluation of the effect the type of species in the experiment 1, topographic position in post mining sites and for effect wireworms on roots of *C. epigejos*. For evaluating differences between individual treatments, Fisher's LSD test was used. Paired *t*-test was used to compare differences between initial and final weight of belowground plant parts in Microcosm experiment 1. These analyses were performed in program STATISTICA 9.0 (Statsoft, Tulsa).

3. Results

3.1. Field observation

The Fisher test showed differences in coverage of *C. epigejos* among different topographic positions. The coverage of *C. epigejos*

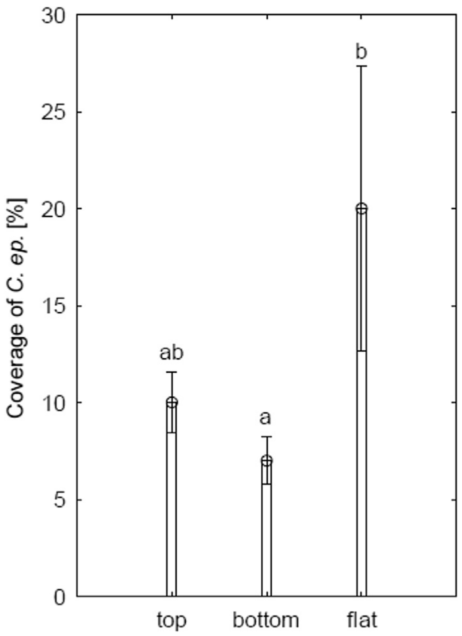

Fig. 1. The coverage of *C. epigejos* at different topographic positions. Results of one-way ANOVA: F(2,11) = 3.016, p = 0.090, Fisher LSD test. Error bars show standard error of mean (SE).

was the highest at flat sections (Fig. 1). At this position, the lowest numbers of wireworms were found (Fig. 2). At the depressions, the numbers of wireworms were highest and the coverage of *C. epigejos* was lowest out of all positions. Our assumptions are supported by correlation test graphically presented in Fig. 3., where the negative correlation between coverage of *C. epigejos* and abundance of wireworms at each topographic position is shown.

3.2. Microcosm experiment 1

In a preference experiment, wireworms were consuming *C. epigejos* rhizomes more than *C. epigejos* roots and then roots of other plant species, although the results of one-way ANOVA were not statistically significant. T-test of differences between initial and final biomass of belowground parts however shows significant results for *H. pilosella* roots and *C. epigejos* rhizomes, as shown in Table 1. Wireworms removed in average 40% of the initial biomass of *C. epigejos* underground shoots in 1 month. Soft and fleshy roots of *T. farfara* were also consumed by wireworms; about 30% of their original biomass was removed, although the difference (*t*-test) was not significant. In *H. pilosella* the biomass removal was less obvious but consistent across all treatments - 22%. Average biomass loss of belowground parts of all plants are shown in Fig. 4.

3.3. Microcosm experiment 2

In the greenhouse experiment, wireworms reduced the biomass of *C. epigejos* rhizomes by more than 50%, whilst in the wireworm-free treatment the biomass of rhizomes was 10% higher than the original weight (Fig. 5). The difference was statistically significant (F(1,6) = 9.720, p = 0.021). The photos in Fig 6 show the differences in length and biomass of rhizomes between the two treatments. The total biomass of *C. epigejos* increased by 60% in the control treatment during the experiment, while in the wireworm treatment its biomass decreased by 5%, this difference was statistically significant (*t*-test, t = −3.12, p = 0.02). Results are shown in Table 2. Biomass of *T.* sect. *ruderalia* and *T. farfara* decreased during the

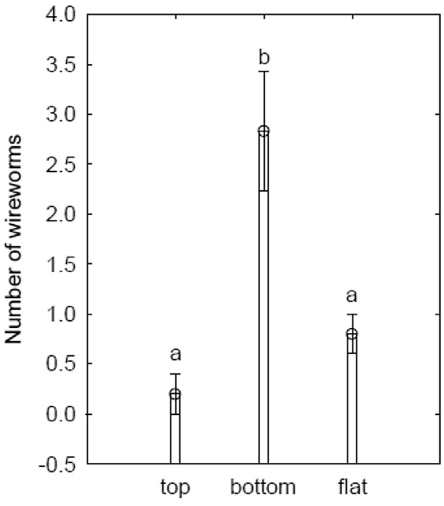

Fig. 2. Numbers of wireworms at the post mining site at different topographic positions. Results of one-way ANOVA: F(2,13) = 11.144, p = 0.002 with Tukey HSD test. The error bars show standard error of mean (SE).

Table 1
The results of *t*-test of differences between initial and final weight of belowground organs of selected plant species from the spoil heaps from the Microcosm experiment 1. Numbers in bold sign significant differences.

	Diff.	Std. Dv.	t	p
Centaurea	−0.0003	0.0796	−0.0079	0.9941
Tussilago	0.2462	0.2812	1.9575	0.1219
Calamagrostis - root	0.0385	0.0568	1.5141	0.2046
Calamagrostis - rhizome	0.0432	0.0281	3.4446	**0.0262**
Hieracium	0.0472	0.0301	3.5115	**0.0246**
Festuca	0.0258	0.0595	0.9682	0.3878

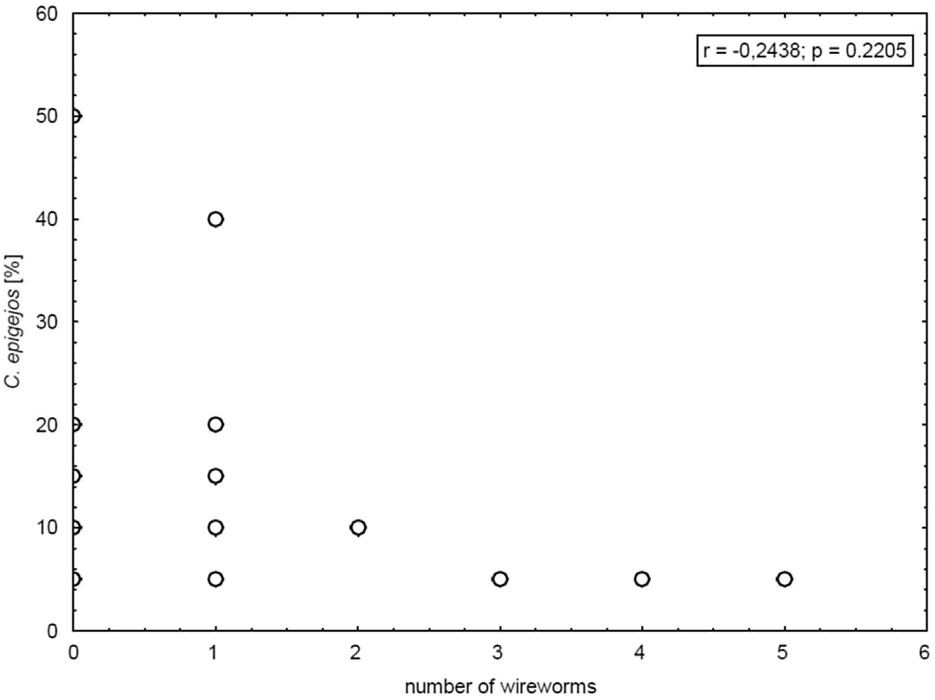

Fig. 3. XY graph. Correlation (r = 0,2438) between *C. epigejos* coverage and number of wireworms. Axis X: number of wireworms, axis Y: coverage of *C. epigejos*.

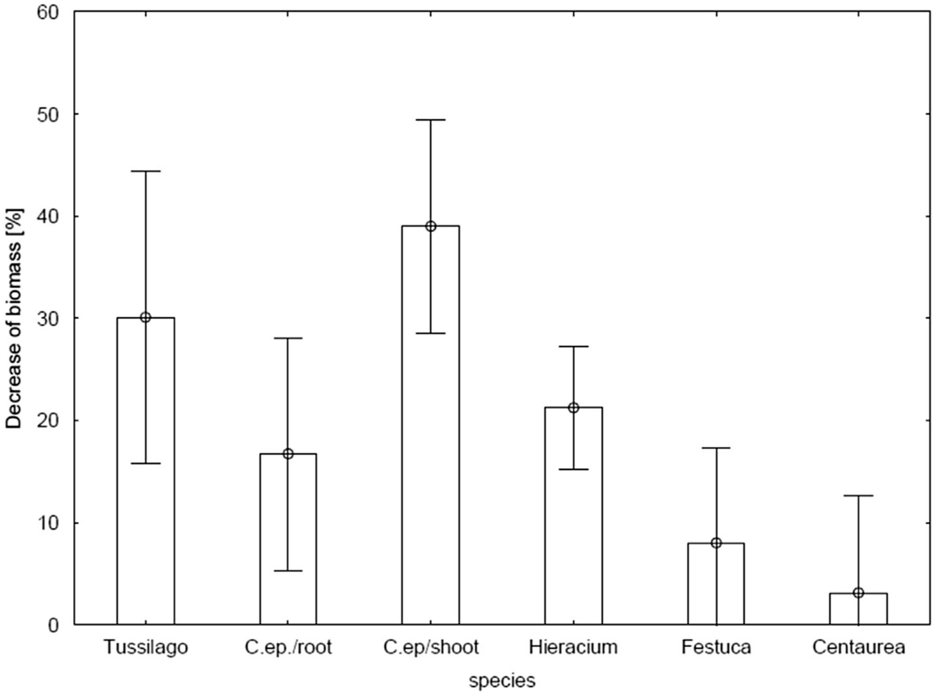

Fig. 4. Decrease root or rhizome biomass left at the end of the Microcosm experiment 1, after 1 month of wireworm presence in the microcosms. Results of one-way ANOVA: F(5,24) = 1.6496, p = 0.18527. The error bars show standard error of mean (SE).

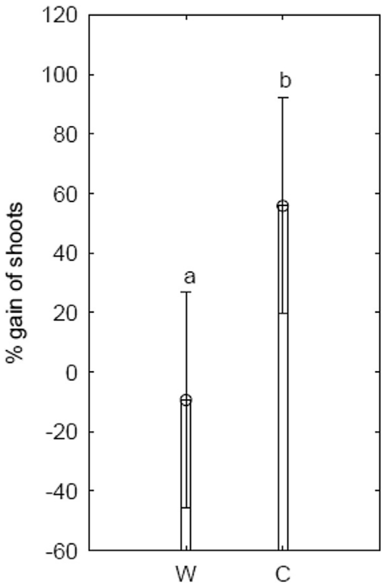

Fig. 5. Shows % gain in length of rhizomes of *C. epigejos* in treatments with wireworms (W) and control treatments (C) during the 4 month duration of the experiment (Microcosm experiment 2). Results of one-way ANOVA: F(1,6) = 9.720, p = 0.021. The error bars show standard error of mean (SE).

experiments, irrespective of the presence of wireworms and biomass of *C. stoebe* increased by 14% in the control treatment and decreased (2,6%) in the wireworm treatment, although this difference was not statistically significant.

