UNIVERZA V LJUBLJANI
BIOTEHNIŠKA FAKULTETA

Mitja SKUDNIK

MAHOVI KOT KAZALCI VNOSA DUŠIKOVIH SPOJIN V NARAVNE EKOSISTEME SLOVENIJE IN PRIMERJAVA Z NEKATERIMI DRUGIMI METODAMI BIOINDIKACIJE

DOKTORSKA DISERTACIJA

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MOSSES AS INDICATORS OF NITROGEN INPUT INTO THE NATURAL ECOSYSTEMS OF SLOVENIA AND COMPARISON WITH SOME OTHER METHODS OF BIOINDICATION

DOCTORAL DISSERTATION

Ljubljana, 2016

Doktorska disertacija je zaključek univerzitetnega podiplomskega študija Varstvo okolja Univerze v Ljubljani.

Na podlagi statuta Univerze v Ljubljani ter po sklepu senata Biotehniške fakultete in sklepa 19. seje komisije za doktorski študij (po pooblastilu 30. seje senata Univerze) z dne 6. 7. 2011 je bilo potrjeno, da kandidat Mitja Skudnik izpolnjuje pogoje za neposreden prehod na doktorski univerzitetni podiplomski študij varstva okolja ter opravljanje doktorata znanosti. Doktorsko delo je bilo opravljeno na Gozdarskem inštitutu Slovenije. Za mentorja je bil imenovan prof. dr. Franc Batič in za somentorico doc. dr. Damijana Kastelec.

Komisija za oceno in zagovor:

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JI sl/en
AI Vzorci mahu vrste štorovo sedje (*Hypnum cupressiforme* Hedw.) so bili nabrani na 103 lokacijah v gozdovih Slovenije in analizirani, da bi ugotovili vsebnost dušika (N) in vrednost izotopske sestave dušika (δ^{15}N). Na vsaki lokaciji so bili mahovi nabrani na dveh mestih: pod drevesnimi krošnjami ter v bližnji gozdni vrzeli. Mahovi, nabrani v gozdnih vrzelih, odražajo atmosferske usedline N; nismo pa odkrili značilne povezave med sestojnimi usedlinami N in vsebnostjo N v mahovih, ki so bili nabrani pod drevesnimi krošnjami. Vrednost δ^{15}N v mahovih je bila značilno odvisna od razmerja amonija in nitrata v atmosferskih usedlinah na odprtem, vendar samo v primeru, da smo izključili lokacije, ki so imele manj kot 1000 mm povprečnih letnih padavin. Rezultati kažejo, da je obremenjenost z N-spojinami pod drevesnimi krošnjami v gozdu večja kot v gozdnih vrzelih. Vsebnosti N v mahovih, nabranih najmanj tri metre stran od najbližje projekcije krošnje dreves, so bile v povprečju za 41 % manjše kot pod drevesnimi krošnjami. Rezultati kažejo, da mahovi, nabrani v gozdnih vrzelih, odražajo značilnosti okoliške rabe tal in posledično glavne vire emisij N. Za mah, nabran pod drevesnimi krošnjami, so značilnosti gozda na lokaciji bolj pomembne od glavnih virov emisij N zunaj gozda. Izdelali smo regresijske modele za napovedovanja vsebnosti N v mahovih v gozdnih vrzelih v odvisnosti od vsebnosti N v mahovih pod drevesnimi krošnjami in drugimi okoljskimi spremenljivkami. Prostorska korelacija je obstajala le pri N v mahovih, ki so bili nabrani v gozdnih vrzelih. V tem primeru se je za prostorsko interpolacijo podatkov uporabil osnovni kriging. Prostorska korelacija ni bila odkrita za N v mahovih, ki so bili nabrani pod krošnjami, niti za vrednosti δ^{15}N pri mahovih, nabranih na obeh vzorčevalnih mestih (pod krošnjami/v vrzeli). V tem primeru je bila prostorska interpolacija podatkov narejena kot vsota regresijske napovedi in utežne inverzne razdalje ostankov regresijskega modela. Karte N za obe mesti nabiranja mahu (pod krošnjami/v vrzeli) so pokazale podobna območja s povečanimi vsebnostmi N. Edina izjema je bila, da je bilo mogoče z mahovi, nabranimi pod drevesnimi krošnjami, identificirati tudi nekatere lokalne onesnaževalce z NO_X. Mejna statistično značilna povezava je obstajala med vsebnostjo N v mahovih in foliarnim N v listavcih in poraslostjo s skorjastimi lišaji. Pri bolj negativnih vrednostih δ^{15}N v mahovih je bila osutost dreves manjša.

KEY WORDS DOCUMENTATION (KWD)

DN Dd
DC FDC 173.2+114.521.7(043.3)=163.6
CX Biomonitoring/bioindication/moss/*Hypnum cupressiforme*/nitrogen/nitrogen isotope/canopy influence/spatial statistic/atmospheric deposition/lichens/defoliation/dependence/environmental modelling
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AB Samples of moss Hypnum cupressiforme Hedw. were collected at 103 locations in the forests of Slovenia and analysed for nitrogen (N) and isotopic composition of nitrogen (δ^{15}N value). At each location, mosses were collected at two sites: under the tree canopy and in a nearby forest clearing. Mosses collected within forest clearing reflect the atmospheric deposition of N; but we have not found a significant relationship between throughfall deposition of N and N concentration in mosses that were collected under canopies. δ^{15}N value in moss depended significantly on the ratio of ammonium and nitrate in precipitation in the open, but only under condition that we excluded the locations which had less than 1000 mm of average annual precipitation. The results show that N concentrations under the canopy are larger than in the forest clearings. N concentrations in mosses collected at least three meters away from the nearest tree canopy projections were on average 41% lower than under the canopy. The results show that moss collected in forest clearings reflects the characteristics of the surrounding land use and, consequently, the main sources of N emissions; while for moss collected under the canopy forest, characteristics at the collecting location are more important than the main emission sources of N. Regression models were established for predicting N concentration in mosses in forest clearings depending on N concentration in mosses collected under a canopy and other environmental variables. The spatial correlation existed only for N in mosses, which have been collected in forest clearings. In this case, the ordinary kriging was used for spatial interpolation of the data. Spatial correlation was not found for N in mosses that were collected under the canopy, nor δ^{15}N value in mosses collected at both sampling sites (under the canopy / in the open). In this case, the spatial interpolation of data was done as the sum of the regression prediction and inverse distance weighted interpolation of regression residuals. Both maps (N concentration in moss collected under the forest canopy and in the clearings) show similar areas with elevated N concentrations. The only exception was that with mosses collected under a canopy of trees, where also some local emitters of NO_x were exposed. Limited statistically significant correlation existed between the N concentration in mosses and foliar N and crustose lichens cover. With more negative δ^{15}N values in mosses the tree defoliation was lower.

KAZALO VSEBINE

KAZALO ZNANSTVENIH DEL

KAZALO SLIK

KAZALO PRILOG

OKRAJŠAVE IN SIMBOLI

N – dušik

N_2 – elementarni dušik

N_{total} – celokupni dušik

mg/g – miligrami elementa na gram suhe snovi

$\delta^{15}N$ – izotopska sestava dušika

N_{open} – vsebnost dušika v mahovih, nabranih v gozdnih vrzelih (zunaj območja horizontalne projekcije krošnje)

N_{canopy} – vsebnost dušika v mahovih, nabranih pod drevesnimi krošnjami (znotraj območja horizontalne projekcije krošnje)

$\delta^{15}N_{open}$ – izotopska sestava dušika v mahovih, nabranih v gozdnih vrzelih (zunaj območja horizontalne projekcije krošnje)

$\delta^{15}N_{canopy}$ – izotopska sestava dušika v mahovih, nabranih pod drevesnimi krošnjami (znotraj območja horizontalne projekcije krošnje)

N_r – reaktivne dušikove spojine

NO_x – dušikovi oksidi

NH_y – dušikovi vodiki

N_2O – didušikov oksid

NO_3^- – nitrat

NO_2 – nitrit

NH_3 – amonijak

NH_4^+ – amonijev ion

NH_2NH_2 – hidrazin

HNNH – diazen

1 PREDSTAVITEV PROBLEMATIKE IN HIPOTEZE

1.1 UVOD

Okolje je podvrženo vse hitrejšim spremembam in se sooča z vse večjim številom znanih in neznanih onesnažil, ki vzajemno s klimatskimi spremembami in manjšanjem biotske pestrosti ogrožajo naravne ekosisteme (Markert in sod., 2003b). Ti kompleksni sistemi interakcij in medsebojnih odnosov otežujejo pridobivanje informacij o stanju in trendu onesnaženosti okolja, zato je nujna vzpostavitev sistema monitoringa, ki omogoča trajno spremljanje stanja ekosistemov (Pavšič-Mikuž, 2005). Samo dolgoročen monitoring okolja nam omogoča pravočasno ukrepanje, tako da koncentracije onesnažil ne dosežejo ali presežejo kritične vrednosti ali zaradi nenehne prisotnosti ne vplivajo negativno na katerega od procesov v ekosistemu.

Po letu 1980 so zakonske zaostritve na področju varstva okolja dosegle zmanjšanje onesnaženosti zraka z žveplovimi spojinami in prašnimi delci v številnih evropskih državah, kar pa ne velja za različne dušikove (N) spojine (Adams, 2003), na količino katerih vplivamo predvsem z vse večjo porabo fosilnih goriv ter vnosom gnojil v ekosisteme (Vitousek in sod., 1997). Povečane koncentracije N-spojin so tako v zadnjem času vse pogosteje označene kot kritična obremenitev okolja na globalni ravni (Adams, 2003; Hauck, 2010; Krupa, 2003; Vitousek in sod., 1997), saj pomembno vplivajo in spreminjajo številne procese v okolju (Bobbink in sod., 2003; de Vries in sod., 2009; Fangmeier in sod., 1994; Krupa, 2003; Stevens in sod., 2010; Vitousek in sod., 1997).

Dušik se v nižjih plasteh atmosfere pojavlja v različnih oblikah in se na zemeljsko površje odlaga v obliki suhih ali mokrih usedlin (Solga in sod., 2005). Različne oblike pojavljanja, različni načini odlaganja ter velika heterogenost vnosa N v ekosisteme so razlogi, ki otežujejo podajanje natančnih ocen količin atmosferskih usedlin N. Posledično so informacije o količinah skupnega N omejene na terenske meritve za izbrane lokacije (Solga in sod., 2005). Z namenom ugotavljanja stanja atmosferskega useda N-spojin so bili vzpostavljeni različni sistemi spremljanja stanja onesnažil, nekateri temeljijo na fizikalnih in kemijskih meritvah atmosferskih usedlin ter kakovosti zraka (Erisman in sod., 2005), drugi pa uporabljajo različne biološke kazalnike (npr. mahovi, lišaji itd.) za posredno oceno odlaganja onesnažil (Markert in sod., 2003b). Slednje, tako imenovane metode biomonitoringa, so običajno metodološko enostavnejše in cenejše od meritev mokrih ali suhih atmosferskih usedlin; posledično je mogoče z metodo biomonitoringa doseči bistveno večjo gostoto vzorčenja (Harmens in sod., 2011). Gostota vzorčenja pa je zelo pomembna pri elementih, kot je N, saj so lahko nekatere N-spojine, še zlasti NH_3, prostorsko in časovno zelo spremenljive (Asman in sod., 1998).

1.2 DUŠIKOVE SPOJINE V NARAVNEM OKOLJU

Dušikove spojine so glavna sestavina proteinov, nukleinskih kislin in drugih sestavin celic ter s tem ključni sestavni del vseh živih organizmov na Zemlji. Največje zaloge elementarnega N_2 so v ozračju (79 % atmosfere), a kljub velikim količinam je N v plinastem stanju (N_2) praktično neuporaben za večino živih organizmov, razen za nekatere bakterije in cianobakterije. Razlog je predvsem zelo močna trivalenta vez, ki veže oba atoma N, za cepitev te vezi pa je potrebno izredno veliko energije (160 kcal za vsak mol N).

V naravi N neprestano kroži med atmosfero, tlemi, rastlinami in živalmi. Postopek pretvorbe oz. kroženja med nereaktivnim N in reaktivnimi oblikami N (N_r) se imenuje »dušikov cikel« in ga delimo na štiri procese (Sl. 2) (Smith in Smith, 2001):

1. fiksacija in asimilacija – pretvorba N iz plinastega stanja v NO_3^- ali NH_3;
2. mineralizacija ali amonifikacija – pretvorba aminokislin v organski snovi v NH_3;
3. nitrifikacija – oksidacija NH_4^+ v NO_2 in nato v NO_3^-;
4. denitrifikacija – pretvorba NO_3^- v atmosferski N (N_2).

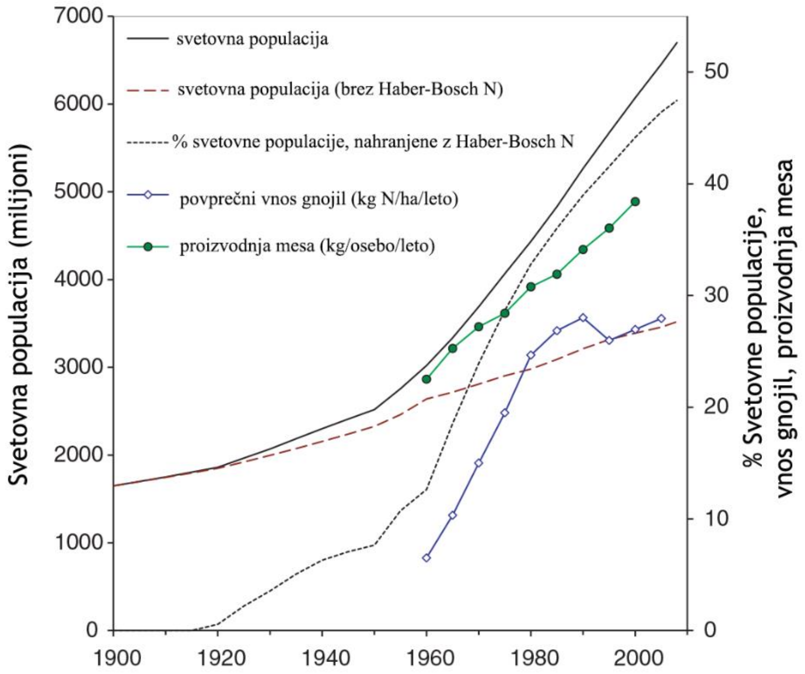
N_r (reaktivni dušik) je izraz, ki opisuje številne oblike N, ki zelo burno reagirajo v atmosferi. Glede na vir lahko N_r delimo na oksidirane N-spojine (npr. dušikovi oksidi – NO_x, didušikov oksid – N_2O, nitrat – NO_3^- in nitrit – NO_2^-), reducirane N-spojine (npr. amonijak – NH_3, amonijev ion – NH_4^+, hidrazin – NH_2NH_2, diazen – HNNH) in na organske N-spojine (npr. proteini, aminokisline), ki pa imajo različne stopnje oksidacije.

Živim organizmom predstavlja glavni vir N-spojin mineralizacija organskega N. V tem procesu glive in bakterije razgradijo proteine odmrlih rastlin ali živalskega materiala v aminokisline. Slednje pa nato razpadejo v ogljikov dioksid, žveplovodik, vodo in amonijak, ki je potem neposredno dostopen živim rastlinam (vgradnja v aminokisline) (Bothe in sod., 2007). Pri tem procesu mineralizacije ne nastajajo nove spojine N, ampak gre za kroženje N med živimi organizmi in odmrlo organsko snovjo. V naravi lahko poteka pretvorba N iz plinastega stanja v NO_3^- ali NH_3 samo prek visokoenergijske in/ali biološke fiksacije N (Burns in Hardy, 1975; Ridley in sod., 1996). Od tega slednja proizvede kar 90 % vsega fiksiranega N na Zemlji in po ocenah za naravne ekosisteme to znaša od 1,4 do 7,0 kg N/ha/leto (Smith in Smith, 2001).

1.3 ONESNAŽENOST Z DUŠIKOVIMI SPOJINAMI

Dušik je torej zaradi svojih lastnosti ključno mineralno hranilo za rast rastlin. Kljub veliki aktivnosti N-cikla se za predelavo hrane za vse hitreje rastočo populacijo ljudi po naravni poti proizvedejo premajhne količine N (Sl. 1). Z iznajdbo Haber-Bosch procesa v začetku dvajsetega stoletja je postalo mogoče iz neomejenih zalog atmosferskega N umetno sintetizirati NH_3. Posledično se je v zadnjem stoletju močno povečala uporaba NH_3 v kmetijstvu, kar je omogočilo povečanje proizvodnje hrane in s tem povečanje svetovnega prebivalstva. Tako se je število ljudi, ki se lahko prehranijo z živili, zraslimi na enem hektarju obdelovalne površine, povečalo z 1,9 v letu 1908 na 4,3 v letu 2008 (Sl. 1) (Erisman in sod., 2008). Kmetijstvo je skupaj z vse večjimi potrebami po izkoriščanju fosilnih goriv glavni vzrok za pojav nekaterih novih okoljskih problemov na lokalni in tudi globalni ravni (Smith in Smith, 2001).

Ljudje s svojimi dejavnostmi različno vplivamo na N-cikel in posledica je zmanjšanje ali povečanje v naravi razpoložljivega N. Spremembe rabe tal iz gozda ali travišč v polja povzročijo izgubo N v tleh. Predvsem mešanje teksture tal (npr. oranje) povzroča, da se organska snov hitreje razgrajuje in s tem se N izpira v nižje plasti tal. Pobiranje pridelka in paša povzročijo dodatne iznose N. Na drugi strani pa prihaja zaradi intenzivnega gnojenja (umetnega in živalskega), odlaganja človeških odpadkov (predvsem kanalizacije) in izpušnih plinov (proces zgorevanja) do vse večjih vnosov N v okolje (Smith in Smith, 2001).



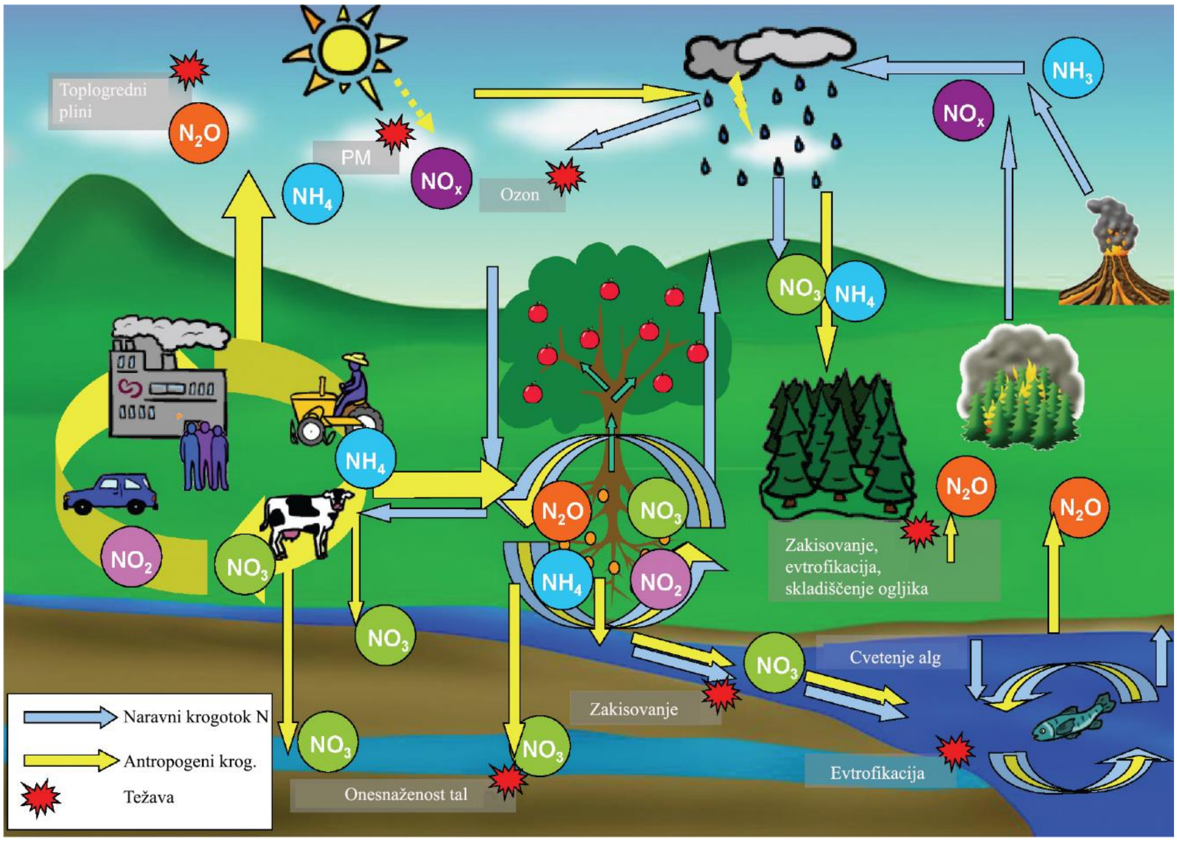
Sl. 1: Trend človeške populacije in uporaba N skozi dvajseto stoletje (povzeto po: Erisman in sod., 2008)

Fig. 1: Trends in human population and N use throughout the twentieth century (after: Erisman et al., 2008)

Človek spreminja naravno pot N-cikla na tri načine (Sl. 2) (Sutton in sod., 2011):

1. sežig fosilnih goriv za pridobivanje energije – izpusti NO_x kot posledica oksidacije N_2 ali fosilnega organskega N v gorivu;
2. proizvodnja gnojil in različnih kemikalij – postopek poteka predvsem preko Haber-Bosch procesa, ki ustvari NH_3 z reakcijo N_2 in H_2;

3. s sajenjem rastlin, ki so specializirane za fiksacijo dušika (npr. stročnic) – spreminjanje N_2 v NH_3, vgrajenega v organsko snov.



Sl. 2: Krogotok reaktivnega N in glavnih fluksov N (povzeto po: Sutton in sod., 2011)

Fig. 2: The reactive N cycle and the main fl uxes (after: Sutton M. A. et al., 2011)

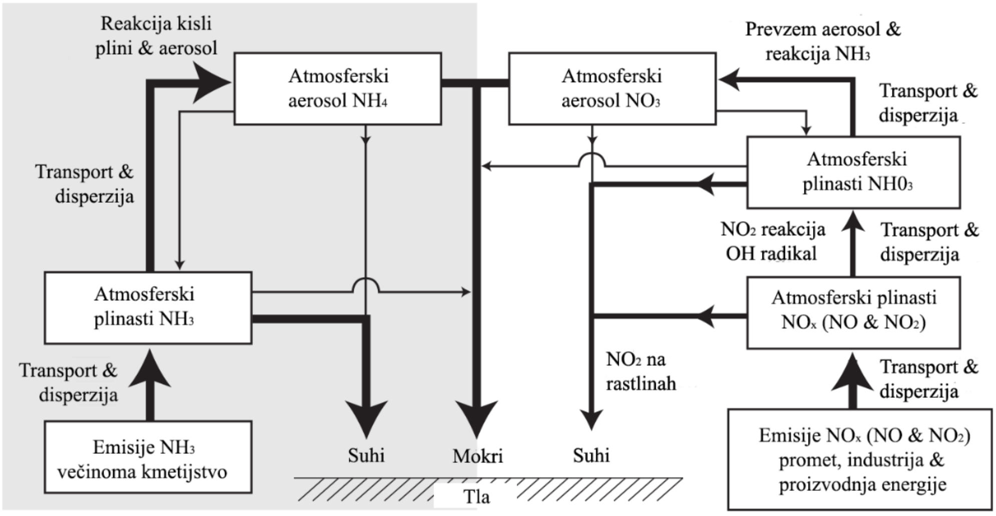
Po ocenah Erismana in sod. (Erisman in sod., 2005) je bilo v letu 2000 od celotnega reaktivnega N v EU kar 74 % antropogenega izvora (41 % Haber-Bosch gnojila N in industrija, 7 % biološka fiksacija N v kmetijstvu, 14 % vnos z živalskim gnojem, 11 % sežig v industriji in transportu), samo 28 % od skupno dostopnega N pa je bilo posledica naravne biološke fiksacije.

Velik del tega reaktivnega N konča v različnih vrstah ekosistemov, kar pogosto privede do njihove evtrofikacije in zakisanja. Koncept se imenuje kritična obremenitev (»*critical level*« – CL) in je bila uvedena z namenom prepoznavanja tveganj vnosa velikih količin reaktivnega

N v okolje. Izraz CL je bil prvič opredeljen leta 1988 kot največji še dovoljeni vnos kislih usedlin v ekosistem, ki še ne povzroči dolgoročnih škod na strukturo in funkcije ekosistema (Nilsson in Grennfelt, 1988). V zadnjem desetletju se koncept CL pogosto uporablja pri razvoju politik za nadzor nad emisijami v Evropi (Skeffington, 1999).

Emisije N v zrak so sestavljene iz oksidiranih (NO_x) in reduciranih oblik (NH_y). Tako so izpusti reaktivnega N v zrak večinoma v treh oblikah: NH_3, NO_x in N_2O, z manjšimi količinami je med izpusti prisoten tudi organski N, kot je npr. amin (derivat amonijaka) (Sl. 3). Kemične reakcije obeh oblik v atmosferi so različne, s tem pa se razlikuje tudi njihov transport in način useda. NO_x so lahko transportirani na večje razdalje in zaradi tega predstavljajo spojine NO_x večji problem na globalni ravni, medtem ko so lahko NH_y (predvsem NH_3) bolj problematični na lokalni ravni, v bližini vira izpustov N. Z razliko od NH_3 se NH_4^+ v atmosferi lahko poveže z aerosoli, kar mu zelo podaljša obstojnost v atmosferi in posledično se lahko, podobno kot NO_x, transportira na razdalje, večje od 1000 km (Hertel in sod., 2011). Iz atmosfere se N odlaga na zemeljsko površje v obliki mokrih in suhih usedlin (Sl. 3) (Fowler in sod., 2009). Plinaste oblike N se odlagajo kot suhe usedline, višje v atmosferi se N spoji z aerosoli in se kasneje odlaga na zemljo v obliki mokrih usedlin; tj. različnih oblik padavin, kot so dež, sneg, rosa itd. (Sl. 3).

Glede na Sutton in sod. (2011) lahko posledice povečanih vnosov N v okolje razdelimo v šest skupin: (i) izpiranje N v tla in podtalnico, (ii) odvajanje odpadnih vod v površinske vode, (iii) evtrofikacija in zakisanje kopenskih ekosistemov, (iv) evtrofikacija morskih ekosistemov, (v) globalno segrevanje (emisije N_2O in druge posledice N) ter (vi) vpliv N na zdravje ljudi.



Sl. 3: Prikaz različnih poti reaktivnega N v atmosferi. Leva stran slike prikazuje pot reduciranih oblik N (NH_y) (v plinastem stanju je amonijak (NH_3), v aerosolnem stanju amonijev ion (NH_4^+)). Desna stran slike prikazuje pot oksidiranih oblik N (NO_x in njegovi produkti). NH_y je večinoma izpust kmetijske panoge, medtem ko NO_x nastaja pri prometu, industriji in proizvodnji električne energije (povzeto po: Hertel in sod., 2011)

Fig. 3: Illustration of N_r path in the atmosphere. In left side of the picture the path of reduced N compounds is presented (in gas phase is ammonia (NH_3), in aerosol phase ammonium (NH_4^+)). In right side the path of oxidised N is presented (NO_x and his products). NH_y is mainly produced by the agriculture, while the NO_x is produced by transport, industry and power production (after: Hertel O. et al.,, 2011)

1.4 OKOLJSKI MONITORING

1.4.1 Konvencije in zakonodaja

Na vzpostavitev sistemov spremljanja onesnaženosti okolja je pomembno vplivala Konvencija o onesnaževanju zraka na velike razdalje preko meja (»*Convention on Long-range Transboundary Air Pollution*« – CLRTAP), ki jo je leta 1979 v Ženevi podpisalo 34 vlad in Evropska komisija (UNECE, 1979). Slovenija je Konvencijo CLRTAP ratificirala leta 1992 in se s tem zavezala, da bo spremljala, poročala in zmanjšala raven onesnaženosti zunanjega zraka. V okviru Konvencije je bil izpogajan tudi tako imenovani Gothenburški protokol o zmanjšanju posledic zakisanja, evtrofikacije in prizemnega ozona (UNECE, 1999), ki je določil zgornje meje emisij za žveplov dioksid (SO_2), dušikov oksid (NO_x), hlapne organske spojine (VOCs) in amonij (NH_3). Z namenom spremljanja atmosferskih usedlin onesnažil je UNECE leta 1983 znotraj Konvencije ustanovila kooperativni program za monitoring in oceno daljinskega transporta onesnaženega zraka za Evropo s kratico EMEP (»*Cooperative Programme for Monitoring and Evaluation of the Long-range Transmission of Air Pollutants in Europe*«), leta 1980 pa še delovno skupino za proučevanje vplivov onesnaženosti na recipiente (»*Working Group on Effects*« – WGE), ki jo sestavlja šest mednarodno usklajenih programov sledenja in proučevanja učinkov onesnaženega zraka na recipiente (»*International Cooperative Programmes*« (ICP)) – ICP Forest, ICP Waters, ICP Materials, ICP Vegetation, ICP Intergrating Monitoring, ICP Modeling and Mapping. Med njimi so za spremljanje učinkov onesnažil N najpomembnejši ICP Gozd (»*ICP Forest*«), ICP Vegetacija (»*ICP Vegetation*«) ter ICP Modeliranje in Kartiranje (»*ICP Modeling and Mapping*«).

Za države članice EU velja enotna zakonodaja, ki ureja področje spremljanja onesnaženosti okolja in kakovosti zunanjega zraka. Področje spremljanja onesnaženosti okolja opredeljuje tako imenovana NEC Direktiva 2001/81/EC (Ur. l. EU L309/22), ki določa zgornje meje emisij za določena onesnažila in predpisuje, da morajo države članice poročati o emisijah Evropski okoljski agenciji (EEA) in Evropski komisiji (EC). Dokument, ki določa standarde o spremljanju kakovosti zunanjega zraka, je Direktiva 2008/50/ES Evropskega parlamenta in sveta z dne 21. maja 2008 (Ur. l. EU L152/1). Slednja je v skupni dokument združila večino do takrat veljavnih direktiv in zakonov: Okvirna direktiva 96/62/EC (Ur. l. EU L296/55), 1-4 hčerinske direktive 1999/30/EC (Ur. l. EU L163/41), 2000/69/EC (Ur. l. EU L313/12), 2002/3/EC (Ur. l. EU L67/14), 2004/107/ES (Ur. l. EU L23/3) in odločitev o izmenjavi informacij 97/101/EC (Ur. l. EU L35/14). Z vidika onesnaženosti voda s spojinami N je pomemben dokument Nitratna Direktiva 91/676/EGS (Ur. l. ES (L375/1)), katere namen je zmanjšati onesnaževanje voda, ki ga povzročajo nitrati iz kmetijskih virov in določiti območja, ki so občutljiva na povečane vnose N.

Glede na načelo subsidiarnosti je vsa EU-zakonodaja implementirana tudi v zakonodajo posamezne države članice. Osnova slovenske zakonodaje na področju spremljanja stanja okolja je Zakon o varstvu okolja (v nadaljevanju ZVO) (Ur. l. RS št. 41/2004.) z dopolnitvami (Ur. l. RS št. 20/2006, 70/2008). Različne uredbe, pravilniki in sklepi pa podrobneje predpisujejo, katera onesnažila v okolju je potrebno spremljati, kakšne so njihove mejne, ciljne, opozorilne ter alarmne vrednosti itd. Uredbe predpisujejo tudi metode spremljanja stanja zunanjega zraka in način letnega poročanja.

Za Slovenijo je v veljavi sledeča zakonodaja s področja kakovosti zunanjega zraka in onesnaženosti okolja, povezana z N-emisijami:

- Uredba o kakovosti zunanjega zraka (Ur. l. RS, št. 9/2011),
- Uredba o ukrepih za ohranjanje in izboljšanje kakovosti zunanjega zraka (Ur. l. RS, št. 52/2002),
- Uredba o žveplovem dioksidu, dušikovih oksidih, delcih in svincu v zunanjem zraku (Ur. l. RS, št. 52/2002),
- Uredba o emisiji snovi v zrak iz nepremičnih virov onesnaževanja (Ur. l. RS, št. 73/1994, 31/2007),
- Pravilnik o ocenjevanju kakovosti zunanjega zraka (Ur. l. RS, št. 55/2011),
- Sklep o določitvi območij in stopnji onesnaženosti zaradi žveplovega dioksida, dušikovih oksidov, delcev, svinca, benzena, ogljikovega monoksida in ozona v zunanjem zraku (Ur. l. RS, št. 72/2003).

Zakonodaja pa ne določa samo spremljanja zunanjega zraka, ampak tudi spremljanje posledic onesnaženosti na okolje. Spremljanje posledic onesnaženosti na gozdove določa Pravilnik o varstvu gozdov z dopolnitvami (Ur. l. RS št. 92/2000, 56/2006, 114/2009).

Za ugotavljanje posledic so bile tako razvite različne analizne metode, ki so običajno dražje in zaradi tega omejene na posamezno lokacijo, ter posredne metode monitoringa, ki so cenejše in jih je posledično mogoče izvajati na več lokacijah, zaradi česar je lahko rezultat boljša prostorska informacija o razporeditvi onesnažil.

1.4.2 Fizikalno-kemijske metode za oceno kakovosti zraka

Glede na Pravilnik o ocenjevanju kakovosti zunanjega zraka (Ur. l. RS, št. 55/2011) izvaja spremljanje kakovosti zunanjega zraka v Sloveniji Agencija republike Slovenije za okolje (ARSO). ARSO upravlja meritve na t. i. »državni merilni mreži za spremljanje kakovosti zunanjega zraka« (DMKZ) že od leta 1968. V letu 2013 je bilo v DMKZ vključenih 19 merilnih mest. To mrežo merilnih mest dopolnjujejo še nekatere meritve ob večjih industrijskih in energetskih objektih, kot sta npr. Termoelektrarna Šoštanj in Termoelektrarna Trbovlje. Na teh lokacijah se v zraku spremlja žveplov dioksid, prašni delci, ozon itd. Od N-spojin meritve beležijo skupno količino vseh NO_x in NO_2^-. Podatki meritev so dostopni na spletni strani ARSO in v letnih poročilih (Cegnar in sod., 2014).

ARSO izvaja tudi meritve kakovosti padavin, in sicer na petih merilnih mestih DMKZ. Od teh so štiri v naravnem okolju (Iskrba, Rakičan, Rateče in Škocjan) in eno v mestnem (Ljubljana). Na teh lokacijah se spremlja količina padavin, pH padavin, električna prevodnost, osnovni kationi in anioni; na merilnem mestu Iskrba pa dodatno še težke kovine in policiklični aromatski ogljikovodiki (PAH-i) (Cegnar in sod., 2014).

Merilno mesto Iskrba je, edino v Sloveniji, vključeno tudi v program EMEP, ki je bil ustanovljen pod okriljem Konvencije CLRTAP (glej poglavje 1.4.1) z namenom meddržavnega spremljanja onesnaženosti zunanjega zraka. Pod okriljem Konvencije so bile ustanovljene tudi delovne skupine, ki spremljajo vplive atmosferskih onesnažil na različne ekosisteme. Ena izmed teh delovnih skupin je tudi ICP Gozd, ki spremlja vplive atmosferskih onesnažil na procese v gozdnih ekosistemih (de Vries in sod., 2003). Glede na Pravilnik o varstvu gozdov (Ur. l. RS št. 92/2000, 56/2006, 114/2009) je za upravljanje teh meritev pristojen Gozdarski inštitut Slovenije (GIS), ki je v letu 2010 izvajal meritve kakovosti padavin na sedmih lokacijah, v letu 2014 pa zaradi krčenja sredstev samo še na štirih (Brdo, Borovec, Tratice in Rožnik) (Simončič in sod., 2015). Mreža opazovanj se imenuje »intenzivni monitoring gozdnih ekosistemov« (ploskve IMGE). Na vsakem merilnem mestu se kakovost padavin oz. usedlin spremlja na dveh mestih, in sicer na odprtem (»*bulk deposition*«) in znotraj sestoja (»*throughfall deposition*«) (Sl. 4). Vse meritve potekajo v skladu z ICP Gozd protokoli (Clarke in sod., 2010). Rezultati meritev pa so predstavljeni v letnih poročilih o stanju gozdov, ki so dostopni tudi na spletni strani GIS-a (Simončič in sod., 2015).

Sl. 4: Prikaz vzorčevalnikov za usedline na ploskvah IMGE znotraj sestoja (leva slika) in na odprtem (desna slika)

Fig. 4: Deposition samplers on IMGE plots for throughfall deposition (left picture) and bulk deposition (right picture)

1.4.3 Metode biomonitoringa in bioindikacije

Pomemben korak v spremljanju stanja onesnaženosti naravnega okolja je bil razvoj različnih oblik pasivnega monitoringa (Fränzle, 2006; Solga in sod., 2005). Markert in sod. (2003b) ločijo med bioindikatorji in biomonitorji. Bioindikator je organizem (del organizma ali skupina organizmov), ki vsebuje informacijo o kakovosti okolja ali delu okolja. Biomonitor pa je organizem (lahko tudi del organizma ali skupina organizmov), ki vsebuje kvantitativno informacijo o kakovosti okolja oz. o delu okolja. Vsak biomonitor je tudi bioindikator, vendar pa vsak bioindikator ne izpolnjuje vedno pogojev biomonitorja.

Uporaba različnih metod biomonitoringa in bioindikacije je predstavljena v knjigah Arndt in sod. (1987) ter Markert in sod. (2003b) in v prispevku Fränzle (2006). Vsem metodam je skupno, da se onesnaženost okolja ocenjuje oz. meri posredno prek izbranih organizmov, ki so lahko rastline, glive, živali ali ljudje. Takšen pristop je pogosto metodološko enostavnejši in cenejši ter omogoča pridobivanje podatkov na večjemu številu vzorčevalnih lokacij.

V Sloveniji so onesnaženost zraka s posrednimi oblikami spremljali že leta 1979, ko so Batič in sod. (1979) za območje mesta Ljubljane ocenili onesnaženost zraka s pomočjo epifitske lišajske flore. Kasneje so bili v Sloveniji lišaji kot indikatorji onesnaženosti zraka pogosto uporabljeni, tako na lokalni (Batič in Martinčič, 1982; Jeran, 1995; Jeran in sod., 1995; Poličnik in sod., 2005; Poličnik in sod., 2008) kot na nacionalni ravni (Batič in Kralj, 1995; Batič in Kralj, 1989; Batič in sod., 2003; Batič in Mayrhofer, 1996; Jeran in sod., 2002; Jeran in sod., 1996a; Jeran in sod., 1996b; Jeran in sod., 2007a).

V okviru programa ICP Gozd so se poleg fizikalno-kemijskih metod, predstavljenih v poglavju 1.4.2, za oceno stanja gozdov oz. odziv gozdov na onesnaženost uporabljale tudi posredne oblike spremljanja stanja onesnaženosti. Program se je v Sloveniji začel izvajati leta 1987 (Batič in sod., 1999), ko so vpliv onesnaženega zraka na stanje gozdov začeli ocenjevati na podlagi indikatorja osutosti. Osutost predstavlja na oko ocenjen delež manjkajočih asimilacijskih organov v primerjavi z normalno vitalnim drevesom istega socialnega položaja, iste drevesne vrste in z enakega rastišča (Eichhorn in sod., 2010). Da bi bila vzorčna drevesa enakomerno razporejena po celotni Sloveniji, so bila v popis vključena drevesa, rastoča na ploskvah na sistematični mreži 16 km ×16 km oz. občasno na mreži 4 km × 4 km. Kasneje se je, z namenom celovitega spremljanja stanja slovenskih gozdov na teh lokacijah, spremljalo še številne druge indikatorje (gozdna tla, različni dendrometrijski parametri, funkcije gozda itd.), sistematično mrežo ploskev pa se je poimenovalo »*Monitoring gozdov in gozdnih ekosistemov*« (ploskve MGGE) (Kovač in sod., 2014a). Metodologija popisa s številnimi referenčnimi fotografijami osutosti dreves je podrobneje predstavljena v terenskih navodilih »*Monitoring gozdov in gozdnih ekosistemov*« (Kovač in sod., 2014b). Leta 2007 se je na ploskvah MGGE popisovala tudi lišajska obrast. Na vsaki ploskvi se je znotraj vzorčne mrežice, ki je bila pritrjena na deblo drevesa, ocenilo kolikšen delež površine je poraščen s skorjastimi, listnatimi in grmičastimi oblikami lišajev. Popis je bolj podrobno predstavljen v prispevku Batič in sod. (2011).

Tako se še danes v Sloveniji na ploskvah IMGE in MGGE stanje gozdov ocenjuje posredno preko indikatorjev osutosti, pritalne vegetacije, sprememb kemijskih lastnosti tal in asimilacijskih organov dreves (foliarne analize), fenologije itd. (Božič in sod., 2015). Vsi na terenu pridobljeni podatki so predstavljeni v letnih poročilih o stanju gozdov in hkrati se podatki posredujejo v skupno bazo podatkov o stanju gozdov ICP Forest, ki jo trenutno vzdržuje inštitut Johann Heinrich von Thünen v Eberswaldu (Nemčija). Za Slovenijo so v poglobljenih študijah Kovač (1996) ter Hočevar in sod. (2002) predstavili indikator osutosti. Za leto 2000 so poročali, da se je zdravstveno stanje od zadnjega popisa izboljšalo in da je osutost dreves v Sloveniji primerljiva z evropskim povprečjem. Kutnar (2006) ter Kutnar in Martinčič (2008) so z uporabo različnih multivariatnih tehnik pokazali, da je lahko pritalna vegetacija dober indikator rastiščnih in sestojnih razmer. Rezultate analiz asimilacijskih organov dreves (listov/iglic) na ploskvah ICP Forest je predstavil Simončič (Simončič, 1997), ki je na mreži 16 × 16 km v letu 1995 ugotovil razmeroma slabe razmere glede vsebnosti N v listih in iglicah dreves. Foliarne analize so bile kasneje uporabljene še za ugotavljanje onesnaženosti zraka v okolici Termoelektrarne Šoštanj (Al Sayegh-Petkovšek, 2013; Al Sayegh-Petkovšek in sod., 2008; Batič in sod., 1995; Ribarič-Lasnik in sod., 1996; Simončič in sod., 2003).

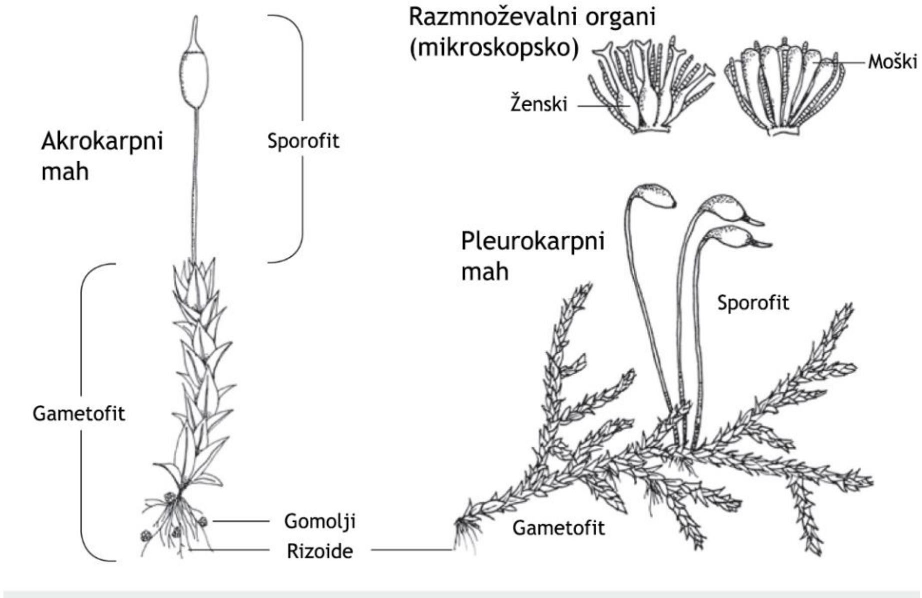
1.5 MAHOVI KOT BIOMONITORJI STANJA OKOLJA

1.5.1 Ekologija mahov

Mahove taksonomsko delimo v tri skupine: listnate mahove (Bryophyta), jetrenjake (Marchantiophyta) in rogačarje (Anthocerotophyta) (Atherton in sod., 2010). Taksonomijo listnatih mahov v Sloveniji je predstavil Martinčič (2003). Mahovi so rastlinska skupina, ki je ne moremo šteti niti med steljčnice (Thallophyta) niti med brstnice (Cormophyta). Njihovo telo je le navidezno diferencirano v stebelce in lističe, ki pa po notranji zgradbi ne ustrezajo listom in steblu pravih brstnic, sploh pa nimajo razvitih korenin. Namesto teh imajo razvite tanke, nitaste, koreninicam le malo podobne rizoide. Te so enostavno grajene in služijo predvsem za pritrjevanje na podlago. Najpomembnejšo funkcijo korenin – črpanje vode in v njej raztopljene mineralne snovi – opravljajo mahovi najpogosteje z vso površino (Boedijn, 1978). Ker torej nimajo koreninskega sistema in imajo nerazvito kutikulo, spadajo med tako imenovane ektohidrične vrste (Glime, 2006).