4. Discussion

In the Microcosm experiments, wireworms significantly reduced the biomass and length of underground shoots of *C. epigejos*. They also reduced the total biomass of *C. epigejos* when grown in pots with other plant species, while in the control treatment *C. epigejos* dominated the plant community, which is in agreement with the results of Roubickova et al. [19], who found that wireworm presence negatively affected both aboveground and belowground biomass of *C. epigejos* during a greenhouse experiment. Also Sonnemann et al. [27] found that feeding by wireworms caused a significant reduction in both belowground and aboveground biomass of certain grassland species. On the contrary Brezina et al. [26] have not noted any difference in overall aboveground biomass of *C. epigejos* ramets after mechanically cutting the rhizomes, although it decreased the survival of smaller ramets due to the stress involved. However, in our feeding preference experiment wireworms reduced only the biomass of rhizomes of *C. epigejos*, not the roots themselves. This means that the process of nutrient uptake by the plant is not affected, only the flow of nutrients between the mother plant and the young tillers [10,11], which may decrease the competitive ability of the plants and slow down the vegetative reproduction [19].

Therefore we can conclude, that the mechanism of wireworm impact on *C. epigejos* is through reduction of the vegetative reproduction, rather than through direct plant deterioration due to biomass removal. Wireworms preferred feeding on rhizomes of *C. epigejos*, which is most probably a result of high nutrient content and low levels of protection, as these organs are softer and juicier, in comparison with roots [28]. This theory is supported by the results of Johnson et al. [29], who found a negative correlation between root toughness in *Nicotiana tabacum* and susceptibility to attack by wireworms; and Hemerik et al. [17] have noted that in a food preference experiment the larvae of *A. obscurus* preferred plant species from nutrient-rich grasslands. Sonnemann et al. [27], on the other hand, found that the level of damage to plant roots by wireworms was determined mostly by the size of the root system, suggesting that wireworm foraging is only extensive and random,

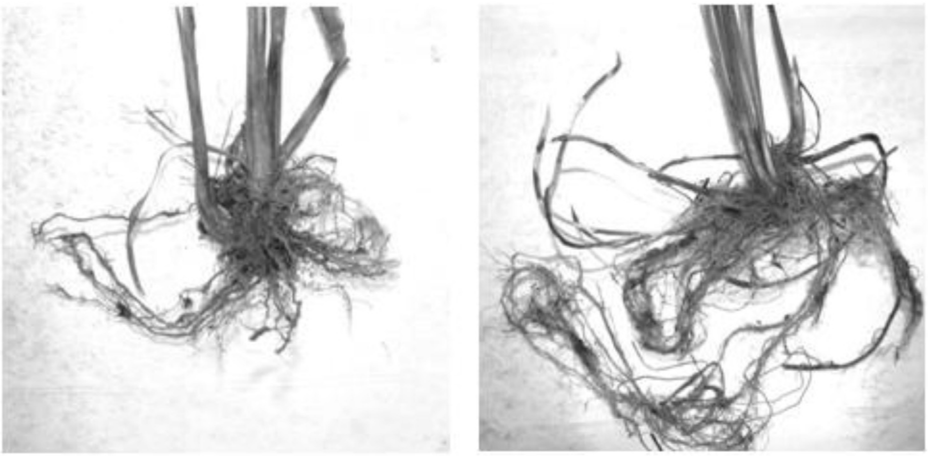

Fig. 6. Photographs of belowground parts of *C. epigejos* plants in the Microcosm experiment 2. On the left is a plant from microcosm with wireworms, on the right a plant from the control treatment.

Table 2
Shows mean % differences in biomass (final biomass - initial biomass) of the 4 plant species grown together in pots with and without wireworms in the Microcosm experiment 2. Numbers in bold sign statistically significant differences between treatments (*t*-test, t = −3.12, p = 0.02).

	Wireworms	Control
Calamagrostis	**−5.934**	**60.973**
Taraxacum	−17.98	−36.33
Centaurea	−2.585	14.393
Tussilago	−20.91	−27.00

however these studies did not take into account the adult behaviour and oviposition, which could take place in vicinity of the most preferred host plants [30].

Our assumptions are supported by a correlation test, the results of which can be seen in Fig. 3. Here the negative correlation between *C. epigejos* coverage and abundance of wireworms at each topographic position can be clearly seen. The fact that the mean coverage of the *C. epigejos* at the post-mining site with spontaneous succession was lowest in the depressions, although the total plant coverage was not different from the other sites, indicates there is a factor that negatively affects this plant species whilst it does not have any effect on other plant species. *C. epigejos* is a plant with a large ecological amplitude, being found in all types of grassland and anthropogenic ecosystems. It thrives especially at stands rich in nitrogen, which is the case for depressions at spontaneously revegetated spoil heaps near Sokolov [31]. One of the possible explanations of low numbers of *C. epigejos* ramets and negative correlation with wireworm numbers here is belowground herbivory, which prevents the grass from spreading. Roubickova et al. [19] found, that after a year-long application of an insecticide, which suppresses wireworm presence, to the soil, the belowground biomass of *C. epigejos* was higher in comparison with the surrounding soil.

Wireworms reached highest numbers at the depressions at the spontaneously revegetated site, which is likely to be caused by higher organic matter and moisture accumulation and subsequent lower temperature fluctuations. The soils at the spoil heaps are very rich in swelling clay (montmorillonite) [32] which is subject to swelling and shrinking, so at the early-successional sites, where the soil is not ameliorated by organic matter, it presents hostile conditions for plant roots as well as for soil fauna and may reduce their migration. Wireworms have been reported to prefer soils with moisture levels around 15–20% and temperatures around 10–20 °C with mild fluctuations [33,34], therefore the depressions present the most suitable conditions at the spoil heaps for these larvae.

The fact that the rhizomes of *C. epigejos* grow in greater depths, in comparison with the roots of most meadow plant species [35], may also affect the rate of consumption by wireworms. These larvae, despite high chitin incrustation, are sensitive to high temperatures and drying out of soil, so their activity is generally limited to spring or autumn months and in the other periods, they migrate to greater soil depths [36], where only *C. epigejos* rhizomes are present. Higher activity of wireworms in spring months may also play a crucial role in the plant-herbivore relationship; in the spring rhizomes of *C. epigejos* have the highest nutrient content [11] and therefore would be most attractive to herbivores, in comparison with roots and rhizomes of other plant species. However, the period of most intense growth of *C. epigejos* is in the summer months [28], when wireworm activity is generally low, therefore it does not interfere with the aboveground biomass as much as with the rhizome biomass [19].

Wireworms also reduced the biomass of *T. farfara*, whose roots are fleshy and without a strong cuticle and serve as vegetative reproduction organs as well as nutrient uptake organs, same as rhizomes of *C. epigejos* [37], however the results were nott significant and in the field experiment *T. farfara* biomass did not show any interaction with wireworm presence. *T. farfara* is a common plant species at the post-mining sites, often dominating some of the early-successional stages [1], however, it had the highest density at the depressions at our study site, where also the wireworm abundance was high, so the negative effect of wireworms on this plant species is not supported by field observations.

Thanks to the high mobility of the adult click-beetles, wireworms are one of the several soil fauna groups that colonize the spoil heaps in the early stages [38]. In later stages, as the sites get dominated by tree species, the Elaterid beetles become less abundant and so do their larvae in the ground, therefore they no longer have the potential to alter the plant community composition. However, we hypothesize that in the early-successional stages at the spontaneously revegetated sites, wireworms play an important role in plant community composition establishment and that they prevent the spreading of *C. epigejos*. This effect is mediated by the spatial variability of these spoil heaps, especially by the undulating surface which causes accumulation of wireworms and as result areas with a lower density of the *C. epigejos*.

5. Conclusions

We found that the fleshy rhizomes of the *C. epigejos* are a preferred food source for wireworms at the early stages of succession at spontaneously revegetated post-mining sites. In areas with high wireworm densities these root herbivores have a potential to reduce the numbers and coverage of *C. epigejos*, as the damage done to the rhizomes negatively affects the vegetative reproduction of this plant. Further research is needed to establish whether the wireworms have a potential to reduce *C. epigejos* abundance on large scale and to find out whether there is potential in utilizing wireworms in biological control of this expansive plant species.

Acknowledgements

This research was funded from IGA grant n. 20164226 from the Czech University of Life Sciences. We would like to thank Marek Vach for great help during the fieldwork, Ondřej Mudrák and Jan Frouz for their advice and support.

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V.

Using expansive grasses for monitoring heavy metal pollution in the vicinity of roads

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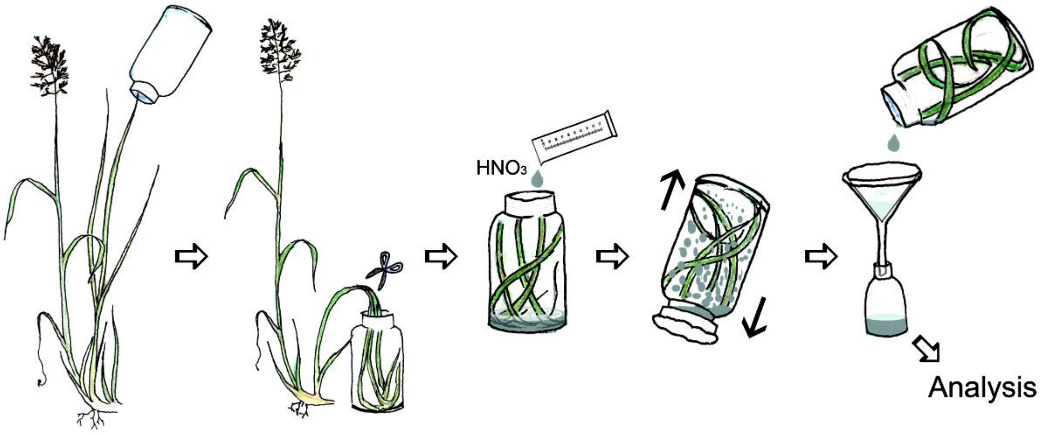
Environmental Pollution

10.1016/j.envpol.2017.05.069



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Using expansive grasses for monitoring heavy metal pollution in the vicinity of roads[☆]

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ARTICLE INFO

Article history:
Received 6 September 2016
Received in revised form
8 March 2017
Accepted 24 May 2017
Available online 2 June 2017

ABSTRACT

We propose a method for monitoring heavy metal deposition in the vicinity of roads using the leaf surfaces of two expansive grass species which are greatly abundant. A principle of the proposed procedure is to minimize the number of operations in collecting and preparing samples for analysis. The monitored elements are extracted from the leaf surfaces using dilute nitric acid directly in the sample-collection bottle. The ensuing steps, then, are only to filter the extraction solution and the elemental analysis itself. The verification results indicate that the selected grasses *Calamagrostis epigejos* and *Arrhenatherum elatius* are well suited to the proposed procedure. Selected heavy metals (Zn, Cu, Pb, Ni, Cr, and Cd) in concentrations appropriate for direct determination using methods of elemental analysis can be extracted from the surface of leaves of these species collected in the vicinity of roads with medium traffic loads. Comparing the two species showed that each had a different relationship between the amounts of deposited heavy metals and distance from the road. This disparity can be explained by specific morphological properties of the two species' leaf surfaces. Due to the abundant occurrence of the two species and the method's general simplicity and ready availability, we regard the proposed approach to constitute a broadly usable and repeatable one for producing reproducible results.