V razvoju mahov se redno izmenjujeta dve generaciji; spolna (gametofit) in nespolna (sporofit). Sporofit se pri mahovih razvije na gametofitu in ostane z njim vedno povezan (Sl. 5). Življenjski krog vsakega mahu se prične z enoceličnim trosom. Ob kalitvi tros sprejema vodo in nabreka dokler ne poči ter skali v nitasto, razvejeno rastlinico steljkaste zgradbe. To je predkal ali protonema, ki raste z delitvijo temenskih celic in se širi po podlagi. Predkal zaključi svoj razvoj s tvorbo klice, ki zraste v gametofit. Gametofit nosi gametangije (arhegonij – ženski in anteridij – moški spolni organ), ki so pri nekaterih vrstah na isti rastlini, pogosteje pa so ločeni vsak na svoji rastlini. Ko monoploiden sprematozoid, ki nastane v anteridiju, doseže arhegonij, se združi z monoploidno jajčno celico. Ob združitvi nastane diploidna celica, ki se začne deliti in nastane embrio. Iz njega se razvije nov, mnogoceličen organizem, trosovnik ali sporogon. Sporogon predstavlja nespolno generacijo ali sporofit. Iz dela sporofita, ki je orientiran navzgor, se razvije organ, v katerem nastajajo trosi. Ko dozori, se diferencira v nitasti del, seto, in v okrogli ali valjasti del, puščico. Sporofit zaključi svoj razvoj s tvorbo trosov, ki nastanejo v notranjosti puščice. Trosi so monoploidni in ko dozorijo, jih veter iztrese iz puščice (Boedijn, 1978).

Glede na razrast gametofita, mahove ločimo na akrokarpne in pleurokarpne. Slednji se od akrokarpnih razlikujejo po tem, da je njihov gametofit plazeč in se razrašča vertikalno (Sl. 5).



Sl. 5: Prikaz glavnih organov mahov ter delitve glede na obliko rasti na pleurokarpe in akrokarpe (povzeto po: Atherton in sod., 2010)

Fig. 5: Presentation of main moss organs and division on two main groups pleurocarps and acrocarps (after: Atherton et al., 2010)

1.5.2 Uporabnost mahov kot biomonitorjev onesnaženosti z dušikovimi spojinami

1.5.2.1 Mahovi kot biomonitorji in primeri uporabe

Večino mahov je torej ektohidričnih, zaradi česar sprejemajo večino mineralnih snovi in vode preko vse površine, neposredno iz tal pa le v majhnem obsegu (Zechmeister in sod., 2003). Zaradi omenjene lastnosti so jih raziskovalci že zelo zgodaj prepoznali kot potencialne indikatorje oz. monitorje za spremljanje nekaterih onesnaževal v atmosferskih usedlinah (Rühling in Tyler, 1968).

Poznamo dva pristopa uporabe mahov kot biomonitorjev; aktivni biomonitoring in pasivni biomonitoring (Markert in sod., 2003a). Pri aktivnem biomonitoringu mahove fizično prenesemo (transplantiramo) iz neonesnaženega okolja v okolje, ki ga želimo spremljati (uporaba t. i. »moss bags«). Metodologija je uporabna predvsem za spremljanje onesnaženosti zraka v urbanih območjih, kjer so naravno prisotni mahovi redki. Pri pasivni obliki biomonitoringa z mahovi se za analizo uporabljajo mahovi, ki naravno rastejo v analiziranem območju. Takšna oblika se uporablja za ekstenzivne študije na večjih površinah (regionalne oz. nacionalne analize) (Ares in sod., 2012).

Pasivna oblika biomonitoringa z mahovi se je za oceno onesnaženosti s težkimi kovinami prvič pojavila že v poznih šestdesetih letih prejšnjega stoletja (Rühling in Tyler, 1968). Leta 1980 se je na pobudo Švedske pričel vzpostavljati sistematični monitoring mahov, da bi ugotovili količine težkih kovin v naravnem okolju. Leta 1990 je bila ustanovljena EU-mreža monitoringa težkih kovin z uporabo mahov (Rühling, 1994). Izvajanje monitoringa je leta 2000 prevzel program ICP Vegetacija (WGE CLRTAP) (glej poglavje 1.4.1), od leta 2005 v njem sodeluje že 28 evropskih držav. Tako je bilo od leta 1990 dalje vzorčenje mahov izvedeno v 5-letnih intervalih (Harmens in sod., 2013).

V Evropi so mahove za prikaz obremenjenosti okolja z N-spojinami na lokalni ravni uporabili v Helsinkih (Manninen in sod., 2013), severni Italiji (Gerdol in sod., 2002; Gerdol in sod., 2014), spodnji Saški (Mohr in sod., 2009; Pesch in sod., 2008) ter v Veliki Britaniji ob izbranih cestah (Pearson in sod., 2000) in piščančjih farmah (Pitcairn in sod., 1998).

Na nacionalni ravni pa so v Evropi mahove za prikaz obremenjenosti okolja z N-spojinami uporabili v Veliki Britaniji (Pitcairn in sod., 1995), Avstriji (Zechmeister in sod., 2008), na severuzahodu Nemčije (Pesch in sod., 2008), na Finskem (Poikolainen in sod., 2009), na severu Španije (González-Miqueo in sod., 2009), v Galiciji (Varela in sod., 2013) in pri nas (Harmens in sod., 2008; Jeran in sod., 2007b). Na evropski ravni so rezultate za območje EU predstavili Harmens in sod. (2011; 2015).

V Sloveniji je v letu 2010 potekala četrta ponovitev nabiranja mahu (pred tem še v letih 1995, 2000 in 2005) (Jeran in sod., 2003; Jeran in sod., 1998; Pavšič-Mikuž, 2005). V vseh letih je raziskavo vodil Inštitut Jožef Stefan (IJS) – Odsek za znanosti o okolju. Terenski del nabiranja mahu pa je deloma pokrival GIS, saj so bile lokacije nabiranja mahu priključene mreži ploskev IMGE in MGGE. Na GIS-u (Oddelek za gozdno ekologijo) so bile opravljene analize vsebnosti N v tkivih mahu z analizatorjem LECO-CNS 2000. V letih 2005 in 2010 se je v mahovih določala tudi izotopska sestava dušika, ki pa je bila opravljena na IJS z masnim spektrometrom za analitiko stabilnih izotopov lahkih elementov (IRMS) – Europa Scientific 20-20 s preparacijskim nastavkom za trdne in tekoče vzorce ANCA SL (Preston in Owens, 1983).

1.5.2.2 Ustreznost uporabe mahov kot biomonitorjev atmosferskih usedlin N-spojin

Z vprašanjem, ali so mahovi primerni za spremljanje usedlin N-spojin v okolje, so se ukvarjali v številnih raziskavah. Primernost uporabe so ugotavljali na podlagi odvisnosti med vsebnostjo N v tkivu mahu in vsebnostjo N v mokrih (dež, rosa itd.) oz. suhih (plini in aerosoli) usedlinah. Glede na način pridobivanja podatkov o atmosferskih usedlinah lahko raziskave delimo v tri skupine:

- uporaba modeliranih vrednosti o vsebnosti N v usedlinah (prostorska interpolacija podatkov o usedu iz merilnih postaj);
- uporaba podatkov iz merilnih postaj (dejanske izmerjene vsebnosti N v mokrih ali suhih usedlinah) ter
- podatki laboratorijskih poskusov škropljenja mahov z vodo, v kateri so bile raztopljene različne koncentracije N-spojin.

Pitcairn in sod. (1995) so med prvimi primerjali vsebnosti N v mahovih z modeliranimi količinami usedlin N v padavinah. Za različne vrste mahu iz osmih lokacij v Veliki Britaniji je bila odvisnost statistično značilna in linearna (r^2 = 0.63). Pozneje so ugotovili, da je odvisnost boljša na lokacijah, kjer prevladujejo suhe usedline N-spojin. Na lokacijah, kjer so prevladovale mokre usedline N, so bile odvisnosti med vsebnostjo N v mahovih in usedlinami boljše za spojine NH_3 in NH_4^+ (r^2 = 0.63) in slabše za celokupni N (r^2 = 0.27). Primerjavo z modeliranimi vrednostmi N v usedlinah padavin so predstavili tudi Stevens in sod. (2011), ki so za vrsto nasršeni resnik (*Rhytidiadelphus squarrosus* (Hedw.) Warnst.) iz 148 lokacij znotraj Atlantske biogeografske regije EU predstavili značilno, a šibko linearno odvisnost (r^2 = 0.10). Leith in sod. (2005) so za 32 lokacij v Veliki Britaniji predstavili šibko, a signifikantno linearno korelacijo s celokupnim N v padavinah (r^2 = 0.26) ter nekoliko močnejšo za N v NH_4^+ (r^2 = 0.48) in N v NO_3^- (r^2 = 0.37). Harmens in sod. (2011) pa so ugotovili, da povezava med vsebnostjo N v različnih vrstah mahov iz 2928 lokacij v 16 državah in usedlinami celokupnega N v padavinah (modelirane usedline N za 50 x 50 km mrežo – merilne postaje EMEP) ni linearna, ampak asimptotična (r^2 = 0.37).

Ker pa je zanesljivost modeliranih anorganskih usedlin N za nekatera območja slabša (Simpson in sod., 2006), so raziskovalci uporabnost mahov kot indikatorjev atmosferskih usedlin N ugotavljali tako, da so mahove vzorčili v neposredni bližini merilnih postaj in primerjali vsebnosti N v mahovih z izmerjenimi količinami N v atmosferskih usedlinah. Solga in sod. (Solga in sod., 2005) so na 8 lokacijah v bližini ploskev Level II UN/ECE ICP Forest programa v Nemčiji vzorčili mahove vrste *Pleurozium schreberi* (Brid.) Mitt. (v nadaljevanju *P. schreberi*) in *Scleropodium purum* (Hedw.) Limpr. (v nadaljevanju *S. purum*). Za obe vrsti mahu so odkrili značilno linearno odvisnost med vsebnostjo N v mahu in količino celokupnega N v padavinah. Odvisnost je bila boljša pri mahu vrste *P. schreberi* (r^2 = 0.43) kot pri vrsti *S. purum* (r^2 = 0.34). Podobne rezultate so za 35 lokacij (20 lokacij UN/ECE ICP Forest in 15 lokacij nacionalne meteorološke mreže) v Avstriji predstavili Zechmeister in sod. (2008), ki so vzorčili pet različnih vrst mahu (50 % vrsta *Hylocomium splendens* (Hedw.) Schimp., 30 % *P. schreberi*, 10 % *Abietinell abietina* (Hedw.) Fleisch., 6 % *H. cupressifrome* in 4 % *S. purum*) in ugotovili, da je bila odvisnost linearna (r^2 = 0.41). Vsebnost N v tkivih mahov vrst *P. schreberi* (5 lokacij) in *Hypnum cupressiforme* Hedw. (9 lokacij) v odvisnosti od usedlin N_{total} pa so za Švico v nacionalnem poročilu predstavili Thöni in sod. (2008) (r^2 = 0.91). Podatke različnih evropskih držav (97 lokacij) so zbrali Harmens in sod. (2014), ki so pokazali, da je povezava statistično značilna, a asimptotična. To pomeni, da nastopi saturacija N v tkivu mahu, če so količine atmosferskih usedlin N večje od 20 kg/ha na leto.

Vendar pa nekatere študije poročajo o šibki odvisnosti N v mahu od N v atmosferskih usedlinah. Predlagana so bila številna pojasnila za te šibke korelacije; vključujoče naslednja:

i. mahovi uravnavajo N v tkivih, ker ima N pomembno vlogo pri presnovi mahu (Arróniz-Crespo in sod., 2008);
ii. različne vrste mahu imajo različne sposobnosti prevzema atmosferskega N (Harmens in sod., 2014; Solga in sod., 2005; Varela in sod., 2013);
iii. poleg atmosferskega N tudi druge okoljske spremenljivke vplivajo na vsebnost N v mahovih (Schröder in sod., 2014 in vključeni viri);

iv. z izbiro lokacije nabiranja mahu lahko vplivamo na vsebnosti N v mahovih, zlasti v bližini drevesnih krošenj (Kluge in sod., 2013; Samecka-Cymerman in sod., 2010).

1.5.2.3 Izotopska sestava dušika v mahovih in primeri uporabe

Meritve izotopske sestave dušika z masnim spektrometrom sta predstavila Preston in Owens (1983). Rezultati meritev izotopske sestave dušika so izraženi z δ^{15}N-vrednostmi v promilih (‰), relativno glede na odklon od mednarodnega standarda. Izračuna se po naslednji formuli:

$$\delta^{15}N = 1000 \times \left(\frac{\left(\frac{^{15}N}{^{14}N}\right) vzorec}{\left(\frac{^{15}N}{^{14}N}\right) standard} - 1 \right) [‰]$$

(1)

Glavni vir antropološko proizvedenega atmosferskega N predstavljata reducirana (NH_y) in oksidirana (NO_x) oblika N-spojin (Vitousek in sod., 1997). Te imajo različno izotopsko sestavo (vrednost δ^{15}N). Ker mahovi privzemajo N neposredno iz ozračja, predpostavljamo, da njihova δ^{15}N-vrednost odraža izotopsko sestavo N v atmosferi (Pearson in sod., 2000). Majhne vrednosti δ^{15}N (bolj negativne) v tkivu mahu kažejo, da je glavani vir N v atmosferskih usedlinah NH_y-N, medtem ko večje vrednosti δ^{15}N (manj negativne) pomenijo več NO_x-N (Gerdol in sod., 2002; Liu in sod., 2008b; Solga in sod., 2006). Na podlagi teh informacij je mogoče razlikovati med območji, kjer so glavni vir antropološkega N fosilna goriva, in območji, kjer je glavni vir intenzivno kmetijstvo (uporaba gnojila).

Metodo izotopske sestave N za ugotavljanje vira N v tkivih mahov so uporabili Bragazza in sod. (2005), Solga in sod. (2005) ter Solga (2007), Liu in sod. (2007), Zechmeister in sod. (2008), Xiao in sod. (2010).

1.5.2.4 Vpliv drugih okoljskih dejavnikov na vsebnost N v mahovih

Splošna primernost uporabe mahov za spremljanje atmosferskih usedlin N-spojin je bila torej predstavljena v številnih študijah (Harmens in sod., 2014 in vključeni viri) (glej poglavje 1.5.2.2). Poleg atmosferskih usedlin pa tudi drugi okoljski dejavniki (npr. nadmorska višina, vrsta mahu, količina padavin, okoliška raba tal itd.) vplivajo na variabilnost vsebnosti N v mahovih (Harmens in sod., 2011; Schröder in sod., 2010). Schröder in sod. (2014) so ugotovili, da se lahko ti vplivni dejavniki med pokrajinami z različnimi ekološkimi značilnostmi razlikujejo. Pitcairn in sod. (1995) ter González-Miqueo in sod. (2009) so npr. poročali, da vsebnost N v mahovih narašča z nadmorsko višino lokacije nabiranja, medtem ko Schröder in sod. (2010) niso odkrili statistično značilnih povezav.

O vplivih okoljskih spremenljivk na variabilnost vrednosti $\delta^{15}N$ v mahovih so poročali Zechmeister et al. (2008) in Liu in sod. (2007). Zechmeister in sod. (2008) so prikazali značilno pozitivno povezavo med vrednostmi $\delta^{15}N$ v mahovih in nadmorsko višino ter količino dežja. Nasprotno, torej negativno, povezavo za nekatere druge zelnate rastline, so predstavili Männel in sod. (2007). Liu in sod. (2007) so ugotovili, da so zaradi vpliva krošenj bližnjih dreves vrednosti $\delta^{15}N$ v mahovih bolj negativne.

Pomemben dejavnik, ki vpliva na vsebnost N v tkivu mahu lahko predstavljajo torej tudi krošnje dreves. Študije atmosferskih usedlin kažejo, da so lahko količine ionov, ki se usedejo na gozdna tla, pod krošnje dreves, povečane ali zmanjšane za nekatere ione, odvisno od ionske reaktivnosti in vrste gozda (De Schrijver in sod., 2007; Houle in sod., 1999). Posledično lahko zato mahovi, ki so nabrani pod drevesnimi krošnjami zaradi spiranja s krošenj dreves v gozdnih sestojih, prejmejo dodatne količine N (Kluge in sod., 2013; Samecka-Cymerman in sod., 2010). Da bi lažje primerjali podatke o vsebnosti N v mahovih med različnimi državami, ki sodelujejo v evropskem monitoringu N z mahovi, je v protokol vzorčenja mahov vključena tudi zahteva, da naj bo najkrajša razdalja med mestom nabiranja mahu in projekcijo najbližje drevesne krošnje vsaj 3 m (ICP Vegetation Coordination Centre, 2010). Ker pa se podnebje, struktura gozda in načini gospodarjenja z gozdovi po Evropi zelo razlikujejo; pojavnost določenih vrst mahu pa je odvisna od značilnosti mikrohabitata (npr.

senca, ki jo povzroča krošnja drevesa) (Glime, 2006; Sabovljević in sod., 2014), je za nekatere vrste mahov nemogoče upoštevati 3-metrsko oddaljenost. Poleg tega so se nekatere države namensko odločile, da bodo nabirale mah pod drevesnimi krošnjami, znotraj vplivnega območja skozi krošnje prepuščenih padavin (Pesch in sod., 2008).

Poznavanje vplivov okoljskih dejavnikov na vsebnosti elementov v mahovih bi lahko omogočilo bolj dosledno primerjavo vsebnosti med državami in tudi znotraj države. V ta namen pa je treba pripraviti metapodatkovni protokol, ki opisuje okoljske dejavnike, značilne za lokacijo nabiranja mahu (Pesch in sod., 2008; Schröder in sod., 2010).

Do nedavnega ni bilo narejenih podrobnih analiz o tem, kako se okoljski dejavniki, ki vplivajo na vsebnost N in vrednosti δ^{15}N v mahu razlikujejo, če so mahovi nabrani na isti lokaciji, vendar na dveh različnih mestih – v gozdni vrzeli in pod drevesnimi krošnjami. Poleg tega niso bili raziskani okoljski dejavniki, ki bi pojasnili razlike med vsebnostjo N v mahovih, nabranih na različnih mestih, vendar na isti lokaciji. Prav tako ostaja odprto vprašanje, ali vsebnosti N v mahu, nabranem pod drevesnimi krošnjami in v gozdni vrzeli, kažejo podobne vzorce onesnaževanja z N in ali so ti vzorci skladni med različnimi območji.

1.5.2.5 Prostorska interpolacija N v mahovih

Karte ocen atmosferskih usedlin se pogosto uporabljajo pri spremljanju stanja emisij in za pripravo emisijske zakonodaje (Fagerli in Aas, 2007). Karte onesnaženosti so za uporabnike običajno laže berljive in predstavljive, saj je informacija o onesnaženosti na voljo za celotno območje in ne samo za izbrane lokacije. Za izdelavo takšnih kart so na voljo različne tehnike prostorske interpolacije podatkov. Vsem je skupno, da želijo napovedati količine onesnažila na nevzorčenem območju na podlagi izmerjenih količin v vzorčenih območjih.

Danes so v Evropi za spremljanje območij s povečanimi zračnimi usedlinami N-spojin v uporabi karte resolucije 50 km × 50 km, ki so izdelane na podlagi modela EMEP MSC-W (Simpson in sod., 2012). Vhodni podatki v model so izmerjene vsebnosti N v mokrih in suhih usedlinah na merilnih mestih EMEP. V nekaterih delih Evrope je število merilnih mest EMEP majhno in posledično je prostorska natančnost kart slabša (Nyíri in sod., 2010).

Doslej so bila objavljena številna regionalna in mednarodna poročila ter znanstveni članki, ki prikazujejo karte onesnaženosti s težkimi kovinami. Te karte so bile oblikovane na podlagi podatkov biomonitoringa z mahovi (Aboal in sod., 2006 in vključeni viri). Do zdaj pa je bilo objavljenih le nekaj člankov, ki so predstavili prostorsko interpolacijo vsebnosti N v mahovih. Avtorji so pri tem uporabili naslednje tehnike prostorske interpolacije: osnovni kriging (Kapusta in sod., 2014; Pesch in sod., 2007; Pesch in sod., 2008; Zechmeister in sod., 2008), regresijski kriging (Schröder in sod., 2011) in nedoločena oblika kriginga (González-Miqueo in sod., 2009; Gonzalez-Miqueo in sod., 2010; Mohr in sod., 2009; Schröder in sod., 2007). Prostorsko interpolacijo vrednosti δ^{15}N so predstavili Zechmeister in sod. (2008 – osnovni kriging) in Varela in sod. (2013 – nedefinirana oblika linearne interpolacije). Pri večini teh študij, z izjemo Pesch et al. (2007; 2008) ter Kapusta in sod. (2014), je bilo nabiranje mahov izvedeno po navodilih ICP Vegetation (2010). V skladu s temi smernicami so bili mahovi nabrani na odprtem (vsaj 3 m od najbližje projekcije drevesne krošnje) in zato karta prikazuje oceno atmosferskih usedlin N. Z vidika gozdne ekologije je to ocena usedlin N na krošnje dreves oz. na tla v gozdnih vrzelih. Na podlagi pregleda literature pa smo našli le nekaj študij, kjer so avtorji z analizo mahov želeli oceniti količino atmosferskih usedlin na gozdna tla pod drevesnimi krošnjami. Študije o prepuščenih padavinah oz. padavinah pod drevesnimi krošnjami so pokazale, da so lahko v skladu z značilnostjo krošnje količine nekaterih ionov pod krošnjami povečane ali zmanjšane (De Schrijver in sod., 2007; Houle in sod., 1999). Te informacije so zelo pomembne, kadar se ugotavlja, v katerih gozdnih ekosistemih je presežena kritična obremenitev z N (Lorenz in sod., 2008).

Točnost kart onesnaženosti je odvisna od gostote vzorčevalnih mest, točnosti izbrane analitske metode, značilnosti onesnažila (transport in kemijske reakcije v atmosferi) in kakovosti prostorske interpolacije izmerjenega podatka. Vendar je v literaturi, z izjemo Varela in sod. (2013) ter Pesch in sod. (2007), malo pozornosti usmerjene na predstavitev i) vrste uporabljene tehnike prostorske interpolacije podatkov, ii) obrazložitvi, zakaj se je takšna tehnika uporabila, in iii) predstavitvi natančnosti kart, izrisanih z izbrano tehniko prostorske interpolacije. Vsa ta vprašanja so zelo pomembna, ker lahko izbira načina prostorske interpolacije močno vpliva na končni rezultat oz. izrisano karto (Margalho in sod., 2014).

1.5.2.6 Primerjava različnih metod biomonitoringa oz. bioindikacije

Fizikalno-kemijske meritve vsebnosti N v padavinah in zraku spadajo med bolj zanesljive vire podatkov o količinah usedlin N-spojin. Vendar, kot je že bilo omenjeno, so takšne meritve zaradi potrebne infrastrukture in stroškov analiz pogosto omejene le na nekaj merilnih mest. Metode biomonitoringa z mahovi lahko dopolnjujejo te meritve na večjem številu lokacij in s tem boljšim pregledom nad prostorsko razporeditvijo atmosferskih usedlin N.

V isti koncept tehnik biomonitoringa, kot je biomonitoring z mahovi, je mogoče vključiti tudi analize foliarnih vzorcev (listov oz. iglic), ki so eden od parametrov v okviru spremljanja stanja gozdov (ICP Forest). Vsebnosti N se v teh vzorcih periodično določajo (vsaki dve leti) na izbranih ploskvah Level II, predvsem z namenom spremljanja hranil v drevesu (glej poglavje 1.4.3) (Rautio in sod., 2010).

Drugače kot metode biomonitoringa metode bioindikacije temeljijo na vizualni oceni ali popisu prisotnosti izbranega organizma. Ocenjevanje osutosti krošnje je eden izmed bolj pomembnih bioindikatorjev, ki so bili razviti v okviru programa ICP Forest in se že vrsto let spremljajo na ploskvah Level I in Level II (glej poglavje 1.4.3). Osutost drevesa naj bi bila posledica odziva gozdnega ekosistema na antropogene in naravne dejavnike stresa ter onesnaženost zraka (Vitale in sod., 2014). Naslednji pogosto uporabljeni organizmi za spremljanje onesnaženosti so lišaji, ki se jih lahko po Conti in Cecchetti (2001) uporablja kot bioindikatorje (popis vrst lišajev in ocena njihove pokrovnosti) (Gadsdon in sod., 2010) in tudi kot biomonitorje, če tkivo lišajev analiziramo, da ugotovimo vsebnosti določenih elementov (Boltersdorf in sod., 2014; Jeran in sod., 2007a).

V nasprotju s fizikalno-kemijskimi meritvami onesnažil v padavinah in zraku obstajajo različna mnenja o ustreznosti uporabe metod biomonitoringa in bioindikacije za ugotavljanje kvalitete zraka. Tako so raziskovalci predstavili številne prednosti in slabosti metod posrednega spremljanja stanja, različni raziskovalci zagovarjajo uporabo različnih metod. V Sloveniji so tri različne pristope bioindikacije (osutost, lišaje in foliarne analize) med sabo primerjali Batič in sod. (1999) ter poročali o dobri odvisnosti med vsebnostjo S v iglicah in v lišajih, medtem ko so bile druge korelacije manj izrazite. Različne načine popisa lišajske obrasti so med sabo primerjali Poličnik in sod. (2008) ter zaključili, da je podroben popis vrst lišajev bolj uporaben za spremljanje onesnaženosti zraka kot pa popis pokrovnosti lišajskih morfoloških tipov – lišajske obrasti. Dodajajo, da je metoda boljša na drevesih, ki rastejo zunaj gozda, saj je tam vrstna pestrost lišajev večja kot pa v gozdnem sestoju. Tri različne tehnike biomonitoringa (lišaji, mahovi in skorje debel) za ocene atmosferskih usedlin N so predstavili Boltersdorf in sod. (2014), ki so zaključili, da so predvsem lišaji korektno odsevali lokalne onesnaženosti z N-spojinami. Jeran in sod. (2007a) niso našli povezanosti med vsebnostjo kovin, N in S v lišajih vrste *Hypogymnia physodes* (L.) Nyl. ter lišajsko obrastjo listastih lišajev.

Na podlagi podatkov, pridobljenih v okviru programa ICP Forest, so medsebojno odvisnost nekaterih indikatorjev že leta 2003 analizirali de Vries in sod. (2003), ki so predstavili odvisnosti med lokacijami in faktorji stresa ter stanjem gozdov. Podobno so Waldner in sod. (2015) predstavili odvisnosti med atmosferskimi usedlinami N-spojin, količinami bazičnih kationov v talni raztopini in stanju hranil v asimilacijskih organih dreves. V okviru študije so dokazali, da lahko povečani vnosi N in žvepla vplivajo na neravnovesje hranil v drevesu. Veresoglou in sod. (2014) so dokazali, da je osutost segmentno linearno povezana z razmerjem N in fosforja (P) ter da pri večini dreves povečanje N : P razmerja vpliva na povečanje osutosti dreves. V nasprotju z njimi sta Solberg in Tørseth (1997) zaključila, da na Norveškem ni nobene statistično značilne povezave med atmosferskimi usedlinami N in osutostjo smreke. Vitale in sod. (2014) pa so na podlagi metode naključnih gozdov (»*random forest*«) ugotovili, da je osutost pri različnih drevesnih vrstah lahko drugače odzivna na okoljske vplive, kot so starost dreves, onesnaženost, značilnosti lokacije drevesa in klimatske razmere. Odvisnosti med meteorološkimi spremenljivkami, fenologijo in osutostjo dreves so predstavili tudi Vilhar in sod. (2014), ki so ugotovili, da pri dobu večja osutost prispeva k zgodnejšemu nastopu fenofaze prvih listov v naslednjem letu. Osutost bukve je bila povezana s količino padavin in vsebnostjo vode v tleh.

1.6 NAMEN RAZISKAV, CILJI IN DELOVNE HIPOTEZE

Naša raziskava je bila osredotočena na gozdne ekosisteme Slovenije. Mahove smo nabrali na 113-ih lokacijah in jih analizirali za vsebnost N in vrednost $\delta^{15}N$. Na vsaki lokaciji smo mahove nabrali na dveh mestih, in sicer pod drevesnimi krošnjami (N_{canopy} in $\delta^{15}N_{canopy}$) ter v bližnji gozdni vrzeli ali jasi (N_{open} in $\delta^{15}N_{open}$). S tem smo želeli pridobiti dve vrsti informacij, in sicer informacijo o atmosferskih usedlinah N-spojin na gozdna tla (pod drevesnimi krošnjami) ter informacijo o atmosferskih usedlinah N-spojin na krošnje dreves oz. na tla, neporaščena z gozdnim drevjem.

Na jugo srednje Evrope doslej niso obstajale študije o primernosti uporabe mahu za spremljanje atmosferskih usedlin N in posledično niso obstajale študije o ustreznosti uporabe mahov kot biomonitorjev za identifikacijo območij, ki so ogrožena zaradi povečanih vnosov N. Ker smo, v okviru doktorskega dela, hkrati nabrali mahove znotraj gozdnega sestoja in na odprtem, smo dobili informacijo, kako se odlaganje N razlikuje med površinami, poraslimi z gozdim drevjem in z drevjem neporaslimi površinami. Hkrati smo prikazali, kako se razlikujejo dejavniki okolja, ki poleg usedlin N-spojin vplivajo na vsebnosti N in vrednosti $\delta^{15}N$ v mahovih. V okviru študije smo ovrednotili vpliv krošenj na spremembo vsebnosti N in vrednosti $\delta^{15}N$ v mahovih ter s tem prispevali k izboljšavi protokola Evropskega popisa mahov (ICP Vegetation Coordination Centre, 2015). Izrisali smo tudi karte, ki prikazujejo, kateri deli Slovenije so bolj in kateri manj obremenjeni z atmosferskimi vnosi N. Takšne izrise kart smo pripravili za N_{open}, N_{canopy} in za $\delta^{15}N_{open}$. Eden izmed rezultatov disertacije je tudi primerjava različnih metod bioindikacije, kjer smo med seboj primerjali rezultate popisa mahov z osutostjo, lišajsko obrastjo in foliarnimi analizami.

Cilji disertacije (razdeljeni glede na objavljena in povezovalna znanstvena dela):

Objavljeno znanstveno delo 1: Vpliv padavin, prepuščenih skozi krošnje na indikativno vsebnost N, S and δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.):

- Ugotoviti, ali so vsebnosti N in vrednosti δ^{15}N v mahovih, ki so bili nabrani na dveh lokacijah v gozdovih, pod drevesnimi krošnjami in v bližnjih gozdnih vrzelih, odvisne od atmosferskih usedlin N (celokupni N, NH_4^+, NO_3^-), izmerjenih v padavinah na odprtem in v sestoju.
- Ugotoviti, kolikšen delež variabilnosti vsebnosti N in vrednosti δ^{15}N v mahovih lahko pripišemo razlikam znotraj lokacije in koliko razlikam med lokacijami.
- Oceniti vpliv krošnje drevesa na vsebnost N ter vrednosti δ^{15}N v mahovih, nabranih pod drevesnimi krošnjami in v gozdnih vrzelih.

Objavljeno znanstveno delo 2: Potencialni okoljski vplivi na vsebnost N in vrednosti δ15N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.), nabranem znotraj in zunaj območja sestojnih padavin:

- Analizirati prostorsko razporeditev vsebnosti N in vrednosti δ^{15}N v mahovih, nabranih pod drevesnimi krošnjami in v bližnjih gozdnih vrzelih.
- Oceniti vpliv okoljskih dejavnikov na vsebnost N in vrednosti δ^{15}N v mahovih, nabranih pod drevesnimi krošnjami in v gozdnih vrzelih.
- Opredeliti, v kakšnem obsegu je s statističnim modelom mogoče pojasniti povečane vsebnosti N v mahovih, nabranih pod drevesnimi krošnjami, v primerjavi z vsebnostmi N v mahovih, nabranih v gozdnih vrzelih, ob upoštevanju izbranih okoljskih dejavnikov.

Objavljeno znanstveno delo 3: Prostorska interpolacija vsebnosti N in vrednosti δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.), nabranem v gozdovih Slovenije:

- Raziskati različne tehnike prostorske interpolacije vsebnosti N in vrednosti δ^{15}N v mahovih, ki so bili nabrani pod drevesnimi krošnjami in v gozdnih vrzelih.
- Na primeru Slovenije prikazati, katere informacije o uporabljeni tehniki prostorske interpolacije je potrebno predstaviti v rezultatih, če objavimo karte vsebnosti N in vrednosti δ^{15}N, pridobljene z uporabo mahov kot biomonitorjev.

- Interpretirati karte prostorske porazdelitve vsebnosti N in vrednosti δ^{15}N v mahovih na nacionalni ravni ter raziskati povezavo med prostorsko porazdelitvijo N v mahovih in primarnimi viri atmosferskih usedlin N.

Povezovalno delo: Odvisnost osutosti dreves, lišajske obrasti in foliarnih analiz od vsebnosti N in vrednosti δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.), nabranem v gozdovih Slovenije:

- Raziskati odvisnost vsebnosti N v foliarnih vzorcih, osutosti dreves in lišajske obrasti na vsebnost N in vrednosti δ^{15}N v mahovih, nabranih na dveh različnih mestih – zunaj in znotraj območja skozi krošnjo prepuščenih padavin.
- Oceniti, kateri drugi okoljski dejavniki lahko vplivajo na osutost dreves in lišajsko obrast.

Postavili smo naslednje delovne hipoteze:

1. Vsebnost skupnega dušika v tkivih mahov je odvisna od atmosferskega vnosa dušikovih spojin, zaradi česar so mahovi primeren kazalnik (biomonitor) za identifikacijo območij, ki jih ogrožajo veliki vnosi atmosferskih depozitov N.
2. Obremenjenost z dušikovimi spojinami pod drevesnimi krošnjami v gozdu je večja kot v gozdnih vrzelih ali jasah. Učinke krošenj na vsebnost skupnega N v mahovih je do določene mere mogoče pojasniti z zastrtostjo nad vzorčenim mestom in drevesno sestavo (iglavci, listavci).
3. Naravni ekosistemi v Sloveniji so z dušikovimi spojinami različno obremenjeni. Vire dušikovih spojin je možno ugotoviti z uporabo izotopskih metod. Predvidevamo, da so večje količine spojin NO_x v naravnih ekosistemih, ki se nahajajo v bližini večjih urbanih središč, prometnic, večjih industrijskih ter termoenergetskih objektov. Večje količine nitratnih in amonijevih oblik N pa v naravnih ekosistemih, ki so v bližini površin, na katerih se izvaja intenzivno kmetijstvo.
4. Uporaba mahov za spremljanje prisotnosti dušikovih spojin v naravnem okolju je primerljiva z analitičnimi metodami (foliarnimi analizami) in natančnejša od nekaterih drugih bolj integrativnih metod (osutost drevja, lišajska obrast).

2 ZNANSTVENA DELA

2.1 OBJAVLJENA ZNANSTVENA DELA

2.1.1 Vpliv padavin, prepuščenih skozi krošnje, na indikativno vsebnost N, S in vrednost δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.)

Influence of canopy drip on the indicative N, S and δ^{15}N content in moss *Hypnum cupressiforme*

Mitja Skudnik, Zvonka Jeran, Franc Batič, Primož Simončič, Sonja Lojen, Damijana Kastelec
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Vzorci mahu vrste *Hypnum cupressiforme* so bili nabrani na dveh mestih v gozdovih; pod drevesnimi krošnjami in v bližnji gozdni vrzeli, ter analizirani na vsebnost N in S ter vrednosti δ^{15}N. Mahovi, nabrani v gozdnih vrzelih, odražajo atmosferske usedline N; nismo pa odkrili značilne odvisnosti vsebnosti N v mahovih, ki so bili nabrani pod drevesnimi krošnjami, od količin sestojnih usedlin N, niti odvisnosti vsebnosti S v mahovih od količin atmosferskih usedlin S na nobeni izmed obeh lokacij. Pri vsebnosti N in S v mahovih, vzorčenih v gozdnih vrzelih, je variabilnost podatka znotraj lokacije primerljiva z variabilnostjo med lokacijami, za vrednosti δ^{15}N pa je bila variabilnost na lokaciji nabiranja mahu manjša kot med lokacijami. Rezultati kažejo, da so na kratkih razdaljah (<1 m) med mestom nabiranja mahu in horizontalno projekcijo najbližje drevesne krošnje povečane vsebnosti N v mahu. Tako so bile statistično značilno večje vsebnosti N v mahovih, nabranih pod drevesnimi krošnjami.

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Influence of canopy drip on the indicative N, S and δ^{15}N content in moss *Hypnum cupressiforme*

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Samples of *Hypnum cupressiforme* were collected at two types of site in forest areas: within the forest stand and within forest openings, and analyzed for N and S concentrations and δ^{15}N. Mosses sampled within forest openings reflect the atmospheric N deposition; however, no influence of throughfall N deposition on the N in the moss that was sampled within the forest stand was found, nor was any influence of S deposition on the S in the moss found. For the N and S concentrations in the mosses sampled within forest openings, the within-site variability was comparable to the between-site variability, and for the δ^{15}N, the within-site variability was lower than the between-site. The results showed that a short distance (<1 m) between the sampling location and the nearest tree canopy increases the N in the moss, and significantly higher values are found in mosses sampled in areas within the forest stand.

1. Introduction

Complex system interactions and the relationships between pollutants and ecosystems aggravate the collection of needed information on the amount of atmospheric pollutants and their spatial distribution (Pitcairn et al., 2006). For this reason, a number of different monitoring systems have been established, some of which are based on physical and chemical measurements of atmospheric depositions and air quality (Erisman et al., 2005) and others that use different bio-indicators (e.g., mosses, lichens, etc.) to assess pollutants (Markert et al., 2003). The general suitability of using mosses as indicators of atmospheric N deposition has been shown in numerous studies. Most authors have examined the relationship between the N concentration in mosses and the modeled atmospheric N deposition (Harmens et al., 2011; Pitcairn et al., 1995; Schröder et al., 2010; Stevens et al., 2011), and a few have examined the dependence of the N concentration in mosses on the measured deposition in the immediate vicinity of the moss sampling site (Solga et al., 2005; Thöni et al., 2008; Zechmeister et al., 2008). The suitability of this method to estimate S deposition was shown by Novak et al. (2001), Vingiani et al. (2004) and Raymond et al. (2010).

The main sources of anthropologically derived atmospheric N are reduced (NH_y) and oxidized (NO_x) compounds (Vitousek et al., 1997) that have different N stable isotope compositions (δ^{15}N value). Because mosses uptake N directly from the atmosphere, it is assumed that their δ^{15}N signature reflects the composition of N in the atmosphere (Pearson et al., 2000). Low δ^{15}N (more negative) values in moss tissue indicate NH_y–N deposition sources, while higher δ^{15}N (less negative) values indicate NO_x–N (Gerdol et al., 2002; Liu et al., 2008; Solga et al., 2006). Consequently it is possible to distinguish between areas where the main source of anthropologically derived atmospheric N comes from fossil fuel consumption and those areas with intensive agriculture (use of fertilizers).

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The relationship between atmospheric element deposition and its concentration in mosses may be affected by environmental factors, such as surrounding vegetation (Harmens et al., 2011). Deposition studies show that the ion deposition that reaches the ground under the forest canopy can be enriched or reduced for certain ions, depending on the ion reactivity and forest type (de Schrijver et al., 2007; Houle et al., 1999). To eliminate the influence of the surrounding vegetation on the element concentration in the analyzed mosses, ICP-Vegetation moss sampling protocols prescribe that each moss collecting location should be at least 3 m away from the nearest tree crown (ICP Vegetation Coordination Centre, 2010). However, many moss species depend on certain favorable microhabitat characteristics (moisture, substratum, etc.), and some species that are suitable for biomonitoring, especially in drier areas, often thrive in the tree crown shadow (Glime, 2006). Consequently, it is not always possible to sample mosses far away from the tree canopy. *H. cupressiforme* is the 3rd moss species recommended by ICP-Vegetation for biomonitoring (ICP Vegetation Coordination Centre, 2010), and it is common in forests of the southern part of central and south-eastern Europe (Kutnar and Martinčič, 2008; Sabovljević et al., 2009; Skudnik et al., 2013). In contrast to the northern parts of Europe, in which the moss biomonitoring method was developed, the percentage of broadleaved forests in the southern part of Europe is high (EEA, 2007). Slovenia is the third most forested country in Europe, with over 60% forest cover, in which 70% of forest stands belong to beech, fir-beech and beech-oak forests (FAO, 2011). Furthermore, the traditional forest management system is based on close-to-nature management, where natural regeneration methods are used. This means that the logging of trees is conducted in a small area and often at locations where young trees are already present. All of these characteristics further complicate the search for appropriate locations for moss collection.

The aim of our study was to 1) explore how the N and S concentrations and the $\delta^{15}N$ signature in moss that was sampled at two types of sites in forests a) within the forest stand (area inside the projection of the tree canopy and b) within forest openings (area outside the projection of the tree canopy) correspond to site specific atmospheric deposition values of N (N_{total}, NH_4^+, NO_3^-) and S (SO_4^{2-}), 2) to test the within-site and between-site variation of N, S and $\delta^{15}N$ for moss samples collected within forest openings and 3) to evaluate the degree of canopy influence on the N and S concentrations and $\delta^{15}N$ signature in moss sampled within the forest stand and in moss sampled within forest opening.

2. Material and methods

2.1. Collection of moss

In the summer of 2010, our team collected samples of *H. cupressiforme* Hedw. at 113 locations inside of forested area (Fig. 1). Out of 113 locations 91 were distributed on the regular sampling grid of 16 × 8 km, which is a subset of the 4 × 4 km grid used for the national forest inventory (Kušar et al., 2010). A location was included in the sampling if the intersection of the 16 × 8 km grid was located inside the forest area (Fig. 1). Additionally moss samples were collected near locations where the Slovenian national monitoring of deposition is assessed (4 locations) and also near 11 research plots of the Intensive Monitoring Programme (UN-ECE ICP-Forest) in

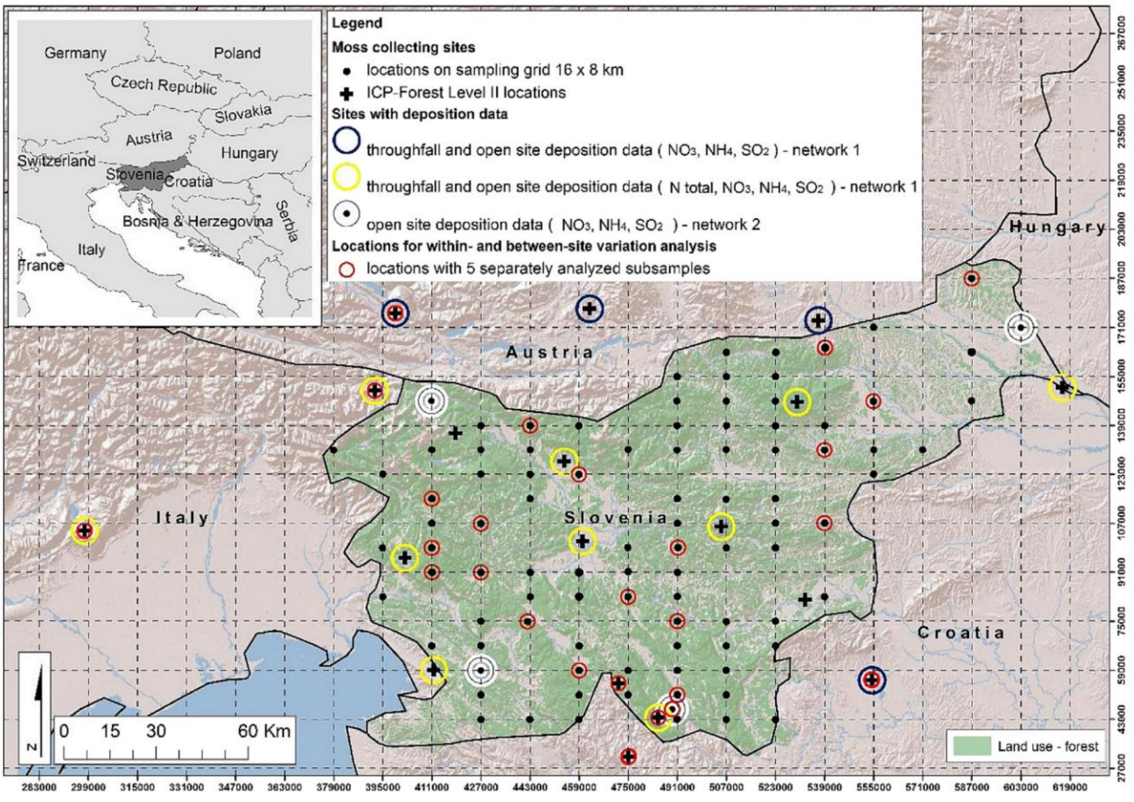

Fig. 1. Map of 113 sampling locations in Slovenia and neighboring countries (coordinates on the map are in the Gauss–Krüger coordinate system).

Slovenia and 7 in neighboring countries (Italy, Austria and Croatia) (Fig. 1). These intensive monitoring plots were installed in the most important forest ecosystems for each country (de Vries et al., 2003).

At each location, samples were taken at two types of site, namely, within the forest stand (inside the area of the tree canopy projection) and within forest openings (outside the area of the tree canopy projection) (Fig. 2). Within the forest stand, one composite sample, which was pooled from five subsamples, was collected. Within forest openings, five subsamples were collected as far as possible from the nearest trees. If there was at least 5 g of cleaned material, the subsamples were analyzed separately (26 locations – Fig. 1); otherwise, the composite sample was pooled after cleaning in the laboratory.

Field sampling was performed according to the guidelines of the UN-ECE ICP-Vegetation program (ICP Vegetation Coordination Centre, 2010), with one adjustment. Within forest openings mosses were collected at least 0.5 m away from the nearest tree canopy projection, not 3 m as proposed by the ICP-Vegetation manual. The distance to the nearest tree canopy projection was recorded for each subsample. The mean distances to the nearest tree canopy values of the five subsamples pooled to composite sample were used for further statistical analysis.

2.2. Chemical analyses

The chemical analyses followed the ICP-Vegetation experimental protocol (ICP Vegetation Coordination Centre, 2010). Mosses were cleaned of obvious contamination particles (soil, litter, etc.) and only the green and brown-green parts of the mosses were taken for further analysis. Bryophyte material was lyophilized for 24 h and homogenized in a ball mill. The N and S concentrations were determined using LECO CNS-2000 at the Department for Forest Ecology at the Slovenian Forestry Institute (SFI).