1. Introduction

Monitoring heavy metal deposition in the vicinity of roads is a frequent topic of interest in areas with various automobile traffic loads. A number of studies have focused on evaluating the content of potentially toxic elements in the soil and dust in the vicinity of roads (Duong and Lee, 2011; Faiz et al., 2009; Christoforidis and Stamatis, 2009; Wei et al., 2010 and many others). Another type of research work uses samples of plants and tree leaves from the vicinity of roads for monitoring heavy metal load. In this case, the metals content reflects both the deposition of particulate matter containing heavy metals and the uptake of these elements from the soil environment. The collected samples of plants or tree leaves are mineralized without previous washing or cleaning (Cicek et al., 2012; Fatoki, 1996; Gautam et al., 2005; Jankowski et al., 2015; Lehndorff and Schwark, 2010; Olajire and Ayodele, 1997; Oliva and Espinosa, 2007; Rossini Oliva and Mingorance, 2006; Sawidis et al., 2011 and others). The content of heavy metals deposited on the surface reflects the amount of deposition and the effect of meteorological factors, in particular precipitation, during the growth period of the analysed plant part. The uptake of elements into biomass is influenced by their content in the soil and corresponds to the long-term pollution levels. This aspect can be eliminated using active biomonitoring methods whereby standardized grass cultures are exposed at the sampling site in pots (Dietl et al., 1996; Klumpp et al., 2009). The proportion of toxic elements contained in the dust particles on the surface of plants relative to their content in the biomass can be specified by splitting the sample. One part is disintegrated and analysed without washing whereas the other is analysed after intensive washing with distilled water (Al-Khashman et al., 2011; Al-Shayeb et al., 1995; Bi et al., 2012; Nabulo et al., 2006; Othman et al., 1997; Serbula et al., 2012) and potentially with dilute acid (Mori et al., 2015b). Capturing and analysing the extraction solution is superfluous in this case. The proportion corresponding to the plant surface is simply defined by the difference in the heavy metals contents between the washed and unwashed sample. However, research on heavy metals pollution near roads can also focus entirely on evaluating dust articles deposited on the surfaces of plants or tree leaves. In such case, it is expedient to analyse directly the particulate matter separated from

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[☆] This paper has been recommended for acceptance by Prof. W. Wen-Xiong.

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http://dx.doi.org/10.1016/j.envpol.2017.05.069
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such surface. A mechanical method of sweeping with a plastic bag and brush was used by Lu et al. (2008). Wet washing of the samples is more frequently done and is performed using a method enabling the use of a precise volume of the extraction solution which can be subsequently analysed. Mori et al. (2015a) washed coniferous needles with distilled water and chloroform, Simon et al. (2011) separated foliage dust by shaking with distilled water in a plastic box. Tomašević et al., (2005) used washing in an ultrasonic bath for separating dust. For the sake of completeness, it should be mentioned that washing is performed also for the purpose of separate gravimetric analyses intended only to quantify particulate matter (PM) deposited on surfaces and trapped in waxes without further analysis of heavy metals and the like within that PM (Dzierżanowski et al., 2011; Przybysz et al., 2014; Sgrigna et al., 2015; Song et al., 2015).

As described above, diverse approaches using a range of sample types which are then processed in various ways are utilized for evaluating heavy metals pollution near roads. The amount and method of handling the samples has a greater or smaller influence on the results of the analyses subsequently performed. In this study, we propose a simple and easy method to enable monitoring the amounts of heavy metals within PM deposited on the surfaces of two types of grasses which often occur in the vicinity of roads. Our objective is to verify and evaluate a well-repeatable approach which practically minimizes the number of operations in collecting and preparing the sample for analysis and which leads to reproducible results.

2. Material and methods

2.1. Species of interest

We monitored the amounts of heavy metals in dust particles deposited on the leaf surfaces in two species of common grasses: *Calamagrostis epigejos* and *Arrhenatherum elatius*. These species have global ecological importance due to their rapid expansion into various biotopes (e.g. Holub et al., 2012; Rebele and Lehmann, 2001).

We selected these species in particular because they are both very frequently present in phytocoenoses along roads. *Calamagrostis epigejos* is a tall grass expanding primarily on anthropogenically influenced locations no longer under management (e.g. Prach and Wade, 1992). Fiala et al. (2003) comment upon its strong competitiveness, high biomass production, and formation of slowly decaying matter. It has been demonstrated that this grass prospers very well even on polluted stands (Mitrović et al., 2008). The ecology of *A. elatius* is very similar to that of *C. epigejos*. It differs in nutrient management (Holub et al., 2012), and it prefers areas with good nutrient supply (Buckland et al., 2001).

2.2. Monitoring sites

Our work focused on verifying collection methods and sample processing for the two selected grass species in the vicinity of roads and not at all to research the monitored sites themselves or the extent of their heavy metal pollution. The specific position of the collection locations was not therefore important for the purpose of our research. The only factor taken into account was the collection spots' distances from the edges of the monitored roads. The samples of *C. epigejos* and *A. elatius* were collected in the vicinity of roads with average traffic intensity and approximately equal proportions of long-distance and local traffic. The studied locations were situated in two regions of the Czech Republic – Western and Central Bohemia. In each region, 10 collection points were chosen in the vicinity of two selected roads. At each collection point, two samples were collected (of the two selected grasses) 5 m from the edge of the road and two samples at distance 20 m from the road. In total, therefore, 80 samples were collected.

2.3. Samples collection, preparation, and chemical analyses

The collection of samples of the selected grasses in the vicinity of roads was carried out to ensure optimal conditions for monitoring the selected heavy metals in dust particles deposited on the surfaces of leaves. An objective was to minimize the number of individual steps, including to limit handling the collected samples, so that the procedure is unambiguous, standardly repeatable, and its results reproducible.

Grass samples representing the upper parts of live leaves (without flowers) were cut using stainless steel scissors and immediately placed into pre-weighed, wide-necked, 1 L polyethylene (PE) bottles. Before use, the collection bottles were treated for the needs of the trace elements analysis by leaching with the solution HNO_3 Suprapur Merck 2 mol.L^{-1} for 12 h and thoroughly washed with bidistilled water prior to use. During collection, an average 15 g of plant matter was deposited into each collection bottle and the bottle was closed. This sample amount seemed optimal for subsequent extraction of dust particles by dilute nitric acid which, was performed directly in the collection bottle without manipulating the sample in any other manner. For extraction, 100 mL of the solution HNO_3 Suprapur Merck 2 mol.L^{-1} was used. The intent was effectively to separate dust particles from the surface of leaves while not disturbing the structure of the plant material and extracting elements from the plant matter itself. Therefore, very intensive hand-shaking of the 100 mL extraction solution in the closed bottle for a relatively short period of 10 min was used (vertical shaking in approximate range of 30 cm with a frequency of about 100 cycles per minute). The extract was then filtered through Filpap KA 1 filtration paper, thereby ensuring separation of particles larger than 15 μm. The filtrate was transferred into a 100 mL PE sample bottle leached with the solution HNO_3 Suprapur Merck 2 mol.L^{-1} and prepared for analysis. The selected volume of the extraction solution consisting of dilute nitric acid (100 mL) appeared optimal for ensuring well-measurable concentrations of the monitored elements with efficiency sufficient for extraction of the monitored elements from the given amount of plant material.

The filtered extract was analysed using atomic absorption spectrometry (AAS) on devices from Agilent Technologies. Determination of Cd, Cr, Cu, Ni, and Pb was performed by atomizing in a graphite tube with a 240Z AA device with Zeeman background correction. Zn was determined by flame AAS on the 55 AA device. The determined contents of the elements identified were subsequently converted to dry weight of collected plant samples. For purposes of determining dry weight, part of the grass sample was inserted into plastic bags (in addition to the PE bottle) at collection and subsequently weighed and dried to constant weight.

3. Results and discussion

Extraction solutions acquired using the proposed procedure contained the monitored elements in concentration limits suitable for direct determination by the AAS method. Aggregate results of the analyses are presented in Table 1 and Fig. 1. Comparing the measured representations of the six monitored elements extracted from the surface of grasses *C. epigejos* and *A. elatius* (Zn > Cu > Pb ~ Ni > Cr > Cd) corresponds to their expected occurrence in the contaminated vicinity of roads. Specific determined amounts of monitored elements on the surface of the two grasses relative to dry weight of plant material are comparable with

Table 1
Elemental amounts extracted from the surface of *C. epigejos* and *A. elatius* relative to dry weight (mg kg^{-1}).

	Calamagrostis epigejos (mean ± SE)			*Arrhenatherum elatius* (mean ± SE)			mass AE/CE
	A distance 5 m	B distance 20 m	amount. ratio B/A	A distance 5 m	B distance 20 m	amount ratio B/A	
Zn	19.62 ± 5.61	10.67 ± 2.88	0.54	22.84 ± 3.98	21.28 ± 1.96	0.93	1.46
Cu	4.34 ± 0.65	2.65 ± 0.72	0.61	5.89 ± 0.79	6.28 ± 0.64	1.07	1.74
Pb	1.45 ± 0.17	1.05 ± 0.17	0.72	1.23 ± 0.21	1.29 ± 0.13	1.05	1.01
Ni	1.05 ± 0.14	0.98 ± 0.15	0.93	1.26 ± 0.17	1.39 ± 0.13	1.10	1.31
Cr	0.496 ± 0.188	0.432 ± 0.177	0.87	0.934 ± 0.202	0.785 ± 0.224	0.84	1.85
Cd	0.165 ± 0.056	0.098 ± 0.015	0.59	0.087 ± 0.008	0.116 ± 0.015	1.33	0.77
mean			0.71			1.05	1.36

results reported in published works stated in mg.kg^{-1} of dry weight for the difference between unwashed and washed leaves and grasses collected in contaminated areas (Bi et al., 2012; Nabulo et al., 2006; Othman et al., 1997; Serbula et al., 2012).