To determine ^{15}N abundance, the moss samples were analyzed at the Department of Environmental Sciences at the Jožef Stefan Institute in Slovenia (IJS) using an isotope ratio mass spectrometer (IRMS) – Europa Scientific 20–20 with an ANCA SL preparation module for solid and liquid samples. The results were reported as relative δ values in per mil (‰), i.e., the difference in parts per mil of the isotopic ratios $^{15}N/^{14}N$ from that of atmospheric N (Air). The accuracy of the $\delta^{15}N_{Air}$ measurements was ±0.2‰. IAEA-N reference materials were used to calibrate the measurements.

Quality control was further ensured by using reference moss material M2 and M3 (Steinnes et al., 1997). The moss standards were analyzed together with the collected moss samples and the results for N concentration (M2: 8.27 ± 0.23 mg/g ($N = 6$); M3: 6.72 ± 0.26 mg/g ($N = 6$)) agreed well with the recommended values (M2: 8.36 ± 0.62 mg/g ($N = 10$); M3: 6.81 ± 0.52 mg/g ($N = 8$)) (Harmens et al., 2010).

2.3. Deposition data

Deposition data for 18 locations was obtained from two monitoring networks: (1) a network within the framework of European Forest Monitoring (UN-ECE ICP-Forest; 14 locations) and (2) a network within a national program for deposition quality (DMKP; 4 locations) (Fig. 1). In monitoring network (1) the deposition of NO_3, NH_4^+ and SO_4^{2-} was measured within forest stands (throughfall deposition) and also at open field sites (bulk deposition). The data on the N_{tot} deposition were available only for 10 locations within network (1). In monitoring network (2) the deposition of NO_3, NH_4^+ and SO_4^{2-} were measured only at open field sites outside the forest (Fig. 1). Hereinafter we use the terms "throughfall deposition" and "open site deposition". The Slovenian deposition sampling methodology has been described by Žlindra et al. (2011) for network (1) and by Šegula et al. (2011) for network (2). The methodologies for Croatian deposition sampling were described by Jakovljević et al. (2013), Austrian sampling was described by Smidt (2007) and Italian sampling was described by Mosello et al. (2002). All deposition data from network (1) followed the quality control measures provided by ICP-Forest and were described by Clarke et al. (2010) and König et al. (2010). The data on deposition for network (2) followed the quality control measure provided by World Meteorological Organization and were described by WMO (2004) and on the country level by Šegula et al. (2011). However, in using data from two different monitoring networks and from different institutions we must be aware of possible differences in data quality. The descriptive statistics of the deposition data are presented in Table 1. The deposition data are positively skewed, and the range is small. Throughfall deposition used in our analysis does not include the stemflow data. Also, mosses collected within forest stands were never collected on tree trunks or in areas with flowing water.

2.4. Statistical analysis

The dependence of N and S concentrations in the moss on the atmospheric N and S were explored using lin-log models. Deposition data were log transformed. Atmospheric deposition data were used as (1) the average N and S deposition over the last three years (2008–2010) and (2) the sum of N and S deposition for the last year before moss sample collection occurred. To check if the chemical composition of N and S deposition affect the element concentration in moss tissue, the following relationships were explored:

- N in moss tissue vs. the amount of precipitation and the N in deposition (N total, NH_4^+–N and NO_3–N);
- S in moss tissue vs. the amount of precipitation and the S in deposition (SO_4^{2-} – S);
- $\delta^{15}N$ values of the moss vs. the amount of precipitation and the ratios of NH_4^+/ NO_3 in the deposition.

For 26 locations (Fig. 1), for which hierarchical sampling was performed (5 subsamples within forest openings), the within- and between-site variations of the element concentration in moss were calculated using linear mixed-effect modeling. Within-site variation shows the variation of element concentration between subsamples (inside an experimental plot) and between-site variation shows the variation of element concentration between experimental plots. In linear mixed-effect model the experimental plots were considered as a random effect and the total variance of the element concentration was divided into two portions, the within location variance and the between location variance. The mean values of the five subsamples were used for further statistical analysis of these 26 sites.

Distances from the moss collection location to the nearest tree crown projection were divided into four categories: within forest stands (i. 0 m) and three categories within the forest openings (ii. <1 m, iii. 1–3 m, iv. >3 m) (Fig. 2). A linear mixed-effect model was used to test the differences between the N, S and $\delta^{15}N$ values in the mosses collected at different distances from the tree crown projection, and the random effect of sample location was considered in the model. The Pearson correlation coefficient was used to test the linear correlation between the element concentration in moss collected within forest stands and the moss collected within forest openings. All statistical analyses were performed with R 3.0 (R Development Core Team, 2013).

3. Results

3.1. Comparison of moss and deposition data

All locations for which cumulative total N deposition data from the open sites were available showed a weak but significant dependence of the N concentration in the moss on the atmospheric

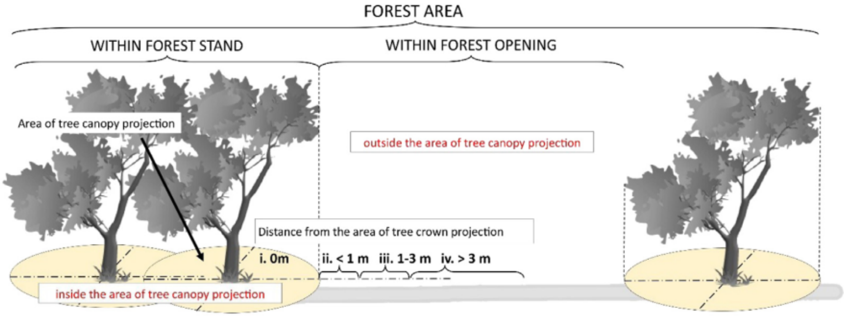

Fig. 2. Schematic representation of locations for collecting moss samples. Within forest stand mosses were always collected inside the area of tree canopy projection but never on tree trunks; within forest opening mosses were always collected outside the area of tree canopy projection.

Table 1
Descriptive statistics for throughfall and open site deposition data (three year average from 2008 to 2010 and one year cumulative from the month of moss collecting). Locations of deposition sites are shown in Fig. 1.

Variable		Precipitation [mm]		NH_4^+–N [kg/ha]		NO_3–N [kg/ha]		N_{tot} [kg/ha]		SO_4^{2-}–S [kg/ha]	
		Open	Thr.	Open	Thr.	Open	Thr.	Open	Thr.	Open	Thr.
2008–2010	Min	776	644	3.53	1.62	2.47	2.45	9.47	7.77	3.29	3.53
	Max	2654	2333	9.52	14.18	9.44	16.92	22.33	36.59	12.15	12.32
	Average	1436	1183	5.22	5.23	4.53	6.59	13.30	16.81	5.35	6.39
	Median	1435	1145	4.70	4.41	4.30	5.89	12.20	15.40	4.88	6.02
1 year cumulative	Min	666	488	2.78	2.26	2.21	2.70	8.86	8.20	1.85	2.26
	Max	2236	1908	8.07	10.37	8.33	12.70	19.19	26.77	11.44	11.95
	Average	1283	904	4.70	4.63	3.86	5.29	11.89	13.46	4.71	4.71
	Median	1291	802	4.10	4.04	3.58	4.51	11.28	12.99	3.93	4.18
Nr. of sites		18	14	18	14	18	14	10	10	18	14

Open – deposition measured at open field.
Thr. – throughfall deposition.

N deposition (Table 2, Fig. 3). However, this dependence was significant with cumulative one-year deposition but not for a three-year average deposition. The influence of the atmospheric N deposition on the N concentration in the mosses was significant for NH_4^+–N and the sum of NH_4^+–N and NO_3–N (Table 2). There was no significant dependence of the N concentration in moss sampled within the forest stand on the throughfall N deposition (14 sites) (Fig. 4). On the other hand, there proved to be boundary significant dependence of the N concentration in the moss sampled within the forest stand on the NH_4^+–N deposition measured in the open sites (Table 2, Fig. 3). It was further found that if we excluded 4 sampling locations out of a total of 18 that received less than 1000 mm of precipitation per year, the dependence of N in the mosses collected within forest openings on the atmospheric NO_3–N deposition increased ($R^2 = 0.34$, $p = 0.028$, nr. sites = 14).

Our study did not observe any dependence of the S concentration in moss tissue on the average or one-year cumulative atmospheric SO_4^{2-}–S deposition (throughfall or open site deposition).

The NO_3 input within the 24 months prior to moss sampling exceeded that of NH_4^+ for all deposition sites. All deposition sites showed no dependence between the $\delta^{15}N$ values of the mosses and the NH_4^+/NO_3 ratio. However if we excluded 4 of the 18 sites with cumulative yearly precipitation of less than 1000 mm, the dependence of the $\delta^{15}N$ values of the moss collected within forest openings and within forest stands on the NH_4^+/NO_3 ratio for the open site deposition was significant for both ($R^2 = 0.53$; $p = 0.005$ for mosses collected within forest openings and $R^2 = 0.47$; $p = 0.014$ for mosses collected within forest stands) (Fig. 5).

3.2. Within- and between-site variation

The results of a linear mixed-effect model for 26 locations with subsampling show that for the samples collected within forest openings the within-site variation was lower than the between-site variation; especially for the $\delta^{15}N$ values, for which there was 27% within-site variation and 73% between-site variation. For the N concentration, the variations were 44% vs. 56%, respectively, and for the S concentration, the variously were 49% vs. 51%, respectively. The N and S concentrations in mosses correlated significantly linearly in the mosses collected within forest openings ($r = 0.91$; $p < 0.001$), as well as within forest stands ($r = 0.93$; $p < 0.001$).

3.3. Influence of canopy drip

By the implementation of moss biomonitoring technique into southern part of central Europe, the problem of finding appropriate moss collecting sites at least 3 m away from the nearest tree crown was exposed. Our results showed that it was possible to collect mosses at least 3 m away from the nearest tree crown projection at only 19 of the 113 total sampling locations (17%); it was possible to collect mosses at a distance between 1 and 3 m away from the nearest tree crown projection in 69% of the locations. The descriptive statistics and results of linear mixed-effect models are presented in Table 3. The differences of both N and S concentrations in the moss tissue that was collected within forest stands and within forest openings were significant (Table 3), but they were not so for $\delta^{15}N$ values. The N and S concentrations in the mosses decreased with increasing distance from the tree canopy projection (Fig. 6). The linear mixed models show that the average N and S concentrations in moss collected inside the area of tree canopy projection were significantly higher than the averages of all three categories of distance from the samples taken outside the area of tree canopy projection ($p < 0.001$, but there is no significant difference between them, as shown in Table 3).

The average relative ratio of the N concentration in the moss, calculated on the basis of the linear mixed model estimates (Table 3), showed that the N concentration in the moss collected within forest stand was, on average, 41% higher than in the samples collected at least 3 m away from the nearest tree crown projection. The samples that were collected within a radius of 1 m from the nearest crown had a N concentration that was, on average, 15% higher than in samples that were collected from at least 3 m away

Table 2
Coefficient of determination (R^2) for the linear regression model describing dependence of the N concentration in moss on the elemental content in a cumulative one-year N deposition (12 months from the week of sampling). Locations of deposition sites are shown in Fig. 1.

Moss element	Moss sampling site	Deposition element	Deposition sampling location	R^2	p-Value	Nr. of sites
N	Within forest opening	log(N total)	In open	0.33	0.081	10
	Within forest opening	log(NH_4^+-N)	In open	0.40	0.005	18
	Within forest stand			0.20	0.063	18
	Within forest opening	log(NO_3–N)	In open	0.23	0.045	18
	Within forest opening	log(NH_4^+–N + NO_3–N)	In open	0.46	0.001	18

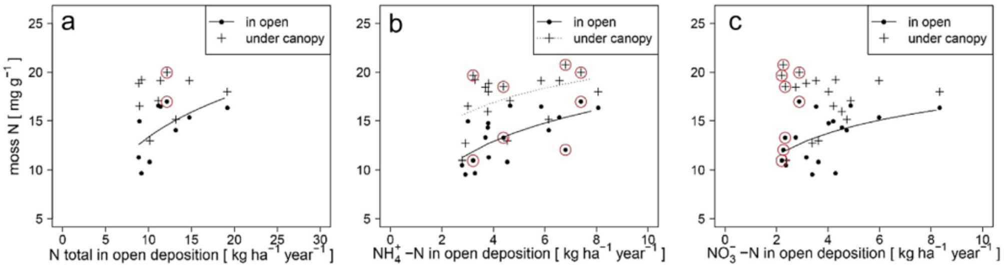

Fig. 3. Dependence of the N concentration in *H. cupressiforme* collected within forest openings and within forest stands on a one-year cumulative of the total N deposition ($n = 10$ sites) (a), NH_4^+–N (b) and NO_3^-–N (c) in the precipitation sampled from the open area ($n = 18$ sites). Regression lines were fitted using the lin-log model. The sites with an average precipitation of less than 1000 mm are marked with red circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

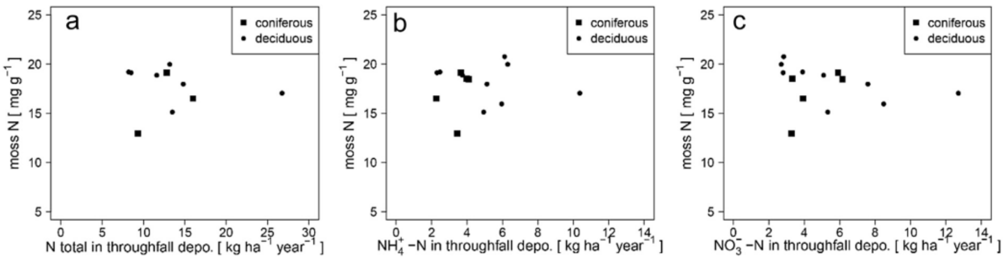

Fig. 4. Dependence of the N concentration in *H. cupressiforme* collected within forest stands on a one-year cumulative of the total N deposition ($n = 10$ sites) (a), NH_4^+–N (b) and NO_3^-–N (c) in the throughfall precipitation ($n = 14$ sites) that was separate for coniferous and deciduous stands.

from the nearest tree crown (Table 3). The S concentration in the moss was, on average, 34% greater in moss collected within forest stands than it was in samples that were collected at least 3 m away from the nearest tree crown (Table 3).

4. Discussion

4.1. Comparison of moss and deposition data

It has been a topic of interest to consider whether the N concentration of moss reliably reflects the atmospheric N deposition (Harmens et al., 2011). While some studies suggest that the moss biomonitoring method can be used to identify more or less polluted sites (Pitcairn et al., 2006; Zechmeister et al., 2008), others have shown models for the estimation of the atmospheric N deposition per area (Harmens et al., 2011; Solga et al., 2005). These models are usually built on the dependence between the N concentration in bulk deposition and the N concentration of mosses collected near deposition measurement sites. This type of dependence has been demonstrated in our study, which shows that *H. cupressiforme*, growing in natural environments in the southern part of central Europe, reflects the measured atmospheric N deposition for this area (Table 2, Fig. 3). Previously species-specific field surveys for five other pleurocarpus mosses (*H. splendens*, *P. schreberi*, *S. purum*, *R. squarrosus* and *T. tamariscinum*) were performed to test their response to N deposition (Hicks et al., 2000; Leith et al., 2005; Mohr, 1999; Raymond et al., 2010; Solga et al., 2005). Similar to other investigations, our results showed relatively weak dependence, and we assume that this may be due to the lack of information on dry deposition, which may strongly influence the N concentration in the moss (Raymond et al., 2010; Skinner et al., 2006). Additionally, a high within-site variability of the N concentration data of the moss may affect the dependence (Table 3, Fig. 6). Last but not least, we must also take into account the low number of deposition measurement sites ($N = 18$).

Our results showed that *H. cupressiforme* reflects the N deposition for the current year but not of the three-year average. Variation in the N concentration of the moss tissue was discussed by Liu et al. (2008), who found differences in the N concentration of the new and old tissues of *H. microphyllum*, with lower values found in the older tissue. These findings suggest that the field sampling campaigns should take place within a short period of time.

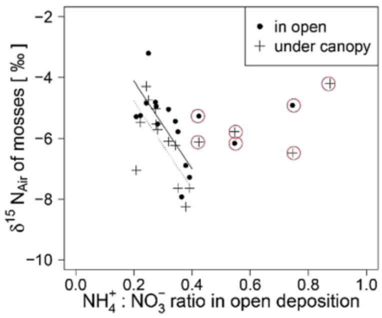

Fig. 5. δ^{15}N signature in *H. cupressiforme* sampled within forest openings and within forest stands and the ratio of NH_4^+/NO_3^- of one-year cumulative open site precipitation. Regression lines were fitted only for sites with average precipitation greater than 1000 mm. The sites with average precipitation of less than 1000 mm are marked with red circle. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Descriptive statistics and linear mixed-effect model results for the N and S concentration in moss for the distance to the nearest tree crown categories.

El.	Sampling site	Dist. to crown [m]	Descriptive stat.				Linear mixed effect model		
			Nr. samp.	Mean [mg/g]	Sd [mg/g]	CV [%]	Estimated mean [mg/g]	Lower 95% CL [mg/g]	Upper 95% CL [mg/g]
N	Within forest stand	0	113	17.54	3.58	20	17.53	16.96	18.11
	Within forest opening	<1	16	14.10	2.31	16	14.37	13.00	15.75
		1–3	78	13.33	2.66	20	13.04	12.37	13.71
		>3	19	11.65	2.15	18	12.45	11.19	13.71
S	Within forest stand	0	113	1.35	0.24	18	1.35	1.31	1.39
	Within forest opening	<1	16	1.13	0.15	13	1.13	1.03	1.22
		1–3	78	1.08	0.19	18	1.06	1.01	1.11
		>3	19	0.97	0.15	15	1.01	0.93	1.10

The influence of atmospheric N deposition on the N concentration in the moss was found to be significant, especially in the case of NH_4^+–N. A higher uptake of NH_4^+–N than of NO_3^-–N has previously been reported in N-uptake experiments for bryophytes (Forsum et al., 2006; Nordin et al., 2006) and is presumably due to the high cation-exchange capacity of mosses (Bates, 1992). We noticed that the percentage of understood data variation between the NO_3^-–N content in the deposition and the N in the moss increased only if locations with more than 1000 mm of rain per year were included in the analysis. In our study, low precipitation sites also had a low atmospheric NO_3^-–N deposition (Fig. 3). One possible explanation is that NO and NO_2 are not very soluble in water, but the product of their reaction, HNO_3, is very soluble. With a higher rate of precipitation, more NO_3^- is washed from the atmosphere, and with low precipitation, HNO_3 reacts with NH_3 and thus changes to NH_4^+ (Asman et al., 1998). The influence of the amount of precipitation on the relationship between the NH_4^+/NO_3^- ratio and the $\delta^{15}N$ value in the moss was even higher. This model was only significant if we excluded the locations with less than 1000 mm of precipitation (Fig. 5). Similar conclusions were also reported by Zechmeister et al. (2008). Additional analysis are needed to determine how the relationships between the atmospheric N deposition and the N concentration in moss changes in areas with less than 1000 mm of yearly precipitation.

Contrary to the results presented by Leblond et al. (2009) we did not find any dependence of the N concentration in the moss sampled within forest stands on the throughfall N deposition; however, for all 14 locations (Fig. 1), the N concentration in the moss collected within forest stand was higher than in moss collected within forest openings, but the N content in the throughfall deposition was higher than it was in the bulk precipitation at only 7 of the 14 locations. In forested areas, precipitation undergoes considerable quantitative and qualitative changes as a result of its interaction with the stand canopy, and the effects of the tree canopy on the concentration of elements in the throughfall varies between conifers and broadleaves, as well as between individual species (Moreno et al., 2001; Nieminen et al., 1999). Fig. 4 suggests a different relationship pattern between the N concentration in the moss and the N content in the throughfall precipitation for coniferous and deciduous stands. The data presented by Leblond et al. (2009) were from 23 locations in France; 18 of these were inside coniferous stands. Higher number of locations in deciduous stands may explain the difference in our results. Under the canopy, throughfall precipitation contains organic as well as inorganic forms of N (amino acids, urea, etc.) (Forsum et al., 2006) and other suspended matter (Gundersen et al., 1998), but it does not contain N from the decaying litter that has fallen on the forest floor (Boxman et al., 2008). The N content from the litter may affect the relationship with the deposition in the deciduous stands and not in the coniferous stands, where the amount of N from the litter is usually lower (Reich et al., 2005).

No relationship between the SO_4^{2-}–S deposition and the S concentration in the moss tissue support the results from Thöni and Seitler (2012). Vingiani et al. (2004) showed that *Sphagnum* species are better for the accumulation and retention of S than some other moss species; one of these species is apparently *H. cupressiforme*. The explanation for there being no dependence could be related to the small variation in atmospheric S deposition data for selected locations (Table 1).

4.2. Within- and between-site variation

The within-site variations of the N, S and $\delta^{15}N$ values in mosses collected within forest openings were lower than those of the between-site variations for all 26 locations (Fig. 1). Our findings show that in this part of Europe, *H. cupressiforme* meets the required biomonitoring conditions presented by Wolterbeek et al. (1996). The within-site variations in N and especially S levels were higher than expected, and we assume that this mainly occurred as a consequence of the difficulty in finding mosses in an open area (>3 m away from the nearest canopy) (Table 3).

4.3. Influence of canopy drip

In the present study, we show that moss tissue collected near or inside the area of the tree canopy projection contains additional inputs of N and S (the N and S concentrations in the moss were, on average, 41% and 34%, respectively, higher within the forest stand

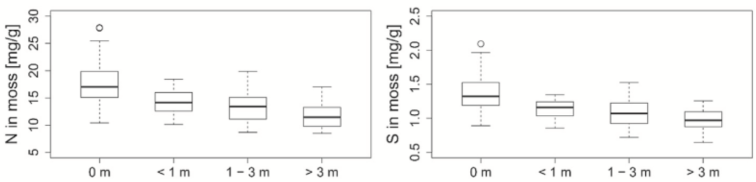

Fig. 6. Boxplots for the elemental concentration in the mosses in different classes of distance to the nearest tree crown projection.

than they were at least 3 m away from the nearest canopy) (Table 3). This is consistent with the findings that forest canopies are efficient sinks for atmospheric gasses and particles that are later leached by rain from the tree canopies to the forest floor (de Schrijver et al., 2008). Forest structure and climate characteristics define the ratio between the N in throughfall and the N in bulk deposition (de Schrijver et al., 2007), but inside the forest, litterfall always provides additional N input to the forest floor (Reich et al., 2005). Canopy drip and litterfall may be the cause of the higher N and S concentrations that have been reported for the southern and eastern parts of Europe (Harmens et al., 2011), where *H. cupressiforme* is the moss most commonly used for biomonitoring (Harmens and Norris, 2008; Sabovljević et al., 2009). The influence of the throughfall composition on the elemental concentration of mosses was also reported by some other authors (Kluge et al., 2013; Pesch et al., 2007; Samecka-Cymerman et al., 2010).

We found that the N and S concentrations in mosses decreases with increasing distance from the nearest tree canopy, but the intervals of the values at 3 m away from the canopy and those at 1 m from the canopy, overlap (Table 3). These findings are important, especially if biomonitoring is performed in dry habitats or in areas with a high percentage of broadleaves, where large gaps among crowns are rare. At these locations, a 1 m threshold would make the methodology more efficient.

Our study results show that it is not meaningful to compare the results between countries or within a country if the mosses are not systematically collected in the open. However, the weak relationship between the N concentration in moss collected within the forest stand and the NH_4^+ found in open site deposition indicates that if collecting moss in the open is not possible, they can be collected within the forest stand. However, the data variability is greater in this situation due to the influence of habitat (Table 3) and thus different models should be used to calculate the amount of atmospheric N deposition (Table 2, Fig. 3).

We could not confirm the findings of Liu et al. (2007), who reported that habitats potentially regulate the $\delta^{15}N$ values of mosses. Our results show that the differences in these values were not significant between samples. Nevertheless, our results also show that the $\delta^{15}N$ values of mosses are more negative within the forest stand. This indicates that the source of N in the moss tissue inside the area of canopy projection is more enriched with reduced N compounds than in the area outside canopy projection. On the contrary, however, Heaton et al. (1997) showed that dry deposition and particulates captured by the tree crown are reflected by higher $\delta^{15}N$ values in the throughfall deposition compared to open site deposition. It should be noted that in the latter study, all analyses were conducted in coniferous forests, which have higher deposition velocities (de Schrijver et al., 2008). Other studies have also showed that the $\delta^{15}N$ value in moss is influenced by altitude and rainfall regimes (Zechmeister et al., 2008) and is species-specific (Liu et al., 2010). These influential factors may be the reasons explaining the differences in the results, and further investigations are needed to examine this issue.

In summary, our study answers some open questions regarding the way different moss growing conditions can bias the information obtained from mosses to assess atmospheric N and S deposition levels. Our results show that *H. cupressiforme* sampled within forest openings reflects the atmospheric level of N but apparently does not reflect the atmospheric S deposition. The results showed that an especially short distance (<1 m) between the sampling location and the nearest tree canopy projection increases the N concentration in the moss, and significantly higher values are found within the forest stand. Since the horizontal and vertical structure of forests varies across Europe significantly and the experimental protocol of the European Moss Survey, especially the distance of the sampling location to the nearest tree canopy, cannot be followed everywhere, we would suggest that a detailed metadata protocol, describing the boundary condition of sampling, is established. This metadata should be evaluated together with the measurement data and with this relate the results of element concentration in moss tissue to the boundary conditions. Additionally this kind of investigations could introduce correction factors in moss surveys and those correction factors could be also applied to past surveys where metadata like the distance to the nearest tree crown projection were recorded.

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2.1.2 Potencialni okoljski vplivi na vsebnost N in vrednosti δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.), nabranem znotraj in zunaj območja sestojnih padavin/skozi krošnje prepuščenih padavin

Potential environmental factors that influence the nitrogen concentration and δ^{15}N values in the moss *Hypnum cupressiforme* collected inside and outside canopy drip lines

Mitja Skudnik, Zvonka Jeran, Franc Batič, Primož Simončič, Damijana Kastelec
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Vzorci mahu *Hypnum cupressiforme* so bili nabrani na 103 lokacijah v gozdovih Slovenije. Na vsaki lokaciji so bili mahovi nabrani na naslednjih mestih: pod drevesnimi krošnjami ter v bližnji vrzeli. Rezultati kažejo, da vsebnosti N v mahovih, nabranih v gozdnih vrzelih, odražajo značilnosti okoliške rabe tal in posledično glavne vire emisij N. Medtem ko vrednosti N vzorcev, nabranih pod drevesnimi krošnjami, odražajo značilnosti gozda na lokaciji in ne glavnih virov emisij N zunaj gozda. Za napovedovanja vsebnosti N v mahovih v gozdnih vrzelih v odvisnosti od vsebnosti N v mahovih pod drevesnimi krošnjami in drugimi okoljskimi spremenljivkami so bili izdelani regresijski modeli. V povezavi z glavnimi viri emisij N je bila v članku razložena prostorska porazdelitev vsebnosti N in vrednosti δ^{15}N v mahovih, nabranih v gozdnih vrzelih in pod drevesnimi krošnjami.

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Potential environmental factors that influence the nitrogen concentration and $\delta^{15}N$ values in the moss *Hypnum cupressiforme* collected inside and outside canopy drip lines

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A B S T R A C T

Samples of the moss *Hypnum cupressiforme* were collected at 103 locations in forests of Slovenia. At each location, samples were taken at two types of sites: under tree canopies and in adjacent forest openings. The results show that the moss collected in the forest openings reflects the surrounding land-use characteristics and, consequently, the main N emission sources. For moss sampled under canopies, the characteristics of the forest at the moss-sampling locations are more important than the main emission sources outside the forest. A regression model was used to provide the nitrogen (N) concentration in moss from the forest openings in relation to the N concentration in moss under canopies and other environmental variables. The spatial distribution of the locations of the N concentrations and $\delta^{15}N$ values in moss collected in the forest openings and under the canopies in relation to main N deposition sources is discussed.

1. Introduction

Increased concentrations of nitrogen (N) compounds have recently been identified as a critical load for the environment on a global scale (Krupa, 2003). Monitoring pollutants is essential to document the current status of and changes in the environment (Paoletti et al., 2010). Using moss as a biomonitor was first proposed in the late sixties (Rühling and Tyler, 1968), when it was used to monitor the atmospheric deposition of trace elements. Biomonitoring is methodologically easier and cheaper than monitoring precipitation or conducting air analyses; consequently, a much higher sampling density can be achieved (Harmens et al., 2011). In 1990, the European moss biomonitoring network was established. Initially, the aim was to determine the spatial patterns of the atmospheric deposition of trace elements (Rühling, 1994). Since 2005–2006, N concentrations have also been determined (Harmens et al., 2008).

Reduced and oxidized N compounds have different ^{15}N isotope signatures ($\delta^{15}N$). Based on the $\delta^{15}N$ values in moss, the N emission sources can be hypothesized (Pearson et al., 2000). If the $\delta^{15}N$ value in moss is low (more negative), then N is mainly derived from agricultural sources (NH_x). If the $\delta^{15}N$ value is high (less negative), then N originates from combustion processes (NO_x) (Gerdol et al., 2014; Heaton, 1986; Larsen et al., 2007).

The general suitability of moss for monitoring atmospheric N deposition has been shown in numerous studies (Harmens et al., 2014 and references therein). Apart from atmospheric deposition, other environmental factors also contribute to the variation in N concentrations in moss (Harmens et al., 2011; Schröder et al., 2010). Schröder et al. (2014) noted that the influential factors could differ among landscapes with different ecological characteristics.

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Environmental factors that can explain the variation in $\delta^{15}N$ values in moss were discussed by Zechmeister et al. (2008) and Bragazza et al. (2005).

An important factor that influences the N concentration in moss tissue is the canopy drip caused by impaction (filtering). Moss that is collected under tree canopies in forest stands receives additional inputs of N (Kluge et al., 2013; Samecka-Cymerman et al., 2010; Skudnik et al., 2014). To obtain consistent comparisons of N concentrations in moss among the countries participating in the European Moss Survey, the experimental protocol of ICP-Vegetation (International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops) stipulated that the shortest distance between the moss sampling location and the nearest tree-canopy projection should be 3 m (ICP Vegetation Coordination Centre, 2010). However, climates, forest structures and forest management vary significantly across Europe; the occurrence of moss species depends on particular microhabitat characteristics (such as tree crown shadows) (Glime, 2006; Sabovljević et al., 2014). Therefore, for some moss species, the 3 m threshold could not be adhered to (Skudnik et al., 2014). Additionally, some countries intentionally decided to collect moss under canopies inside the canopy drip line (Pesch et al., 2008).

A detailed metadata protocol that describes the boundary conditions of moss-sampling locations was suggested for comparing N concentrations in moss among the countries that participated in the European moss survey (Pesch et al., 2008; Schröder et al., 2010; Skudnik et al., 2014). Improved knowledge regarding the impacts of environmental factors on the elemental concentrations in moss could lead to more consistent concentration comparisons among and within countries.

Until recently, no detailed analyses had been conducted regarding how the environmental factors that influence the N concentrations and $\delta^{15}N$ values in moss differ among moss samples taken at the same location but at different sites, i.e., in forest openings and under tree canopies.

Furthermore, environmental factors that could explain the differences among the N concentrations in moss at different sites in the same region had not been explored. It is unknown whether N concentrations in moss collected under canopies and in forest openings show similar N pollution patterns and whether this pattern is consistent among different sampling sites.

Therefore, the aims of this study are as follows: (1) investigate the spatial distribution of N concentrations and $\delta^{15}N$ values in moss collected under canopies and in adjacent forest openings; (2) evaluate the influence of select site-specific environmental characteristics on the N concentrations and $\delta^{15}N$ values in moss sampled under canopies (hereafter N_{canopy} and $\delta^{15}N_{canopy}$, respectively) and in forest openings (hereafter N_{open} and $\delta^{15}N_{open}$, respectively); and (3) identify to what extent the additional N inputs in the moss collected under canopies, compared with the N concentrations in mosses collected in forest openings, could be explained by a statistical model by accounting for the select environmental characteristics.

2. Material and methods

2.1. Moss collection and chemical analysis

Samples of cypress-leaved plait moss (*Hypnum cupressiforme* Hedw.) were collected in the summer of 2010 at 103 locations in forests of Slovenia. The dominant land-use type in Slovenia is forest, which covers 62% of the total area (12,530 km^2 of 20,140 km^2) (FAO, 2011). Of the 103 locations, 91 were located on a 16 km × 8 km systematic sampling grid. A location was included in the sampling area if the intersection of the 16 km × 8 km grid was located inside the forest area. Specifically, we wanted to obtain the N information for forests (more densely forested areas should have a greater number of moss-sampling locations), and we wanted to limit the survey expenses by integrating the field work of the National Forest Inventory (NFI) and the European Moss Survey. Consequently, in areas with a smaller percentage of forest cover, the number of moss-sampling locations is smaller.

Additionally, moss samples were collected in the vicinity of the ICP-Forest Level II research plots (International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests) and near Slovenian national N-deposition monitoring sites. At each location, moss samples were taken from two types of sites: a) under tree canopies (N_{canopy}, inside the area of the canopy drip line) and b) adjacent (<2 km) forest openings/clearings (N_{open}, outside the area of the canopy drip line).

Canopy sampling was conducted at the NFI plots. If there was no *H. cupressiforme* at the plot, then moss was collected in the surrounding area but within a similar forest structure.

For each subsample, the distance to the nearest tree canopy was recorded according to the following four categories: under canopies (i. 0 m) and in forest openings (ii. <1 m, iii. 1–3 m, iv. >3 m). With the exception that the minimum distance between the moss-sampling location and the nearest tree canopy projection should be at least 3 m (a requirement that was not always met), sampling was performed according to the guidelines of the ICP Vegetation Coordination Centre, 2010. For a more detailed description of the

Table 1
Predictors used to investigate the influence of sampling location on element content in moss. Categorical variables are marked with bold letters.

Regressor group	Regressor	Data type	Data provider
Location of moss sampling	**Distance from each moss subsample at each sampling site to nearest tree crown, distance to nearest shrub, growth substrate, topography**, slope, exposition, altitude	Site-specific	Field assessment
Characteristics of surrounding forest	Basal area, **canopy closure, tree species mixture**, top tree height, age of the stand, **development phase**, number of trees per hectare, **type of bedrock**	Site-specific	Slovenian Forestry Institute
Climate data	Average yearly precipitation (1971–2000), number of days with more than 30 mm of rain (1971–2000), average air temperatures (1971–2000), average air humidity (1971–2000), sunshine duration (1971–2000), average snow cover duration (1971–2000), average wind speed 50 m above ground (1994–2001)	Raster map – resolution 100 × 100 m	Slovenian Environmental Agency
Precipitation data	Yearly precipitation (average for period 2008–2010), sum of monthly precipitation, number of days with snow cover (average for period 2008–2010)	Raster map – resolution 100 × 100 m	Slovenian Environmental Agency
Land use – local scale	Forest land use, agricultural land use, urban land, water land (0.5, 1, 5, 8 km radius)	Shape map	Ministry of Agriculture and the Environment
Land use – regional scale	Forest land use, agricultural land use, urban land, water land (40, 80, 100 km radius)	Raster map – resolution 100 × 100 m	European Environment Agency

sampling methodology, see Skudnik et al. (2014).

Chemical analyses of moss followed the ICP-Vegetation protocol (ICP Vegetation Coordination Centre, 2010). N concentrations were determined using an LECO CNS-2000 at the Department of Forest Ecology at the Slovenian Forestry Institute (SFI). The $\delta^{15}N$ values were determined at the Department of Environmental Sciences, Jožef Stefan Institute, using an isotope ratio mass spectrometer (IRMS) - Europa Scientific 20–20, which has an ANCA SL preparation module for solid and liquid samples. The results are reported as relative $\delta^{15}N$ values in per mille (‰), which is the difference (in parts per mille) between the isotopic ratios $^{15}N/^{14}N$ and the atmospheric N (Air).

Quality control was ensured by using moss reference material M2 and M3 (Steinnes et al., 1997) and laboratory standards. The results from the laboratory agreed well with the recommended values (M2 was 8.27 ± 0.23 mg N g^{-1} dry wt; and M3 was 6.72 ± 0.26 mg N g^{-1} dry wt) (Harmens et al., 2014). A more detailed description of the chemical analyses and quality control results are presented in Skudnik et al. (2014).

2.2. Explanatory environmental characteristics

To explore the environmental characteristics that might influence the N concentration and $\delta^{15}N$ values in moss, data on environmental characteristics were collected at all sites. Fifteen site-specific characteristics were assessed directly in the field, and 16 regional characteristics were acquired later with geographic information system tools (Table 1). Field variables describing the characteristics of the sampling locations were recorded in parallel with the moss samples. Additionally, forest characteristics at the NFI plots were assessed according to the national forest monitoring guidelines (Kovač et al., 2014). Information on the types of bedrock was acquired from the NFI for the year 2000 (Kovač et al., 2000). For all sampling sites, information on climate characteristics, including the average wind speed 50 m above ground, was obtained from the Slovenian Environment Agency (ARSO). All maps used are available online (ARSO, 2006). Daily precipitation data for 2008–2010 were acquired from the ARSO, which collects information from 241 metrological stations across the country. Using Universal Kriging for spatial interpolation (Cressie, 1993), monthly raster maps with a 100 m resolution were created, and information from the moss sampling sites was overlaid with the raster maps.

To explore the possible sources of the main N emissions, the proportions of land use were derived from a national land-use map (MKGP, 2010) and from the Corine land cover map of 2006 (EEA, 2006). Because of its superior spatial resolution, the national land-use map was used for the local-scale analysis (radii of 0.5, 1, 5 and 8 km); the Corine land cover map was used for the regional-scale analysis (radii of 40, 80 and 100 km). The radii were based on the known atmospheric transport and deposition of N (Asman et al., 1998; Hertel et al., 2011). All GIS analyses were conducted with ArcGIS 10.0 software (ESRI, 2011).

2.3. Statistical analysis

Multiple linear regression models were used to determine which environmental characteristics explain the variation in the N concentration and the $\delta^{15}N$ values in moss. Linear regression

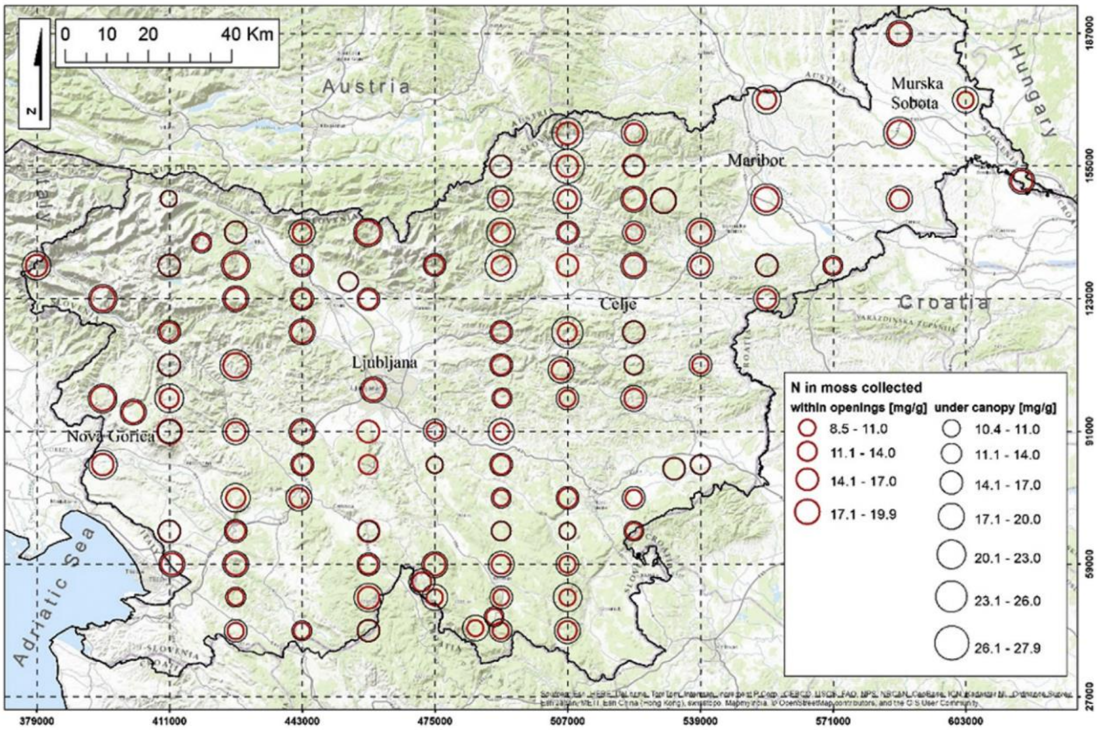

Fig. 1. Spatial presentation of N_{open} and N_{canopy} measurements in moss. The size of the circle in the legend shows higher values for N_{canopy} (coordinates on the map are in the Gausse–Krüger coordinate system).

models were used instead of frequently used Classification and Regression Trees (CART) (Schröder et al., 2014) because the data on N concentrations and $\delta^{15}N$ signatures were normally distributed such that parametric statistics could be applied. The regression diagnostic (analysis of residuals and influential points, heteroscedasticity, linear relationship between explanatory variables and dependent variables) showed that the regression models performed well.

The regressors, which explained most variation in the response variables, were chosen on the basis of hierarchical regression model comparisons with a partial F-test. The chosen regressors were ordered based on the partitioning of the coefficient of determination (R^2) by averaging the ordered values with the metric "lmg" using the "relaimpo" package in R (Grömping, 2006). The relative importance of the regressor indicates its contribution to the variation in the response in a multiple regression model while considering the correlation among all of the regressors in the model. All statistical analyses were performed with R 3.0 (R Development Core Team, 2014).

3. Results

3.1. N concentration and $\delta^{15}N$ values in moss in Slovenia

A spatial representation of the measured N_{open} and N_{canopy} in moss tissue is shown for all 103 locations in Fig. 1. N_{open} in the moss *H. cupressiforme* ranged between 8.5 and 19.9 mg/g, with an average of 13.1 ± 2.7 mg/g. N_{canopy} ranged between 10.4 and 27.9 mg/g, with an average of 17.5 ± 3.7 mg/g. The highest values of N_{open} occurred in the western mountains of Slovenia around large cities, such as Ljubljana, Celje and Maribor and in areas with intensive agriculture in the eastern part of the country. Similar results are also shown on the map of N_{canopy}, with some exceptions, which are depicted as the differences between the red and black circles (Fig. 1). For N_{canopy}, some forests in the southeastern and northeastern part of the country also have high N concentrations. Fig. 1 shows that N_{canopy} was higher than N_{open} at 95 of the 103 locations.

The spatial distribution of $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ was measured

Table 2
Variation in the N concentrations and the $\delta^{15}N$ values in moss explained by the linear models (increase of element concentration in moss – italicized letters, decrease of element – underlined letters, categorical variables – bold letters). Explanatory environmental characteristics are stated according to the relative importance of the regressor (in brackets) in the linear model.

Element	Explanatory variables	R^2
N_{open}	*% of urban land within 80 km radius (19%), sum of 120 days of precipitation (11%),* **distance to the nearest tree (9%),** *altitude (4%), % of agricultural land within 5 km radius (3%)*	0.46 $p < 0.001$
N_{canopy}	**canopy closure* (11%), tree mixture** (9%),** *average wind speed 50m above ground (7%),* % of forestland within 0.5 km radius (6%), *% of urban land within 40 km radius (6%), sum of 120 days of precipitation (2%)*	0.41 $p < 0.001$
$\delta^{15}N_{open}$	**tree mixture** (10%)**, altitude (7%), % of agricultural land within 0.5 km radius (6%), **distance to the nearest tree(5%)**	0.28 $p < 0.001$
$\delta^{15}N_{canopy}$	altitude (12%), **tree mixture** (6%),** % of agricultural land within 5 km radius (3%)	0.21 $p < 0.001$

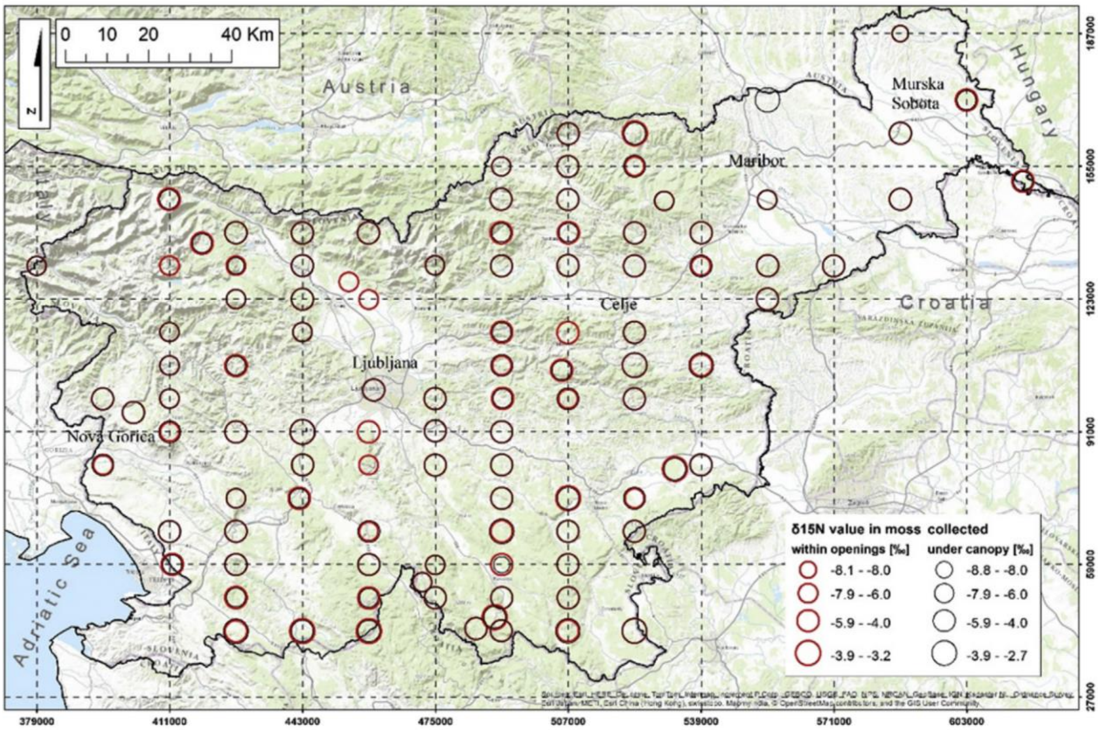

Fig. 2. Spatial presentation of the $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ values in moss (coordinates on the map are in the Gausse–Krüger coordinate system).