In the monitored heavy metals extracted from the surface of *C. epigejos*, there is an apparent decreasing trend with distance from the road in the extracted amounts as determined relative to dry weight. This is as one would assume (e.g. Jankowski et al., 2015). Ratios of B/A, where B represents the amounts of the monitored elements extracted from the surface of *C. epigejos* 20 m from the road and A represents those amounts 5 m from roads are presented in Table 1. The ratios average 0.71. This trend was not determined for the case of *A. elatius*. The latter results indicate that at a distance of 20 m from the road even slightly larger average amounts of Cu, Pb, Ni and Cd were extracted relative to dry weight than at the 5 m distance. The average B/A ratio for the monitored elements was in this case 1.05 (see Table 1), thereby demonstrating in the case of *A. elatius* a practically indifferent relationship between the determined amounts of deposited heavy metals and the distances from the road. This situation is plausible, particularly inasmuch as a similar spatially indifferent distribution of toxic elements had been described by Mori et al. (2015a) on coniferous needles collected at a height of 1–2 m above ground. The results of this study document the possibility for non-decreasing surface accumulation (μg.cm^{-2}) of PM 2.5 small dust particles and thereby also of heavy metals on the needles with increasing distance from roads. These small particles can be spread over wider areas while less influencing the vegetation along roads. Spatial distribution of heavy metals accumulated on plants in the vicinity of roads can therefore be markedly influenced by the variable affinity of their surfaces for capturing the individual size types of dust particles. The amounts and types of dust particles deposited on plants depend on the surface properties of their above-ground organs – surface roughness, pubescence, wax amounts, and leaf area size and orientation. Other factors include frequency of places suitable for dust accumulation (points of connection of leaves to stem, leaf sheaths, etc.) and mobility of the above-ground part (Beckett et al., 2000; Sæbø et al., 2012; Weber et al., 2014). Species growing close to the ground accumulate greater amounts of PM than do taller species (Weber et al., 2014).

The spatial distribution of heavy metals we detected as deposited on the surfaces of the two related species (from the same family) in the vicinity of roads can therefore be interpreted in terms of the different properties of these grasses' above-ground organs. Morphological differences between the leaf surfaces of *C. epigejos* and *A. elatius* are shown in Fig. 2. Microscopic images of leaf cross-section show the different surface structures of the two grasses. The surface of *A. elatius* is rougher in appearance than is that of *C. epigejos* and there is an occurrence of hairs. A rougher surface presents a larger leaf area and should accumulate more dust particles. Our results confirm this assumption. Table 1 states an average ratio between *A. elatius* and *C. epigejos* of elemental amounts relative to dry weight of 1.36 (average from all samples for both distances from the road). Of course, this conclusion is conditioned upon the assumption that the above-ground parts of the compared grasses have very similar composition and dry biomass density. Even the determined spatial distribution of the monitored elements accumulated on the surfaces of both grass species can be interpreted as an effect of morphological differences (with average B/A ratios of 0.71 for *C. epigejos* and 1.05 for *A. elatius*). There is a good possibility that the more structured – and therefore larger – surface of *A. elatius* could have a selectively higher affinity for capturing smaller particles (PM 2.5) in which there is a higher probability of non-decreasing deposition rate in relation to the of 5–20 m magnitude of distances from the road within which the grass samples were collected.

The objective of this study was to develop and verify a procedure allowing efficient monitoring of heavy metal deposition in the vicinity of roads while minimizing the number of operations in collecting and preparing a sample for analysis. We believe that this approach constitutes a broadly usable method for producing reproducible outputs which is generally simple, readily accessible due to its minimal equipment requirements, and well-repeatable. Reproducibility can be of course achieved only when the same grass species is used (e.g. the widely distributed *C. epigejos*). We are aware that expression of the results used in this study (amount of the element extracted from grass surface relative to the dry weight of the plant [mg.kg^{-1}]) is an indirect one. A more appropriate and direct method for expressing the content of elements determined in the washing solution from leaves would be the weight per measured area of washed leaves, i.e. μg.cm^{-2} (Mori et al., 2015a). In other words, expressing content per unit of weight (e.g. mg.kg^{-1}) is adequate in determining elements within mineralized washed or unwashed plant material (Sawidis et al., 2011; Serbula et al., 2012 and many others), and alternatively in analysing dust particles extracted from the leaf surface if the element content would be related to the dry weight of the particles themselves (Simon et al., 2011). The amount of nutrients, soil type or pollution has effect on biomass production (e.g. Holub et al., 2012; Lehmann and Rebele, 2004). However, there is no evidence of the change in SLA (specific leaf area) depending on these parameters. We have measured SLA for leaves collected at five different locations. Though there was some variability within one locality, we have not registered significant difference in SLA between locations ($F(4; 20) = 1.417$; $p = 0.2646$). We believe, however, that expressing the amounts of extracted elements simply relative to dry plant weight is entirely appropriate when considering use of the same grass species having a stable surface-to-dry-weight ratio for the purposes of the proposed method of monitoring current heavy metal pollution in the vicinity of roads.

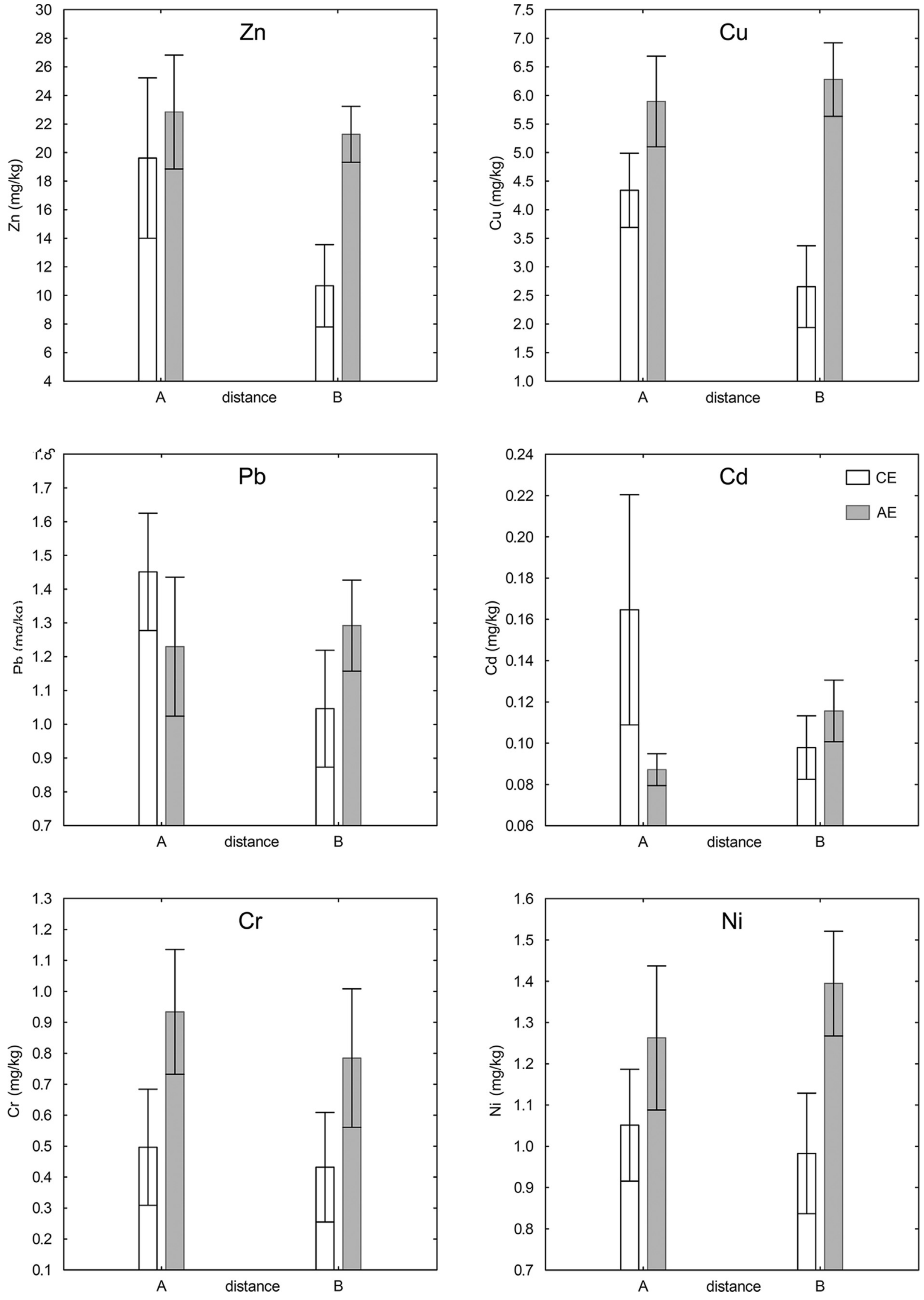

Fig. 1. Amounts of elements extracted from the surface of *C. epigejos* and *A. elatius* relative to dry weight (mg kg^{-1}) at distances of 5 m (A) and 20 m (B) from the road.

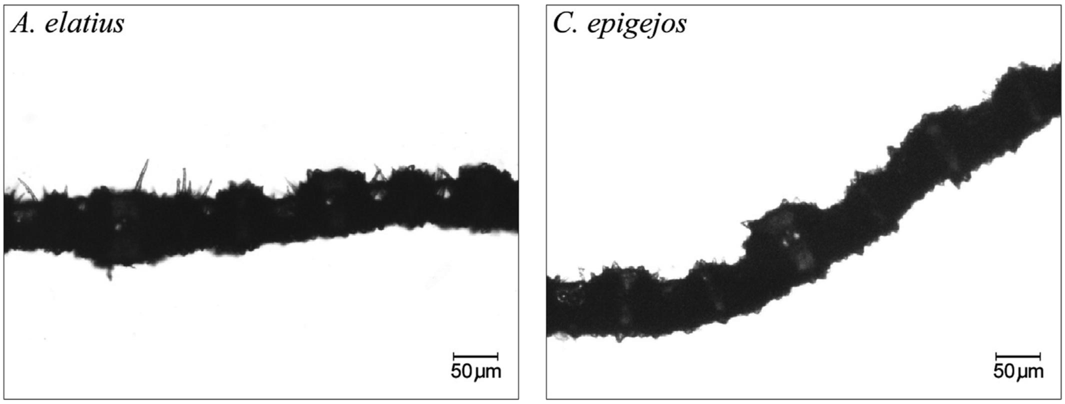

Fig. 2. Morphological differences between leaf surfaces of *C. epigejos* and *A. elatius* in leaf cross-section.

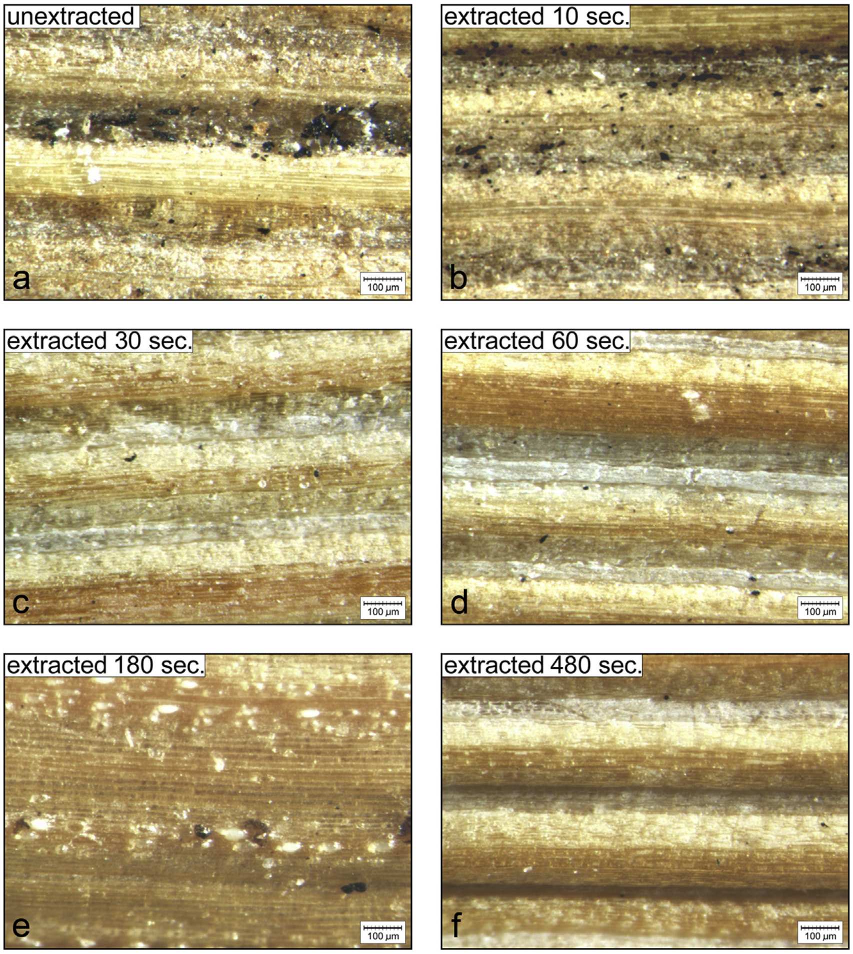

Fig. 3. Microscopic images of the leaf surface of *C. epigejos* corresponding with different times of extraction with diluted HNO_3. The images show a randomly selected leaves of extracted bushgrass.

The proposed procedure minimizing the number of operations in preparing the sample for analysis does not include total (microwave) decomposition of the extracted dust particles. We assume, however, that such total decomposition is not necessary for the described procedure. Intensive shaking with 2 mol.L^{-1} nitric acid leads to effective extraction of material from the surface of the grass leaves, inasmuch as the surface is disrupted by the acid although the plant is not decomposed.

Fig. 3 shows the state of the leaf surface of *C. epigejos* after a variable period of extraction with diluted HNO_3 in sampling bottle by procedure described in Part 2.3. The sample for this verifying experiment was collected in the vicinity of the road with high traffic intensity. On the image of the unextracted leaf surface - Fig. 3a - we can see a large amount of dust particles. Ten seconds of extraction process lead to a reduction of dust particles amount on the leaf surface. However, this wasn't a significant proportion of the total amount of paarticles, see Fig. 3b. The effect of half minute extraction (Fig. 3c) is already evident. Continued extraction with duration 1, 3 and 8 min does not lead to striking changes of leaf surface state, see Fig. 3 d, e and f. Fig. 3 presents a sequence of microscopic images of randomly selected leaves parts of extracted bushgrass sample, which confirms the high efficiency of the proposed method for extraction of dust particles from the surface of *C. epigejos* leaves. A substantial proportion of heavy metals fixed on the dust particles is accordingly dissolved in the acid solution. A number of authors have demonstrated that trace elements, and particularly metals in soft dust elements emitted by automobile transport and other anthropogenic sources, are mainly (up to 90%) (Voutsa and Samara, 2002) present in unstably bound forms (Kyotani and Iwatsuki, 2002) the solubility of which markedly increases in conditions of decreasing pH (Fernández Espinosa et al., 2002). The effectiveness of the proposed procedure was also verified by monitoring the content of selected toxic elements in extraction solution depending on the time of extraction. We used a mixed sample of *C. epigejos* leaves collected at two sites in the vicinity of roads with high traffic intensity. The extraction was interrupted at selected time intervals. At each break, half of the extraction solution volume (50 mL) was separated for analysis (AAS used - see part 2.3) and diluted HNO_3 was added to make 100 mL of solution. The concentration values of elements measured in individual time intervals were subsequently recalculated to take into account the dilution made and concentration corresponded to the total amount extracted. Fig. 4 shows the dependence of the relative contents of evaluated elements in the filtered extraction solution

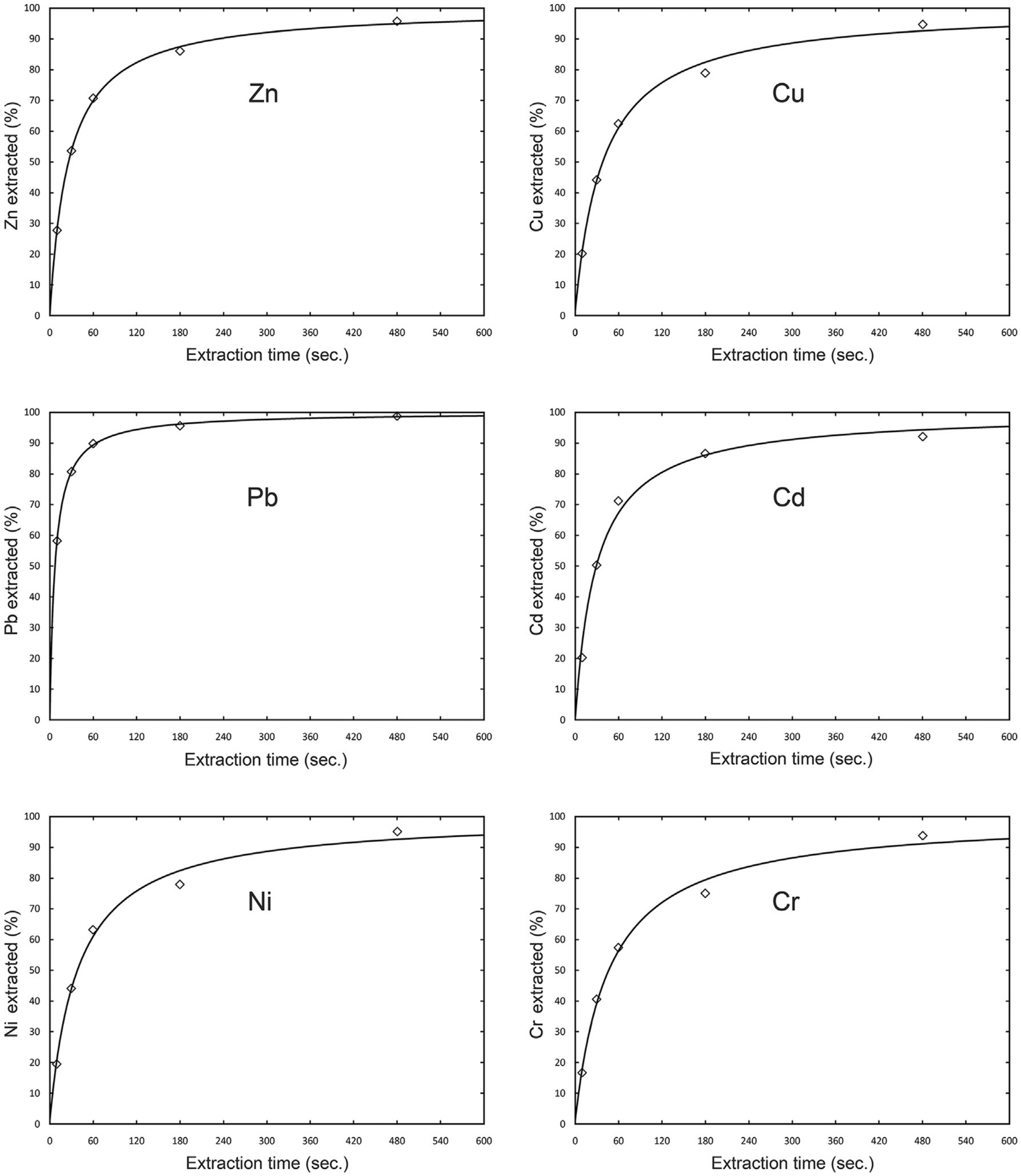

Fig. 4. Extracted shares of monitored toxic elements in dependence on the duration of extraction. Percentages are related to the expected total amount of each element extractable from the leaf surface of *C. epigejos*.

on the duration of extraction. Presented percentage values are related to the expected total amount of each element contained in the dust particles deposited on the surface of the bushgrass leaves. The estimated total amounts of monitored elements are derived as the limit (value a) of used regression function $x = a - b/(c + t)$, a, b and c are regression coefficients (optimized by gradient method), t is the independent variable - time. We chose this general nonlinear function suitable for the given time dependencies, because the order of kinetic process of elements extraction from the leaf surface is not known. With respect to the varying degree of fixation of each type of dust on the leaves, the first order process etc. cannot be automatically assumed. The results presented on Fig. 4 document the effectiveness of the chosen procedure in accordance with Fig. 3. The graph shows that the extraction time 8–10 min leads to the release of monitored toxic elements from the surface of the leaves of bushgrass at a rate exceeding 90%. 47 s are sufficient for the release of 50% of the slowest extractable element - Cr, while in the case of Pb, it is less than 10 s. It is evident that the individual elements are contained within the particles unevenly and they are extractable by different speeds. In this context, there are evident similarities between Cu, Ni and Cr. Zinc and cadmium is another pair with similar extraction properties, rapid release lead is specific.