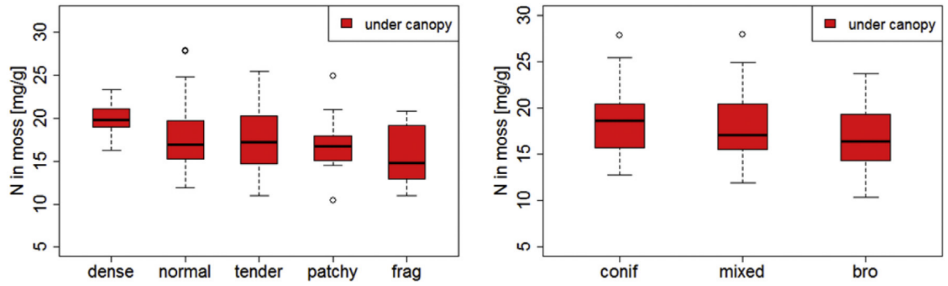

Fig. 3. Boxplots of the influence of canopy closure* and tree mixture** on N_{canopy} (*canopy closure describes the intensity of interaction of the tree crowns in a stand: dense – crowns crowded and in close contact, normal – no or only slight mutual contact or influence, tender – only small gaps in the canopy, patchy – gaps in canopy large enough to insert single crowns or several crowns, fragmented – area stocked with individual single trees that have no contact with each other; ** tree mixture describes the percent of area that is covered with coniferous or broadleaved trees: conif – coniferous > 75%, mixed, bro – broadleaved > 75%).

in moss and is shown in Fig. 2. The mean $\delta^{15}N_{open}$ was −5.4‰ (min = −8.1‰, max = −3.2‰, and sd = 1.0‰) and the mean $\delta^{15}N_{canopy}$ was −5.5‰ (min = −8.8‰, max = −2.7‰, and sd = 1.1‰). The $\delta^{15}N_{open}$ values were more negative in the mountainous parts of Slovenia and in lowlands with intensive agriculture. Less negative $\delta^{15}N_{open}$ values were measured in locations around city of Ljubljana and in the southeastern part of the country. There were no differences in the spatial patterns between the $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ values; the measures were similar at all but a few locations. No significant correlation existed between the N concentrations and $\delta^{15}N$ values.

3.2. *Influence of environmental characteristics on the N concentration and $\delta^{15}N$ value in moss*

The environmental characteristics that significantly influence N_{open}, N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ in moss are presented in Table 2. For N_{open}, the most important explanatory variable is the percent of urban land within an 80 km radius of the moss-sampling location. Considering all other regressors in the model, this variable accounts for 19% of the N_{open} variability explained by the model (46%). With increasing precipitation, altitude and percent of agricultural land within a 5 km radius, the value of N_{open} also increases. In contrast, with greater distance from the nearest tree, N_{open} decreases (Table 2).

The most important factor controlling the variability in N_{canopy} is the forest structure (i.e., the canopy closure and tree species composition) rather than the emission source. The model for N_{canopy} explained 41% of the data variability. N_{canopy} was higher in forests with dense canopy closure under coniferous trees (Fig. 3). N_{canopy} also increased with higher wind velocities 50 m above the ground and with higher amounts of precipitation in the three months leading up to the moss sample collection. A higher percentage of forest land within a 0.5 km radius of the moss-sampling location corresponds to a smaller N_{canopy}; in contrast, a higher percentage of urban land within a 40 km radius corresponds to a higher N_{canopy} (Table 2).

The three variables (tree species composition, altitude and percentage of agricultural land within 5 km of the moss-sampling location) only explain 28% of the variability in $\delta^{15}N_{open}$ (Table 2). High altitudes (Fig. 4 – left) and nearby agricultural land result in a more negative $\delta^{15}N$ values in moss. Additionally, $\delta^{15}N$ depends on

Table 3
Results of the linear model for the prediction of N_{open} on the basis of N_{canopy} and additional environmental variables that significantly influence the relationship between the N in moss from both sampling locations.

Predicted variable	N_{open}		
R^2	0.54		
p	<0.001		
Number of pairs	103		
Regressors with relative importance of the regressor (bold values)	Estimate	Lower CI	Upper CI
Intercept	−0.096	−3.653	3.461
N_{canopy} – 18%	0.251	0.089	0.413
% of urban land within 80 km radius – 16%	1.087	0.252	1.922
sum of 120 days of precipitation – 9%	0.005	0.001	0.009
distance to the nearest tree – 7%	0.000	−0.137	2.996
> 3 m	1.429	0.104	4.094
1–3 m	2.099		
0–1 m			
Altitude – 3%	0.002	−0.001	0.004
% of agricultural land within 5 km radius – 2%	0.039	−0.022	0.099

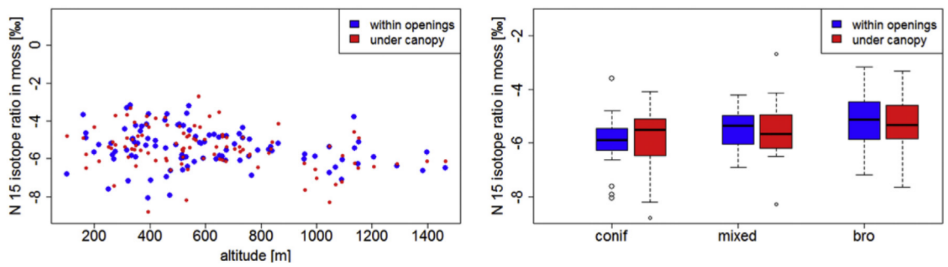

Fig. 4. Relationship between $\delta^{15}N$ values in moss and altitude (left graph); $\delta^{15}N$ values in moss for different tree mixture** (right graph).

the tree species composition; in coniferous stands, the values are more negative (Fig. 4 – right).

3.3. Environmental characteristics that explain N_{open} and N_{canopy} differences

To predict N_{open} at locations where only N_{canopy} measurements exist, a multiple linear model was constructed to account for N_{canopy} and environmental site characteristics (Table 3). The model explained 54% of the N_{open} variability. The most important explanatory variable was N_{canopy}, followed by the percentage of urban land within an 80 km radius, the cumulative precipitation over 120 days and the distance to the nearest tree. The results of the model show that if N_{canopy} increases by 1 mg/g and if all other explanatory variables included in the model are constant, then N_{open} increases by 0.24 mg/g (95% confidence interval from 0.1 mg/g to 0.4 mg/g) (Table 3). If N_{canopy} is excluded from the model, then only those environmental characteristics that explain the variability in N_{open} remain in the model (Table 2).

4. Discussion

4.1. N concentrations and $\delta^{15}N$ values in moss in Slovenia

Regarding N_{open} in moss in Slovenia, most of the investigated sites can be considered rural or background sites. This research design is in accordance with the ICP Vegetation Programme, whose goal is to explore the N concentrations in moss in nonurban areas and to explore long-range transboundary N pollution rather than local N sources (ICP Vegetation Coordination Centre, 2010). The N_{open} mean of 13.1 mg/g (min = 8.5 mg/g and max = 19.9 mg/g) (Fig. 1) was high compared with North European sites, such Finland (Poikolainen et al., 2009) where the N pollution is the lowest in Europe (Harmens et al., 2014), but it is comparable to those values in neighboring Austria (mean = 12.1 mg/g, min = 7.6 mg/g, max = 19.9 mg/g) (Zechmeister et al., 2008).

The mean value of N_{canopy} in Slovenia was 17.5 mg/g (min = 10.4 mg/g and max = 27.9 mg/g) (Fig. 2), which is higher than that of N_{open}. The influence of canopy drip on the N concentration and $\delta^{15}N$ values in moss is discussed in more detail by Skudnik et al. (2014). The greatest difference between N_{open} and N_{canopy} was observed in the southeastern and northeastern parts of the country (Fig. 1). In southern Slovenia, forests are dominant. Other locations with larger differences between N_{open} and N_{canopy} could be observed near two thermal power plants (TE Šoštanj and TE Trbovlje) and along sections of some highways. A comparison of the mean N_{canopy} with the N concentrations in moss reported in other European countries in 2010 indicates that Slovenia has among the highest atmospheric N depositions in Europe (Harmens et al., 2013). This finding shows how important moss-sampling locations are in national and international reports, particularly when the moss samples are collected under canopies.

In Slovenia, the mean $\delta^{15}N_{open}$ value of −5.4‰ (min = −8.1‰ and max = −3.2‰) and the mean $\delta^{15}N_{canopy}$ value of −5.5‰ (min = −8.8‰ and max = −2.7‰) are comparable with the results reported for Spain (mean = −5.6‰, min = −7.9‰, and max = −3.3‰) but are less negative than those reported for France (mean = −6.0‰, min = −8.0‰, and max = −3.3‰), Switzerland (mean = −6.9‰, min = −10.8‰, and max = −3.3‰) (Foan et al., 2014) and neighboring Austria (mean = −6.0‰, min = −10.0‰, and max = −2.5‰) (Zechmeister et al., 2008). The results show that, compared with the surrounding countries, Slovenia derives more atmospheric N from NO_x (combustion processes) than from NH_x (agricultural sources) (Beyn et al., 2014). However, the differences among the countries in this part of Europe are still negligible. For example, the Slovenian Environment Agency, which is responsible for the national program for deposition quality, reported higher values for reduced N than for oxidized N in 2012, with average dry depositions of 0.80 µg/m^3 for (NH_3 + NH_4)–N and 0.26 µg/m^3 for (HNO_3 + NO_3)–N and average wet depositions of 3.8 kg/ha year for NH_4–N and 3.0 kg/ha year for NO_3–N (Bolte et al., 2013).

4.2. Influence of environmental characteristics on N concentrations and $\delta^{15}N$ values in moss

Our results show that the environmental factors that influence N_{open} differ from those influencing N_{canopy}. For N_{open}, the percentage of urban land within an 80 km radius is the most important variable, which shows that the N emission sources explain the highest percentage of the data variability. In contrast to N_{canopy}, the main N emission source is overshadowed by the characteristics of the forest at the moss-sampling location (canopy closure and tree species composition) (Table 2). N_{open} decreases with increasing distance from the nearest canopy (Skudnik et al., 2014). Therefore, if we collect moss under canopies within the canopy drip line, then we must consider that forest characteristics also influence the variability in N concentrations in the moss. Deposition studies show that under the forest canopy, the deposition that reaches the forest ground could be enriched or reduced for particular ions, depending on the ion reactivity and forest type (De Schrijver et al., 2007; Houle et al., 1999). The N in moss is higher in forests with more dense stands and with a higher percentage of coniferous trees (Fig. 3); this finding is consistent with other studies that showed that coniferous forest floors receive larger N throughfall depositions then deciduous stands in similar climatic conditions (De Schrijver et al., 2008; Van Ek and Draaijers, 1994; Wuyts et al., 2008). Specifically, coniferous forests have a greater stand (number of trees per hectare) and crown densities (Cole and Rapp, 1981). Wind is one of the most important factors in the transport of air pollutants, particularly for dry deposition and for particles that are eventually captured by tree crowns (i.e., needles), which are efficient at collecting droplets and particles (Erisman and Draaijers, 2003). Our investigation indicates that the forest structure (canopy closure and tree species composition) and climate characteristics (average wind speed) tended to be the most important factors for explaining additional inputs of N_{canopy}. De Schrijver et al. (2007) reported similar environmental factors that defined the ratio between the N in throughfall and bulk deposition.

The importance of the amount of precipitation in the 4 months leading up to the moss collection in the forest openings (Table 2) suggests that moss surveys should be conducted in drier periods of the year to reduce the data variability among the locations. Dependence of N_{open} on altitude was positive (Table 2), which is in accordance with findings that deposition rates of pollutants are greater at higher elevations (Miller et al., 1993). In the literature, the dependence of N concentrations in moss on altitude differed. Some studies found a positive dependence (Baddeley et al., 1994; Zechmeister et al., 2008), whereas others found a negative dependence (Schröder et al., 2010). Hicks et al. (2000) reported a linear increase in the N deposition with altitude but found no relationship for the moss *Hylocomium splendens*. These discrepant results indicate that the dependence could be species- and relief-specific, and further research is needed.

Land-use characteristics that influence the N concentration in moss, in contrast to other environmental characteristics that were explored in this study, address whether moss reliably reflects the main N emission sources. The results of the models (Table 2) are in agreement with other studies, which also identified urban land as an important source of anthropogenic N in Europe (Schröder et al., 2010). The main emissions from urban land are oxidized N (NO_x) and are mainly produced by traffic, industry and power production. NO_x has little impact close to the emission source because it is mainly emitted as N monoxide (NO) and N dioxide (NO_2), with low dry deposition rates (Hertel et al., 2011). The low deposition rates of NO_x close to the source are consistent with the higher radii (80 km for N_{open} and 40 km for N_{canopy}) found by the models and are important for explaining the N concentrations in moss (Table 2). However, reduced N (NH_x), particularly ammonia (NH_3), has a large impact near the source due to the high dry deposition rates (Asman et al., 1998; Dungait et al., 2012). Our model for N_{open} showed similar results; the percentage of agricultural land within a 5 km radius tended to be important (Table 2), but the percentage of agricultural land within a larger radius, such as 40, 80 or 100 km, was not. For N_{canopy}, the percentage of forest cover was important, and the dependence was negative. A higher percentage of forest corresponds to a lower percentage of agricultural land or urban land and therefore less N pollution; additionally, a higher percentage of forest corresponds to a smaller forest edge effect, which could significantly influence higher N element inputs (Wuyts et al., 2008).

The influence of altitude on $\delta^{15}N$ was negative (Fig. 4 – left), which supports the findings for other vascular plants (Männel et al., 2007) and supports the observation that with increasing altitude, the amount of precipitation increases. Because ammonium (NH_4) is removed by precipitation much more efficiently than NO_x (Asman et al., 1998), the reduced N in moss could increase. Compared with other types of coniferous forests, the $\delta^{15}N_{canopy}$ values were more negative (Fig. 4 – left), supporting the open chamber experiment in which the deposition velocity of NH_3 was higher near spruce than near beech (Huber et al., 2002). The negative influence of the percentage of agricultural land within 5 km of the moss sampling site on $\delta^{15}N$ confirms that, despite the influence of environmental factors, the $\delta^{15}N$ values in moss are an indicator of the N source.

4.3. *Environmental characteristics explain the differences between N_{open} and N_{canopy}*

Skudnik et al. (2014) noted that in some areas, it is not possible to follow the ICP-Vegetation guideline for collecting moss outside the influence of the tree canopies (ICP Vegetation Coordination Centre, 2010) to avoid the canopy leaching effect and higher N concentrations in moss tissue (Kluge et al., 2013; Samecka-Cymerman et al., 2010; Skudnik et al., 2014). With the aim of estimating N_{open} from N_{canopy}, we presented a model showing which environmental characteristics influence N_{open} near N_{canopy} (Table 3). Notably, N_{open} exhibits a better relationship with atmospheric N deposition compared with N_{canopy} (Skudnik et al., 2014).

This type of investigation could introduce correction factors into moss surveys and provide more consistent comparisons among moss-sampling locations. Still, the model explains only 54% of the N_{open} data variation.

Potential users of the proposed model (Table 3) must be aware of the confidence intervals of the modeled results. One deficiency of the present analysis (models) is that, in our survey, it was possible to collect moss as far as 3 m from the canopy at only 14 locations; thus, the correction of N_{open} on the basis of N_{canopy} could still be overestimated. For example, Kluge et al. (2013) showed that the N_{canopy} values were twice as high as those of N_{open} if the moss were collected at least 10 m from the nearest canopy. The proposed model could be improved if the data on the N concentrations in the moss sampled in the same area but at different distances from the tree crown projection were available.

In summary, our map of N_{canopy} exhibits a similar spatial pattern as N_{open} in Slovenia except in the southeastern and northeastern parts of the country. A comparison of the mean N_{canopy} with the N concentrations in moss, as reported by ICP-Vegetation in 2010, indicates that Slovenia has one of the highest N atmospheric depositions in Europe.

In this study, we determined which environmental characteristics, apart from the atmospheric N deposition, influence the N concentrations and $\delta^{15}N$ values in moss. For moss collected in the same area but at different sites (under canopies and in forest openings), the N concentration and $\delta^{15}N$ values agree with the scientific knowledge that is based on monitoring precipitation or atmospheric analyses. N_{open} reflects the surrounding land-use characteristics and, consequently, the main N emission sources. For N_{canopy}, the main emission sources are overshadowed by the characteristics of the forest at the moss-sampling location.

Our findings indicate that knowing whether the moss was collected inside or outside the canopy drip line is important. The information should be included in national reports and should be considered when comparing moss-sampling locations within or among countries.

In specific areas where it is not possible to collect particular moss species outside of the tree canopy projection to avoid the canopy drip effect (i.e., where a higher N concentration in moss exists) (Kluge et al., 2013; Samecka-Cymerman et al., 2010; Skudnik et al., 2014), models could be used to convert N_{canopy} to N_{open}.

A detailed analysis on which environmental characteristics, other than atmospheric N deposition, influence the N concentration and $\delta^{15}N$ values in moss should be conducted for different ecological landscapes. Important descriptive site data should then be collected and included in the interpretation of the results.

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2.1.3 Prostorska interpolacija vsebnosti N in vrednosti δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.), nabranem v gozdovih Slovenije

Spatial interpolation of N concentrations and δ^{15}N values in the moss *Hypnum cupressiforme* collected in the forests of Slovenia

Mitja Skudnik, Zvonka Jeran, Franc Batič, Damijana Kastelec
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Mahovi so bili nabrani na 103 lokacijah in analizirani za vsebnost N in vrednosti δ^{15}N. Na vsaki lokaciji so bili mahovi, nabrani na dveh mestih: pod drevesnimi krošnjami ter v bližnji gozdni vrzeli. Prostorska korelacija je obstajala le pri N v mahovih, ki so bili nabrani v gozdnih vrzelih. V tem primeru se je za prostorsko interpolacijo podatkov uporabil osnovni kriging. V nasprotju s tem prostorska korelacija ni bila odkrita za N v mahovih, ki so bili nabrani pod krošnjami, niti za vrednosti δ^{15}N pri mahovih, nabranih na obeh vzorčevalnih mestih (v gozdni vrzeli ali pod krošnjami). V tem primeru je bila prostorska interpolacija podatkov narejena kot vsota regresijske napovedi in interpoliranih ostankov regresijskega modela. Za interpolacijo ostankov regresijskega modela smo uporabili matematično metodo, kjer se interpolirana vrednost izračuna kot linearna kombinacija vrednosti v okolici. Karte za N za obe mesti nabiranja mahu (pod krošnjami/v vrzeli) prikazujejo podobna območja s povečanimi vsebnostmi N. Edina izjema je bila, da so bili z mahovi, nabranimi pod drevesnimi krošnjami, izpostavljeni tudi nekateri lokalni onesnaževalci z NO_x.

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Spatial interpolation of N concentrations and $\delta^{15}N$ values in the moss *Hypnum cupressiforme* collected in the forests of Slovenia

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ABSTRACT

Mosses were collected from 103 locations and analyzed for N concentration and $\delta^{15}N$ values. At each location, the samples were collected from two types of site: under the tree canopies and in the adjacent forest openings. A spatial correlation was detected, and ordinary kriging could be applied only to the N in the mosses that were collected in the openings. In contrast, a spatial correlation was not detected for N in the mosses that were collected under the canopies or for the $\delta^{15}N$ of the mosses at either type of site. For those, the spatial interpolation was calculated as the sum of the regression predictions and the inverse distance weighted interpolation of residuals. The maps for the N at both site types (canopy/open) identified similar areas with increased N concentrations. The sole exception was the mosses collected under the canopy, for which some local emitters of NO_x were also identified.

1. Introduction

Historically, the availability of reactive nitrogen (N) compounds has been one of the primary factors that limit human activities (Sutton et al., 2011). After the Industrial Revolution, the emission of N into the atmosphere increased because of anthropogenic activities such as food (Rockstrom et al., 2009) and energy production (Galloway et al., 2008). Much of this new reactive N led to eutrophication and acidification in various types of ecosystems (Erisman et al., 2007). In recent years, the pollution with N has been problematic. It is not only a problem in highly industrialized countries and in those with intensive agriculture. It also has the potential to be problematic in countries such as Slovenia that are predominantly forested (Andelov et al., 2014).

Maps of atmospheric deposition patterns are often used to develop emission control policies (Fagerli and Aas, 2008) because the pollution maps are typically easy to interpret and the information on the selected pollutant is available for an entire area and not only for the selected locations. To construct this type of pollution map, different interpolation (prediction) techniques are available. The techniques share a common aim, which is to predict the concentration of the pollutant in a non-sampled area based on the concentrations measured in the sampled areas.

Until recently, 50 km × 50 km grid maps based on precipitation and dry deposition data (Simpson et al., 2012) were produced by the European Monitoring and Evaluation Programme (EMEP) transport model to identify the areas with high depositions of N in Europe (Posch et al., 2013). In some parts of Europe, the number of EMEP stations is low; consequently, the spatial accuracy of the modeled EMEP data is low (Nyíri et al., 2010). The low number of stations for deposition measurements is often explained by the high costs of establishing and maintaining this type of monitoring system. To gain additional spatial information on the deposition of N, numerous biomonitoring techniques were developed, including the European moss biomonitoring survey, which in recent years, was coordinated within the ICP-Vegetation framework (International Cooperative Program on Effects of Air Pollution on Natural Vegetation and Crops) (Harmens et al., 2011, 2015). The advantages of this type of monitoring are that the methodology of data

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collection is comparatively simple and the chemical analyses are less expensive; consequently, the sampling density can be much higher (Markert et al., 2003). The increase in sample density is indeed highly important for the monitoring of N pollution because some N compounds, particularly NH_3, may be highly spatially variable (Asman et al., 1998). The general suitability of mosses for identifying areas at risk for high atmospheric deposition of N has been demonstrated by numerous studies (Harmens et al., 2011, 2014, and the included references). However, some studies have reported only a weak dependence of N in moss tissue on the N in deposition. Numerous explanations for these weak correlations have been discussed; they include the following:

(i) Mosses regulate the N tissue loads because of the important role of N in moss metabolism (Arróniz-Crespo et al., 2008).
(ii) Different species of moss have different abilities to bind atmospheric N (Harmens et al., 2014; Solga et al., 2005; Varela et al., 2013).
(iii) In addition to the atmospheric N, other environmental variables also influence the N concentrations in the mosses (Schröder et al., 2014, and the included references; Skudnik et al., 2015);
(iv) The N concentrations in the mosses can be affected by the selection of the locations to sample the mosses, particularly in the zone of canopy drip (Kluge et al., 2013; Samecka-Cymerman et al., 2010; Skudnik et al., 2014).