Part of the proposed procedure is filtering out the separating particles larger than 15 μm. These large, undissolved particles can comprise grains of sand or other mineral materials, and it cannot be presumed that they would bind a more substantial proportion of heavy metals than is contained in the significantly smaller particles (Park and Kim, 2005; Samara and Voutsa, 2005). Filtration also removes the broken parts of collected grass leaves which are carried out in the extraction solution when pouring out the collection bottle. Smaller particles (up to 15 μm in size) which could eventually bind some remaining share of the heavy metals remain in the filtrate and are further analysed. We have tested the effect of filtering on the results of the determination of monitored elements in extraction solution. Half of the extraction solution volume taken during the kinetic experiment (described in previous text) was filtered through a Munktell paper grade 1288 (12–15 μm pore size), residual volume was analysed without filtration. The filtration does not affect Zn, Cu, Pb and Cd. The concentrations of these elements measured in the individual fractions of the filtered and unfiltered extraction solution showed no significant difference. In contrast, the concentrations of Ni and Cr measured in the filtered extraction solution were, on average, two-thirds compared to the values measured in the unfiltered solution. It shows that Ni and Cr may be more fully contained within the larger and slightly soluble particles. Potential presence of undiluted particles therefore creates no (mechanical) problem in dispensing filtrate into the graphite cuvette of the atomic absorption spectrometer. It can be assumed that there occurs effective disruption and atomization even in this undissolved fraction. We can add that AAS with electrothermal atomization also allows atomizing solid material in direct solid sampling. Similar assumptions can be stated also for the case of flame AAS, which must be used for determining Zn (small particles with size up to 15 μm do not cause obstruction of the intake tube). Nevertheless, it certainly would be possible to use an even finer filter (if, for example, inductively coupled plasma mass spectrometry were to be used for analysing the filtrate) and to separate out even substantially smaller particles than just those larger than 15 μm. In such case and taking into account the facts described above, the effect on results of the analyses should be negligible for Zn, Cu, Pb, Cd and specific for Ni and Cr.

The reproducibility of results from a method utilizing plant surfaces for evaluating heavy metal deposition is certainly limited also by meteorological factors, and particularly the amount and intensity of rainfall. This influence, however, should not be paramount. Tomašević et al. (2005) had determined that accumulation of deposited dust particles on leaves of two tree species during the vegetation period corresponded to the fact that a large part of PM is not washed away by rainfall. We have tested the possible influence of rainfall on the amounts of monitored elements contained in dust particles deposited on the leaves of *C. epigejos*. We tried to extract the sample of bushgrass leaves by shaking them with distilled water in the sampling bottle for 1 min. Subsequently, the whole volume of water was taken for the analysis and next the sample was 8 min extracted with diluted HNO_3. The contents of Cu, Pb and Cd found in the water extract solution were less than 1% of the estimated total extractable quantity contained in the dilute acid extract solution for Zn and Cr, it was about 2% and 10% for Ni. It can be seen that the influence of rainfall is not significant for the content of the elements on the surface of leaves of *C. Epigejos*. Nickel is a specific element, however in this case the influence of rain isn't crucial. Accordingly, it is apparent that measured values of heavy metal deposits, for example in the vicinities of two different roads, can be compared if grass samples are collected in a similar stage of the vegetation period. The selected species *C. epigejos* and *A. elatius* are suitable for monitoring deposition of heavy metals not only in consideration of their abundant occurrence but also because, being expansive grasses, they colonize entire areas in the vicinity of roads and there occurs practically no influence from spatial distribution of PM due to shading by other species.

The verification experiments were performed on species *C. epigejos* because its leaves are also usable at other times than the growing season. But we expect similar extraction properties for species *A. elatius*.

Acknowledgments

Finalization of this paper was supported by the projects IGA 20164226 funded by the Internal Grant Agency of the Czech University of Life Sciences. We would like to thank Alena Walmsley for language help and Tereza Dvořáková - Březinonvá, Lucie Součková, Jan Růžička and Pavel Jakubec for their instrumental support.

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5. Komentář k publikovaným výsledkům

5.1. Průběh expanze ve společenstvu s *C. epigejos*

Z **článku I** vyplynulo, že **počet druhů** ve sledovaných čtvercích byl **negativně ovlivněn** nejen vzrůstajícím množstvím ***C. epigejos***, ale také časem. Jednoroční **kosení se pozitivně neprojevilo na počtu druhů** a neovlivnilo ani množství *C. epigejos* v jednotlivých experimentálních čtvercích. I když někteří autoři uvádějí, že po několikaletém sečení se navrací druhová bohatost nebo se alespoň snižuje pokryvnost této trávy (Házi et al., 2011; Rebele et Lehmann, 2002). Důvodem odlišných výsledků může být větší náchylnost experimentálních čtverců k expanzi – experimentální plocha je umístěna v bývalém vojenském cvičišti, které je až na výjimky neobhospodařované - *C. epigejos* zde má velmi vysokou pokryvnost. Druhy *Veronica officinalis*, *Plantago lanceolata*, *Epilobium* sp., *Lotus corniculatus, Potentilla argentea*, *Festuca pratensis, Hypericum perforatum, Thymus chamaedris, Gnaphalium sylvaticum, Trifolium arvense, Trifolium pratense* mizí při expanzi *C. epigejos* nejdříve. Regeneraci těchto druhů brání zastínění způsobené velkým množstvím stařiny (Fiala et al., 2011; Somodi et al., 2008). Ta totiž vytváří fyzickou bariéru pro semena a semenáčky (zachytí světlo, stíní semena a sazenice, snižuje teplotu v půdě). To samozřejmě ovlivňuje utváření společenstva (Facelli et Pickett, 1991).

Druhy, které ustoupily bezprostředně po expanzi *C. epigejos*, byly *P. lanceolata, F. pratensis, Centaurea jacea, Lotus corniculatus, P. argentea, Dianthus deltoides*, *Taraxacum* sect. *Ruderalia* nebo *T. arvense*. *C. epigejos* totiž velmi dobře vytlačuje jiné druhy (Mudrák et al., 2010)

Zvyšující se abundance *C. epigejos* negativně korelovala s abundancí *A. elatius*, který roste především na druhově chudých porostech (Scotton et al., 2014) pozdních sukcesních stádií (Mudrák et al., 2012). Svědčí mu heterogenní neobhospodařované plochy s dostatkem živin (Hejcman et al., 2007). V takovém případě dokáže vykompetovat *C. epigejos* (Tůma et al., 2009, 2005). To potvrzují i naše výsledky. *A. elatius* ztrácí při kosení více dusíku než *C. epigejos* (Holub et al., 2012), protože ho hromadí v nadzemní biomase (Berendse et al., 1992). Potřebuje tedy jeho pravidelnou dotaci (Buckland et al., 2001). Kontrolní, tedy bezzásahový režim vyhovuje především *L. corniculatus, C. jacea, P. lanceolata, F. pratensis,* ale i *A. elatius. L. corniculatus* je druh, který se nevyskytuje na stinných místech, ale má poměrně širokou ekologickou a fytocenologickou amplitudu (Jones et Turkington, 1986) a pozitivně reaguje na dostatek fosforu (Kelman, 2006). *C. jacea* se nejčastěji vyskytuje ve společenstvech třídy *Molinio-Arrhenatheretea*, ale jedná se o častý ruderální druh luk, pastvin a lesních lemů, kde vyhledává živinově bohaté půdy. Tento druh nepreferuje zastíněné lokality stejně jako *F. pratensis a P. lanceolata* (Slavík, 2004). Společenstvo, které by se zde vyskytovalo, pokud by nedošlo k expanzi *C. epigejos*, by dle katalogu biotopů (Chytrý et al., 2001) nejspíše spadalo do kategorie T1.1 – mezofilní ovsíkové louky. Tento předpoklad vznikl na základě informací ze starých map. Nemůže být také zanedbán fakt, že druhy *T. pratense, L. corniculatus, T. arvense, Vicia angustifolia, Holcus lanatus* nebo *C. jacea* jsou pozitivně korelovány s výskytem *A. elatius*.

Během sledovaných let došlo k ústupu druhů jako *H. perforatum, Poa pratensis, P. argentea, V. officinalis, G. sylvaticum T. chamaedris, Bromus sterilis a Epilobium* sp. Společenstvo se měnilo kontinuálně v čase. Velkou roli zde hraje zvyšující se pokryvnost *C. epigejos*. Nejdůležitějším faktorem změny společenstva v čase bude zřejmě přirozená sukcese, která ovšem směřuje k porostu s dominantní *C. epigejos*.

5.2. Reakce *C. epigejos* na dodané živiny

Dotace **živinami se projevila na výšce vegetace již v prvním roce** po jejich použití. Tento výsledek plně koresponduje s výsledky jiných studií o vlivu dostupného dusíku na rychlou tvorbu biomasy. Ovšem všeobecně přijímaný pozitivní **vliv fosforu na množství biomasy** (Hejcman et al., 2010; Suss et al., 2004) zde **nebyl potvrzen**. Výška vegetace ve variantě, kde byl aplikován pouze fosfor, byla prokazatelně nižší, tento rozdíl setrvával ještě nadcházející sezonu roku 2012. To mohlo být způsobeno větším množstvím stařiny, která se v tomto čtverci naakumulovala. Zajímavý je i vývoj kontrolních variant. První tři sledované sezóny byla abundance *C. epigejos* velmi podobná. Ovšem v roce 2013 byl zaznamenán **prudký nárůst** tohoto druhu. Obdobný průběh měly i ostatní varianty, ale tento vývoj neměl statisticky významný charakter.

V prvním roce po aplikaci živin byl zaznamenán nárůst *Arrhenatherum elatius*, *L. corniculatus* a *P. lanceolata*. Vyjma *P. lanceolata* tyto druhy v dalších letech opět ustoupily. I když efekt kosení nebyl prokázán, zdá se, že kosené plochy s dostatkem dusíku jsou náchylnější k expanzi *C. epigejos* než jiné. V roce 2013 nebyl ve výšce vegetace v jednotlivých variantách zaznamenán rozdíl. Bylo zaznamenáno prokazatelné zvýšení vegetace během sledovaných let. Výška vegetace je pozitivně ovlivněna vzrůstajícím množstvím *C. epigejos*.

5.3. Stabilita společenstva s dominantní *C. epigejos*

S cílem vyhodnotit stabilitu společenstva s dominantní *C. epigejos* byly analyzovány dva faktory – abundance *C. epigejos* a počet ostatních druhů pod vlivem managementu. Každá dvojice hodnot reprezentující abundanci *C. epigejos* a počet ostatních druhů je brána jako výsledek interakce dvou protihráčů odpovídající strategiím uplatňovaným v závislosti na managementu. Nashova rovnováha představuje takovou kombinaci strategií každého hráče, která je nejlepší odpovědí na strategii ostatních hráčů (Nash, 1951). Naše výsledky publikované v článku **Stochastic identification of stability of competitive interaction in ecosystems** ukazují, že aplikace navrhovaného postupu nabízí možnost řešení problému rovnovážného optima – **nejlepšího kompromisu mezi pay-off hodnotami**, které jsou pro každý z interagujících subjektů nejvýhodnější. Zadání není triviální, maximum payoff je u obou subjektů dosahováno v rozdílné situaci. Pro *C. epigejos* je nejvýhodnější situace hnojení dusíkem, fosforem, draslíkem a kosení. Nejvyšší počet druhů je v kontrolní, nekosené variantě. Vyhodnocení výchozích payoff hodnot vede k preferenci maxima pro počet ostatních druhů, kde vychází významně vyšší pravděpodobnost výskytu Nashovy rovnováhy interpretovatelná jako míra stability. Tento výsledek odpovídá obecným předpokladům – nejvyšší míra stability je dosahována u situace bez zásahu a tato **stabilita koresponduje s počtem vyskytujících se druhů** – tedy s biodiverzitou a nikoli s abundancí expanzní třtiny. Výsledkem navrhovaného konceptu je spojitá distribuce míry stability vyhodnocovaných situací. Korelace této závislosti se souborem výchozích payoff hodnot vychází jednoznačně ve prospěch výskytu ostatních druhů – **stabilita koresponduje s biodiverzitou**. Určujícím faktorem determinujícím obdržený výsledek je výskyt Nashových rovnováh v čistých formálních strategiích, takže vypočítaná distribuce míry stability není pro oba subjekty významně rozdílná. Z výsledků tedy nelze vyvozovat např. doporučení, která varianta managementu by mohla být stabilnější pro výskyt ostatních druhů oproti abundanci *C. epigejos*. Nicméně i tak mohou obdržené výsledky provedeného vyhodnocení znamenat přínos k údajům majícím praktický význam při řešení problému expanzních a invazních druhů. Na základě provedeného vyhodnocení lze shrnout – **existují varianty managementu vedoucí k významnější eliminaci výskytu *C. epigejos* v rostlinných společenstvech**, např. varianta, kde byl aplikován pouze draslík. Jejich předpokládaná míra stability je však oproti situaci bez zásahu velmi nízká a tyto stavy nemusí být dlouhodobě udržitelné.

Navrhovaný přístup je založen pouze na **porovnávání měřitelných nebo pozorovatelných hodnot kvantitativních parametrů** bez zahrnutí specifických interních faktorů určujících složité interakce ekosystému. Interpretace větší pravděpodobnosti výskytu Nashovy rovnováhy jako vyšší ekologické stability je pouze předpokladem, který nemusí v řadě případů platit. Takto vymezená míra stability je navíc pouze relativní - je větší či menší pouze v porovnání s výsledky obdrženými pro ostatní vyhodnocované situace.

Přístupy založené na teorii her a aplikaci Nashových rovnováh jsou v ekologii, zejm. pak evoluční často užívány (Apaloo et al., 2009; Mcnickle et Dybzinski, 2013; Nowak et Sigmund, 2004; Vincent et Brown, 2005). Zde navrhovaný koncept vycházející ze stochastického herního modelu může být použit především při **aplikaci na rozsáhlejší datové soubory** reflektující např. ekologické parametry většího počtu krajinných prvků, u kterých by mohla být takto vyhodnocována relativní ekologická stabilita. Respektive vyhodnocení pravděpodobnosti výskytu Nashovy rovnováhy by mohlo takovýchto dat vhodně doplňovat standardní statistickou analýzu. Navrhovaný koncept je jistě využitelný v mnoha dalších aplikacích.

5.4. Možnosti eliminace *C. epigejos*

Studie ze Sokolovských výsypek publikovaná pod názvem **Topography of spoil heaps and its role in plant succession and soil fauna presence** ukazuje, že morfologie terénu při tvorbě výsypek má vliv na rozložení živin v půdě, což ovlivňuje složení rostlinných společenstev a přítomnost půdní fauny. Může také urychlit migraci některých půdních živočichů. Zařazení ploch rovných spolu se zvlněnými představuje pozitivní přínos pro rozmanitost stanovišť na místech se spontánní sukcesí.

Celkový obsah dusíku, draslíku, hořčíku a vápníku byl vyšší než průměrné hodnoty z travních porostů České republiky (Kalina, 2005; Mládek et al., 2006). V naší studii byla **nejvyšší hodnota celkového dusíku** měřena **na dně vln** na mladší lokalitě. Vrcholy vln byly živinově chudší, pravděpodobně díky erozi, kolísání teploty a špatnému zadržování vody na vrcholcích (Zhu et al., 2014). Alkalické terciární jíly, které tvoří tyto výsypky, jsou obecně **bohaté na celkový fosfor** (Frouz et al., 2008; Šourkova et al., 2005), ale situace je odlišná pro obsah rostlinou dostupného fosforu - ve srovnání s množstvím **dostupného fosforu** z trvalých travních porostů v České republice (Kalina, 2005) byl zjištěn velmi **nízký obsah**. To s největší pravděpodobností souvisí s vysokým obsahem vápníku, který tvoří nerozpustný fosforečnan vápenatý, který obecně snižuje dostupnost fosforu v půdě (Addiscott et Thomas, 2000; Braschi et al., 2003; Tunesi et al., 1999). Vyšší množství dostupného fosforu na starší lokalitě je výsledkem kombinace biotických a abiotických procesů během sukcese (Ciarkowska et al., 2016).

Složení rostlinných společenstev se lišilo mezi oběma lokalitami. Rostlinná společenstva na vyrovnaných plochách byly podobné těm na vrcholcích vln. Výsypkové půdy jsou velmi bohaté na montmorillonit, který efektivně zadržuje vodu. Pokud se ovšem tato zemina vysuší, stává se velmi tvrdou. Takovéto podmínky jsou velmi nepříznivé pro kořeny rostlin i půdní faunu. Migrace se stává problematickou a některé druhy, které nejsou schopné se těmto extrémním podmínkám přizpůsobit, chybí (Dunger et Voigtlander, 2009; Lavelle et Spain, 2001). *C. epigejos* ovšem dokáže prospívat i za těchto pionýrských podmínek (Rebele et Lehmann, 2001)

Počty půdní fauny byly ovlivněny jak věkem místa, tak topografickou polohou. Drátovci byly nejčastěji v terénních depresích na obou místech, pravděpodobně díky vyšší vlhkosti (Campbell, 1937; Evans, 1944; Finney, 1946; Parker et Howard, 2001) nebo většímu zastínění - méně extrémů. Počet drátovců je nižší na starší lokalitě, která má odlišné složení rostlinného společenstva od mladší plochy.

Zdá se, že **vegetace je lepším prediktorem pro přítomnost drátovců a žížal než měřené faktory prostředí**. Variabilita, kterou vysvětluje vegetace, odráží biotické interakce mezi půdní makrofaunou a vegetací. Tyto vztahy byly zaznamenány také v jiných studiích (Frouz et al., 2008; Roubíčková et al., 2012, 2009) a lze je vysvětlit přímými nebo nepřímými trofickými interakcemi. Rostliny představují zdroj potravy pro drátovce a žížaly a tím pádem mohou ovlivnit množství a složení půdní fauny (Frouz et al., 2001; Wardle et al., 2005). Na druhé straně žížaly ovlivňují půdní vlastnosti a procesy, jako je tvorba agregátů (Marashi et Scullion, 2003), schopnost udržet vodu, aktivitu mikroflóry smísením rostlinných vrstev a minerální půdy (Frouz et al., 2006) a dostupnost živin (Lavelle et Spain, 2001). Kořenoví býložravci mohou také podstatně ovlivnit abundanci některých druhů rostlin a tím změnit složení rostlinného společenstva (Blossey et Hunt-Joshi, 2003; Brown et Gange, 1992, 1989)

Výsledky předchozí studie nás přivedly na myšlenku otestovat trofické preference drátovců, kteří by mohli významně snižovat abundanci *C. epigejos* a zpomalovat její expanzi. Dva experimenty a jedna studie jsou uvedené v článku **Wireworms suppress spreading of the expansive weed *Calamagrostis epigejos* (L) Roth by feeding on its rhizomes**. Bylo zjištěno, že oddenky *C. epigejos* jsou vyhledávaným zdrojem potravy pro drátovce především v raných fázích spontánní sukcese na výsypkách. V oblastech s vysokými hustotami drátovců mají tito kořenoví býložravci potenciál snížit abundanci *C. epigejos*, protože škody negativně ovlivňují vegetativní reprodukci této rostliny.

V kontrolovaných podmínkách klimaboxu drátovci významně **snížili délky podzemních výhonků** *C. epigejos*. Přestože Sonnemann et al. (2012) zjistili, že drátovci způsobuji snížení nadzemní i podzemní biomasy některých lučních druhů, námi sledované druhy (*Tussillago farfara, Centaurea stoebe, Hieracium* *pillosela, Festuca rubra*) nebyly nijak významně ovlivněné. Drátovci sice snížili biomasu *T. farfara*, jehož podzemní orgány mají stejnou funkci jako podzemní části *C. epigejos* (Barkley et al. 2006), nicméně výsledky nebyly průkazné a ani na studijní ploše výsypky se neprokázaly žádné interakce s drátovci. *T. farfara* je běžný druh rostlin post-těžebních lokalit, který často dominuje v brzkých sukcesních stádiích (Prach, 1987). Jeho nejvyšší abundance byla zjištěna v prohlubních naší studijní plochy (**článek III**), kde byl nalezen i vysoký počet drátovců. Negativní vliv drátovců na růst *T. farfara* tedy nebyl prokázán.

Nejnižší pokrytí *C.epigejos* bylo zaznamenáno v prohlubních vln. Zde bylo také nalezeno **nejvíce drátovců**. Třtina je tráva se širokou ekologickou amplitudou a velmi dobře prospívá v porostech bohatých na dusík. Což je případ prohlubní na spontánně zarůstajících sokolovských výsypkách (Frouz et al., 2011, článek III). Její nízkou pokryvnost můžeme vysvětlovat vyšším počtem nalezených drátovců v těchto typech lokalit.

V našich experimentech **drátovci snížili biomasu podzemních výhonků**, nikoli kořenů. To znamená, že proces příjmu živin není ovlivněn, pouze tok látek mezi jednotlivými rametami (Gloser, 2005; Kavanová et Gloser, 2005), což může snižovat konkurenční schopnost rostliny a zpomalit její vegetativní rozmnožování (Roubíčková et al., 2012).

5.5. Využití *C. epigejos* pro monitoring znečištění těžkými kovy z atmosférické depozice

Cílem této práce bylo rozpracovat a ověřit postup umožňující **efektivní monitoring depozice těžkých kovů v blízkosti silnic** založený na minimalizaci počtu operací při odběru a přípravě vzorku k analýze. Domníváme se, že tento přístup je v souvislosti s celkovou jednoduchostí a dostupností danou minimalizací náročnosti na přístrojové vybavení **dobře opakovatelnou a široce použitelnou metodou** s reprodukovatelnými výstupy umožňujícími porovnatelnost. Reprodukovatelnosti může být samozřejmě dosaženo pouze při využívání stejného druhu trávy – např. hojně rozšířené *C. epigejos*.

Extrakční roztoky získané navrhovaným postupem v článku **Using expansive grasses for monitoring heavy metal pollution in the vicinity of roads** obsahovaly sledované prvky v koncentračních rozmezích vhodných pro přímé stanovení metodou AAS. Porovnání změřených zastoupení šesti sledovaných prvků extrahovaných z povrchu trav *C. epigejos* a *A.elatius* - Zn > Cu > Pb ~ Ni > Cr > Cd odpovídá jejich předpokládanému výskytu v kontaminovaném okolí silnic. Konkrétní zjištěné **množství sledovaných prvků** na povrchu dvou trav vztažené na suchou hmotnost rostlinného materiálu je **srovnatelné** s výsledky uváděnými publikovaných pracích zabývajících se tímto tématem (Bi et al., 2012; Nabulo et al., 2006; Othman et al., 1997; Serbula et al., 2012).

U sledovaných těžkých kovů extrahovaných z povrchu *C. epigejos* je patrný trend poklesu zjištěných extrahovaných hodnot vztažených na suchou hmotnost se vzdáleností od silnic, což odpovídá našim předpokladům. Pro *A. elatius* tento trend nebyl zjištěn. Z výsledků je patrné, že ve vzdálenosti 20 m od silnice byly u Cu, Pb, Ni a Cd naměřeny dokonce mírně větší průměrné extrahované hodnoty než v 5 metrech. Průměrný ratio B/A pro sledované prvky - 1.05 vykazuje pro *A. elatius* prakticky indiferentní závislost zjištěného množství deponovaných těžkých kovů na vzdálenosti od silnice. Nicméně tato situace je možná – podobnou prostorově indiferentní distribuci toxických prvků popisuje Mori et al. (2015a) na jehličnanech odebíraných ve výšce 1-2 m nad zemí. Výsledky této práce dokumentují možnost neklesající povrchové akumulace (µg.cm^{-2}) menších prachových částic PM 2.5 a tím i těžkých kovů na jehlicích s rostoucí vzdáleností od silnic. Tyto menší částice mohou být rozptylovány do větších ploch při menším ovlivnění vegetací podél silnic. Prostorová distribuce těžkých kovů akumulovaných na rostlinách v okolí silnic může být významně ovlivněna proměnnou afinitou jejich povrchu k záchytu jednotlivých velikostních typů prachových částic. Množství a typ prachových částic deponovaných na rostlinách závisí na vlastnostech povrchu nadzemních orgánů - drsnosti povrchu, míře ochlupení, množství vosku, velikosti plochy listu a jeho orientaci (Beckett et al., 2000; Sæbø et al., 2012; Weber et al., 2014). Dalšími faktory jsou také četnost míst vhodných ke kumulaci prachu (místa napojení listů na stonek, listové pochvy apod.) a pohyblivost nadzemní části (Sucharová et Suchara, 1990). Druhy rostoucí při zemi hromadí menší množství PM než druhy vyšší (Weber et al., 2014).

Reprodukovatelnost výsledků metody využívající k vyhodnocování depozice těžkých kovů povrch rostlin je jistě limitována i meteorologickými faktory, zejména množstvím a intenzitou dešťových srážek. Tento vliv by však neměl být převládající. Tomašević et al., (2005) zjistili kumulaci deponovaných prachových částic na listech dvou druhů stromů v průběhu vegetačního období odpovídající tomu, že podstatná část PM není dešťovými srážkami vyluhována. V této souvislosti je zřejmé, že hodnoty naměřených množství těžkých kovů deponovaných v např. okolí dvou různých silnic je možné porovnávat, pokud jsou vzorky trávy odebírány v podobném stadiu vegetačního období. Zvolené druhy ***C. epigejos*** **a** ***A. elatius*** **jsou vhodné pro monitoring depozice těžkými kovy** nejen s ohledem na hojnost výskytu, ale i proto, že jako expanzní trávy obsazují v okolí silnic celistvé plochy a prakticky nedochází k ovlivnění prostorové distribuce PM zastíněním jinými druhy.

6. Závěr

Dlouhodobý a poměrně intenzivní proces přetváření a využívání krajiny v posledních dvou stech letech ustupuje do pozadí. Jednou z hlavních příčin je průmyslový a technický rozvoj, který přinesl změny ve způsobu hospodaření. Důsledkem je, že mnoho biotopů není tak intenzivně využíváno jako v minulosti nebo je zcela ponecháno ladem. Náš polní experiment prokázal, že dostatek živin (především dusíku), značně urychlují expanzi *C. epigejos* do takovýchto stanovišť. Živinová zásoba je na těchto lokalitách právě z dob hospodaření.

C. epigejos má mnoho vlastností, díky kterým dokáže měnit rostlinná společenstva. Změna společenstva v čase je determinována především mírou expanze této trávy. V současnosti je jen málo biotopů, které by nebyly ohroženy jejím nekontrolovatelným šířením. Pokud stoupá její abundance, postupně se snižuje biodiverzita. *C. epigejos* je původním druhem naší flory. Proto má také nezastupitelné místo ve skladbě rostlinných společenstev.

Otázkou stále zůstává, jak dosáhnout relativní stability polopřirozených stanovišť, aniž by se tato tráva stala dominantním druhem. Částečnou odpověď nám přineslo využití principů teorie her. Využití Nashových rovnováh jako faktorů míry stability vedlo k takovému výsledku, kde stabilita koresponduje s počtem druhů. Jednoznačné odpovědi na otázku, který management může vést k eliminaci *C. epigejos* sice nebylo dosaženo, ale na základě našeho vyhodnocení se lze předpokládat, že existují varianty vedoucí k její významnější eliminaci. Tato metoda může být přínosná nejen při řešení problémů s expanzními a invazními druhy, ale také jako podklad pro vyhodnocování relativní ekologické stability.

Stabilita rostlinného společenstva jistě není určena pouze jeho složením a obsahem živin v půdě. Například morfologie terénu při tvorbě výsypek má vliv na rozložení živin v půdě, což ovlivňuje složení rostlinných společenstev a přítomnost půdní fauny. Bylo zjištěno, že topografická struktura stanoviště ovlivňuje distribuci živin a půdní fauny, což determinuje složení rostlinného společenstva. Zároveň, díky vegetaci můžeme predikovat přítomnost drátovců a žížal. Mozaika terénních nerovností kombinovaná s urovnanými plochami představuje také pozitivní přínos pro rozmanitost stanovišť, které jsou utvářené spontánní sukcesí.

Eliminace expanzního druhu jako *C. epigejos* může být ve své podstatě jednoduchá, zjistíme-li, která přirozená složka ekosystému je poškozena nebo zcela chybí. Pokud budeme předpokládat, že původní druhy se začaly rozšiřovat v důsledku nějaké ekosystémové nestability, stačí „pouze“ určit co za nestabilitou stojí a pokusit se rovnováhu obnovit. Byly sledovány trofické preference drátovců a bylo prokázáno, že nejvíce predované jsou výhonky *C. epigejos* – redukuje se jejich podzemní biomasa. V této souvislosti může být snížena konkurenční schopnost rostliny a zpomaleno její vegetativní rozmnožování.

Velmi široký areál *C. epigejos* může přinášet i studijní výhody. Byla navržena metoda, umožňující monitoring depozice těžkých kovů nejen v blízkosti silnic. Listové plochy *C. epigejos* jsou vhodné pro námi navrhovaný postup, který je tak obecně použitelný a opakovatelný při dosažení reprodukovatelných výsledků. Tato metoda minimalizuje počet operací a může být použitelná v širokém spektru stanovišť.

Pochopení důvodů nadměrného rozšiřování původních, ale i invazních druhů, je velice zdlouhavý proces. Komplexní problém, na který bychom se měli dívat s krajinným a možná i historickým nadhledem. Zároveň se možná musíme smířit s faktem, že v nejbližších letech se půda nebude využívat tak intenzivně jako v minulosti a změní se tedy i charakter celých ekosystémů. Pokud ovšem nebudeme chtít investovat finanční prostředky a mnoho sil na udržení vzácných biotopů, které zarůstají expanzní třtinou křovištní, invazními druhy jako je třeba bolševník velkolepý nebo se postupně vrací do stadia lesa.

7. Summary

Long-term and intensive process of transformation and utilization of landscape has receded to backround in the last two hundred years. One of the main causes has been industrial and technical development which brought changes in the way of farming. In consequence, many biotopes are not as intensively used as in the past or they are completely abandoned. Our field experiment has shown that a high content of nutrients (especially nitrogen) accelerates the expansion of *C. epigejos* into such habitats. The nutrient reserve in these biotopes is just from the farming period.

C. epigejos has many properties that have made it possible to change plant communities. Change of the plant community in time has been mainly determined by the degree of expansion of this grass. Currently there are only a few biotopes what aren't threatened by its uncontrollable spread. If its abundance increases, biodiversity is gradually diminishing. *C. epigejos* is a native species of our flora, Therefore, it also has an irreplaceable place in the composition of plant communities.

The question remains, how to achieve relative stability of semi-natural habitats without this grass becoming the dominant species. A partial solution has been found when using the principles of game theory. Use of Nash equilibriums as the degree of stability factors has led to a result where the stability corresponds to the number of species. However, we did not have a clear answer to the question regarding which management can lead to the elimination of *C. epigejos*, but on the basis of our assessment it can be assumed that there are alternatives leading to its major elimination. This method can be beneficial not only for solving problems with expansion and invasive species but also as a possible approach for evaluating relative ecological stability.

The stability of the plant community is not only determined by its composition and nutrients content in the soil. For example, morphology of terrain forming the dumps has an effect on the distribution of nutrients in the soil, which affects the composition of plant communities and the presence of soil fauna. We have found that the topographic structure of the habitat affects the distribution of nutrients and soil fauna, which then determines the composition of the plant community. At the same time, thanks to vegetation, we can predict the presence of worms and earthworms. The mosaic of terrain inequalities combined with settled areas also represents a positive contribution to the diversity of habitats that are formed by spontaneous succession.

The elimination of an expansive species such as *C. epigejos* can be basically simple if we find out which native component of the ecosystem is damaged or completely absent. If we assume that the original species have begun to expand as a result of some ecosystem instability, it is enough to "determine" what the instability is and try to restore the balance. We monitored the trophic preferences of the wireworms and it was proved that the most preferred were the shoots of *C. epigejos* - their underground biomass was reduced. In this context, the plant's competitive ability and its vegetative reproduction may be reduced.

The very wide area of *C. epigejos* distribution can also offer study benefits. We have proposed a method for monitoring the deposition of heavy metals, not limited to the vicinity of roads. *C. epigejos* leaf areas are suitable for our proposed procedure, which is generally applicable, repeatable and leads to obtaining of reproducible results. This method minimizes the number of operations and can be used in a wide range of locations.

Understanding the reasons for the excessive spread of native and invasive species is a very tedious process. It is a complex issue, at which we should look from a landscape and maybe historical perspective. At the same time, we may have to accept the fact that in the next few years the land will not be used as intensively as in the past and the character of the whole ecosystem will change. Unless, however, we want to invest funds and a lot of power into maintaining rare biotopes which are degraded by shrubby invasive species such as the magnificent hogweed or gradually return to the forest stage.

8. Reference

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