Within a survey area, numerous different sources of N emission can occur. These sources are roughly divided into two groups that emit primarily reduced N (NH_y) or oxidized N (NO_x). The $^{15}N/^{14}N$ ratios ($\delta^{15}N$ value) differ between the oxidized and the reduced forms of N; the values are more negative when the N originates as NH_y from agricultural sources such as fertilizer and cattle, and the values are less negative when the N originates as NO_x from combustion processes in the production of energy (power plants and heating) and in transportation (Heaton, 1986; Phillips and Gregg, 2003). Because the uptake of N in mosses is directly from the atmosphere, the ratios of the stable isotopes of N (the $\delta^{15}N$ value) can be used to determine the source of the N in the atmospheric deposition (Bragazza et al., 2005; Gerdol et al., 2014; Liu et al., 2008; Pearson et al., 2000; Skudnik et al., 2014; Solga et al., 2005; Zechmeister et al., 2008) and to identify the primary N emitters in the surroundings of the sample location.

Numerous regional and international reports and scientific articles have been published with maps of heavy metal pollution. These maps were created from moss biomonitoring survey data (Aboal et al., 2006 and references therein). Until recently, however, only a few articles have examined the spatial interpolation of N concentrations in the mosses. For the techniques that are currently in use, descriptions are found for ordinary kriging (Kapusta et al., 2014; Pesch et al., 2007, 2008; Zechmeister et al., 2008), regression kriging (Schröder et al., 2011) and an unspecified type of kriging (González-Miqueo et al., 2009, 2010; Mohr et al., 2009; Schröder et al., 2007). For the spatial interpolation of the $\delta^{15}N$ values, Zechmeister et al. (2008 – ordinary kriging) and Varela et al. (2013 – unspecified type of linear interpolation) provide descriptions of the techniques. In most of these studies, with the exception of Pesch et al. (2007, 2008) and Kapusta et al. (2014) the collection of mosses followed the guidelines of ICP-Vegetation (2010). Compliance with these guidelines implies that the moss samples were collected, if possible, in the open field to obtain the results of atmospheric N depositions, avoiding the canopy drip effect. From a forest ecology perspective, one seeks to obtain estimates of the atmospheric N deposited on the forest overstory or in clearings. In contrast, there were only a few studies in which the authors tried to obtain information on N deposited on the forest floor, beneath the tree canopies.

Throughfall deposition studies have shown that under the forest canopy, the deposition of certain ions can be enriched or reduced (De Schrijver et al., 2007; Houle et al., 1999), and this information could be very important when assessing the possibility that N exceeds the critical load value within forest ecosystems (Lorenz et al., 2008).

The accuracy of pollution maps is dependent on the sample design, the measurement error and the quality of the interpolation. However, with the exception of Varela et al. (2013) and Pesch et al. (2007), little attention has focused on the type of geostatistical technique used for spatial interpolation of the moss data, the reason for the use of a particular geostatistical technique, or the accuracy of the maps produced by a particular technique. These factors are particularly important because the selection of the interpolation technique will influence the results (Margalho et al., 2014).

Therefore, the aims of this study are as follows:

(1) We sought to explore the different techniques for the spatial interpolation of N concentrations and $\delta^{15}N$ values in the mosses that were collected within or outside the area of the canopy drip line (methods given in Sections 2.1 and 2.2, results in Section 3.1).
(2) We aimed to show, with Slovenia as a case study, which information about the spatial interpolation methods used is essential as a topic for discussion and inclusion in the published results when presenting maps of N concentrations and $\delta^{15}N$ values for moss biomonitoring surveys (methods given in Section 2.2, results in Section 3.2).
(3) We aimed to explore the spatial distributions of the N concentrations and the $\delta^{15}N$ values in mosses on a national scale and to determine the connections between the spatial distributions and the primary sources of N deposition (methods given in Section 2.3, results in Section 3.3).

2. Materials and methods

2.1. Collecting moss and chemical analyses

Samples of cypress-leaved plait moss (*Hypnum cupressiforme* Hedw.) were collected in the summer of 2010 at 103 locations in the forests of Slovenia, which cover 62% of the total area of the country (FAO, 2011) (Fig. 1). Ninety-one of the 103 locations were located on a 16 km × 8 km systematic sampling grid, and the other locations were in the vicinity of the Slovenian Forestry Institute research plots (part of the ICP-Forest Level II plots) and near the location at which the Slovenian national monitoring of N deposition was assessed for reporting to the EMEP (Fig. 1). A location was included in the sample design only if the intersection on the 16 km × 8 km grid was located inside a forested area. Specifically, we wanted to obtain information on N for the forests of Slovenia (the more densely forested areas had a greater number of moss-sampling locations). The sampling grid design was used to ensure that the sampling locations uniformly covered the whole study area. Fernández et al. (2005) showed that the grid design was the most suitable design for the national moss biomonitoring surveys. Additionally because mosses were collected together with the fieldwork of the National Forest Inventory (NFI), expenses were lower.

At each location, the moss samples were collected (a) under the tree canopies (N_{canopy}, inside the area of the canopy drip line) and (b) in adjacent (<2 km) forest openings/clearings (N_{open}, outside the area of the canopy drip line). For each subsample, the distance to the nearest tree canopy was recorded. Skudnik et al. (2014) showed that the concentration of N in mosses increased with decreasing distance to the nearest tree canopy and that the influence of the canopy on the N concentration in moss was significantly higher

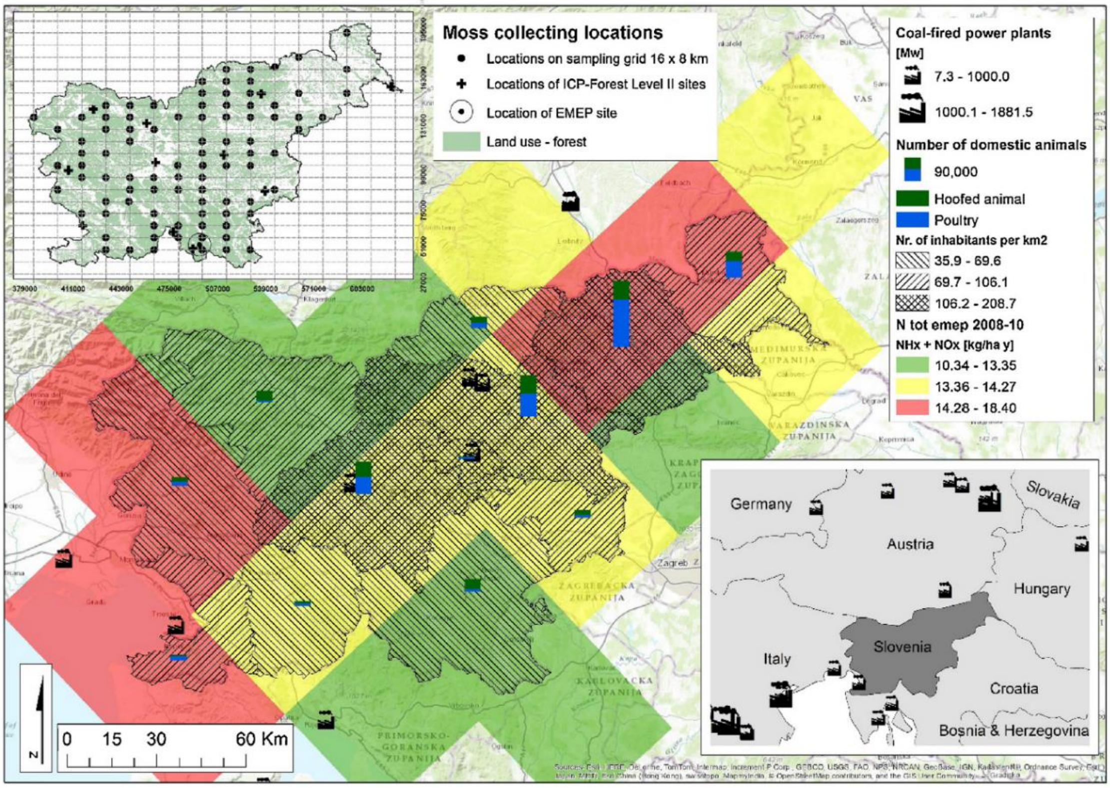

Fig. 1. Map of EMEP modeled deposition of the total oxidized and reduced N for grids 50 km × 50 km, with locations of coal-fired power plants and statistical data for the NUTS 3 regions (Nomenclature of Territorial Units for Statistics) for number of domestic animals, average number of inhabitants and locations of coal-fired power plants. In the upper left corner, the sampling grid design is presented and in the lower right corner, the location of Slovenia in relationship to the neighboring countries.

when the distance between the moss sample location and the nearest tree canopy projection was less than 1 m. It was further reported that the number of locations with such a small distance to the nearest tree crown was 15. Furthermore, for all these 15 locations in the present study, the N_{open} value was lowered by 10%. With such corrections of the N_{open} values, we recalculated the values to the distance of 1–3 m from the nearest tree canopy projection (Skudnik et al., 2014). For the ordinary kriging, the corrected values were used, but in all other analyses, the original data were used (see Section 2.3).

With the exception that, in some cases, the minimum distance between the location of the moss sample and the nearest tree canopy projection was less than 3 m, the sampling was performed according to the guidelines of the ICP Vegetation Programme Coordination Centre (2010). A more detailed description of the sampling methodology and the chemical analyses and quality control results was presented previously in Skudnik et al. (2014, 2015).

2.2. Spatial explanatory variables

The environmental variables that influenced the N concentrations and the δ^{15}N values in the mosses in Slovenia were presented in our previous paper (2015). For the purpose of the present work, the maps were prepared for all those significant variables (Table 1) and used for further work. The maps of the percentage of surrounding land use cover were derived from the national land use map (MKGP, 2010) and the Corine Land Cover map (EEA, 2006). With the "focal statistics" tool in ArcMap (ESRI, 2011), which is designed to calculate, for each cell in a raster file, a statistics of the values within a specified neighborhood around it, we calculated the sum of raster cells for the selected land use type within the selected radius. Because of the higher spatial resolution, the national land use map was used for the analyses on a local scale (radii of 0.5 and 5 km around the moss collecting location), and the Corine Land Cover map was used for the analyses on a regional scale (radii of 40, 80 and 100 km around the moss collecting location) (Table 1). Finally, the percentage of the total area covered by the selected land use type within the selected radius was calculated.

The daily precipitation data for the year 2010 were derived from Slovenian Environment Agency (ARSO), which collected information from 241 meteorological stations across the country (ARSO, 2010). With the use of universal kriging for spatial interpolation (Cressie, 1993), daily raster maps with 100 m resolution were created. The monthly maps were then summed for the four months (120 days) before the date of moss collection. This design was based on the results of our previous work (2015), namely, our finding that the total amount of precipitation in the last four months before sampling has the highest influence on N concentrations in mosses.

With the goal of establishing a relationship between the N concentrations in the mosses and the modeled depositions calculated by the EMEP/MSC-W model (Simpson et al., 2012), we also included in the analyses the spatial information from the maps produced

Table 1
Databases and maps used for spatial interpolation of the data within regression models. All maps were used with a resolution of 100 m × 100 m.

Maps used within regression models	Data type used	Original GIS data type and resolution of the map	Data source
Distance to nearest tree	Calculated	Raster (res. 100 m)	Field assessment – SFI
Tree canopy closure	No spatial map available		
Tree mixture[a]	Original	Raster (res. 100 m)	Corine Land Cover (EEA, 2006)
Altitude	Original	Raster (res. 100 m)	Digital Elevation Model 100 (GURS, 2000)
Sum of 120 days of precipitation	Recalculated	Raster (res. 100 m)	Daily precipitation maps (ARSO, 2010)
Average wind speed 50 m above ground	Original	Raster (res. 1 km)	Map of wind speed (ARSO, 2011)
% of urban land within 80 km radius	Recalculated	Raster (res. 100 m)	Corine Land Cover (EEA, 2006)
% of cropland within 5 km radius	Recalculated	Shape	National Land Use Map (MKGP, 2010)
% of cropland within 0.5 km radius	Recalculated	Shape	National Land Use Map (MKGP, 2010)
% of forested land within 0.5 km radius	Recalculated	Shape	National Land Use Map (MKGP, 2010)
Modeled deposition (total reduced N + total oxidized N) (08–10 average)	Recalculated	Raster (res. 50 km)	EMEP MSC-W deposition model (EMEP, 2004)
Modeled deposition (total reduced N) (08–10 average)	Recalculated	Raster (res. 50 km)	EMEP MSC-W deposition model (EMEP, 2004)
Modeled deposition (total oxidized N) (08–10 average)	Recalculated	Raster (res. 50 km)	EMEP MSC-W deposition model (EMEP, 2004)

[a] Tree mixture describes the percent of area covered with coniferous or broadleaved trees: conif – coniferous > 75%; mixed, bro – broadleaved > 75%.

Table 2
Regression models used within nongeostatistical techniques to explore the spatial structure of the N_{open}, N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ in mosses. For the regression models, the increase of the element concentration in moss is in italics, the decrease of the element concentration in moss is underlined, and the categorical variables are the bold letters. Explanatory environmental characteristics in the regression model are stated according to the relative importance of the regressor (in brackets) in the linear model. Other abbreviations in the regression models are UL – urban land, FL – forested land, and CL – cropland.

Measurement in moss	N_{open}	N_{canopy}	$\delta^{15}N_{open}$	$\delta^{15}N_{canopy}$
Regression function for regression predictions and inverse distance weighted interpolation of residuals	N_{open} = *% of UL within 80 km radius* (15%) + *modeled N_{tot} deposition* (13%) + *precipitation* (9%) + **distance to nearest tree** (7%) + *altitude* (6%) + *% of CL within 5 km radius* (3%)	N_{canopy} = **tree mixture**[a] (7%) + *average wind speed 50 m above ground* (6%) + % of FL within 0.5 km radius (6%) + *modeled N_{tot} deposition* (6%)	$\delta^{15}N_{open}$ = altitude (11%) + **tree mixture**[a] (8%) + % of CL within 0.5 km radius (6%) + modeled NH_y deposition (5%) + *modeled NO_x deposition* (2%)	$\delta^{15}N_{canopy}$ = altitude (12%) + **tree mixture**[a] (5%) + % of CL within 5 km radius (3%)
	R^2: 0.53 ($p < 0.001$)	R^2: 0.25 ($p < 0.001$)	R^2: 0.32 ($p < 0.001$)	R^2: 0.20 ($p < 0.001$)

[a] Tree mixture describes the percent of area covered with coniferous or broadleaved trees: conif – coniferous > 75%; mixed, bro – broadleaved > 75%.

by the regional deposition models for reduced and the oxidized N (years 2008–2010) (Figs. S1 and S2) and for the sum of the total reduced and oxidized N (years 2008–2010) (Table 1 and Fig. 1). The annual EMEP/MSC-W model results for the depositions are available as gridded data online (EMEP, 2004).

It is known (Pesch et al., 2008; Skudnik et al., 2015) that the distance from the moss collecting location to the nearest tree canopy projection is important in explaining the variation of N_{open}. In the linear regression model used in the present study (Table 2), the distance ">3 m" was set as the reference categorical variable, and regression predictions were calculated for this category (Table 1).

All of the maps used in our analyses, except for the map of the categorical variable tree mixture, were polished to avoid sharp edges in the resulting regression map (see Section 2.3). The polishing was performed with the "focal statistic" tool in ArcMap (ESRI, 2011), i.e., for each raster cell; the mean values of 10 neighboring raster cells were calculated.

2.3. Spatial interpolation of the moss data

In this study of the spatial interpolation for the N concentrations and the $\delta^{15}N$ values in mosses, the assumption was that the amount of N in the mosses and the $\delta^{15}N$ values reflected the atmospheric N deposition, which was considered a continuous spatial process (Bivand et al., 2008; Cressie, 1993; Diggle and Ribeiro, 2007). As mentioned previously, the sampling design was adapted to the forest. Consequently, the resulting maps were limited only to the forested land. The presented data (see Sections 2.1 and 2.2) were used in geostatistical and nongeostatistical modeling to predict the values of N concentration and $\delta^{15}N$ in the moss in 100 m × 100 m regular grids across Slovenia. Such a dense grid was used to preserve the spatial resolution of the forest edge in the resulting map. If the spatial interpolation were performed into less denser grid, the forest edge shown on the resulting map would be fragmented and less accurate.

The analyses of the spatial correlation for the N_{open}, N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ were conducted.

(i) First, without consideration of their dependence on the environmental variables;
(ii) second, on the residuals from the multiple regression models considering the environmental characteristics that explained the variation in the N concentrations and the $\delta^{15}N$ values in the mosses (Table 2). These analyses are presented in more detail by Skudnik et al. (2015).

Monte Carlo simulation of sample variograms was used to examine spatial correlation. A sample variogram was calculated as an average of variogram values for different lags of the distance between locations (i.e., the locations at which the measurements were made). In the Monte Carlo simulation, the data were randomly reassigned several times (up to 1000 times) to given spatial locations. For each reassignment, the sample variogram is calculated for the given distance lags. The sample variogram envelope presents the 2.5% and 97.5% percentiles of the calculated sample variograms at each lag. If the original sample variogram values fall outside the envelope, then the spatial correlation is statistically significant. The maximum likelihood method was used for estimation of the parameters of the variogram model based on the empirical variogram cloud (Diggle and Ribeiro, 2007) and not on the basis of

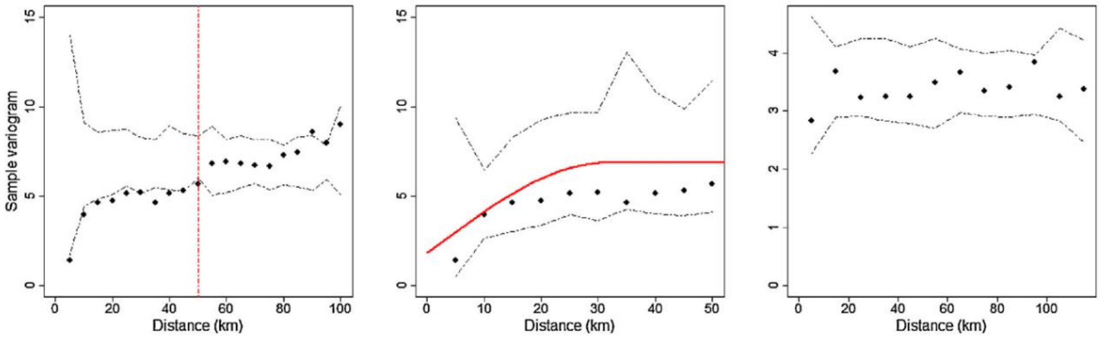

Fig. 2. Sample variogram (points) and envelope of Monte Carlo variogram simulations for N_{open}. The red vertical dashed line shows the approximate maximum distance for the existence of a spatial correlation (left); sample variogram (points), fitted spherical variogram model of N_{open} using maximum likelihood estimation with estimated nugget = 1.82, sill = 5.07, and practical range = 31.59 km, with the envelopes based on simulations considering the chosen variogram model (middle); sample variogram (points) and envelope of Monte Carlo variogram simulations for residuals of the regression model for N_{open} (right). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

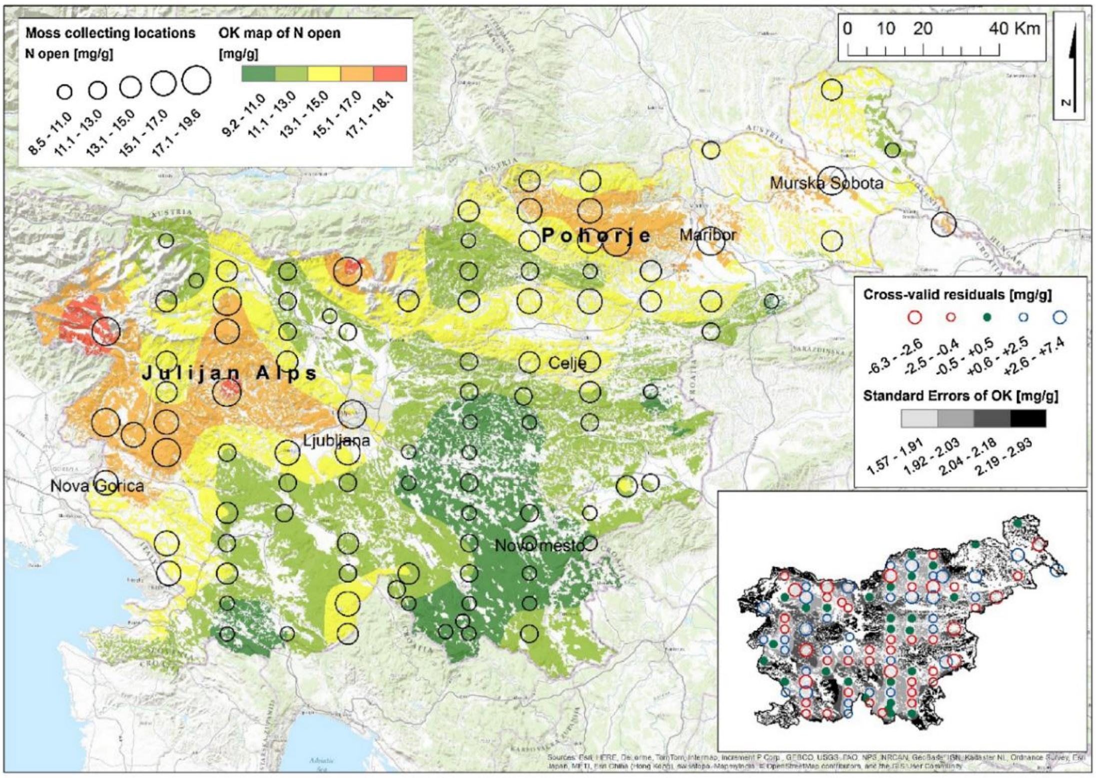

Fig. 3. Map of locations of measured N_{open} in mosses and spatial interpolation of N_{open} using ordinary kriging (OK). Map in the right lower corner shows standard errors of the predictions and the residuals of cross-validation. The cross-validation showed that 25% of variance of N_{open} was explained with the model.

the sample variogram, which is quite dependent on the bin width. Based on the chosen variogram model, simulated values of the spatial process were generated at the data locations to estimate the accuracy of the variogram model. The empirical variogram is computed for each simulation using the same lags used for the original sample variogram. The envelopes are computed by using, at each lag, the maximum and the minimum values of the variograms for the simulated data.

Ordinary kriging was used for the spatial interpolation of the N_{open}. For the N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ or for the residuals of the regression model, no spatial correlation was found, and therefore, a nongeostatistical spatial interpolation technique

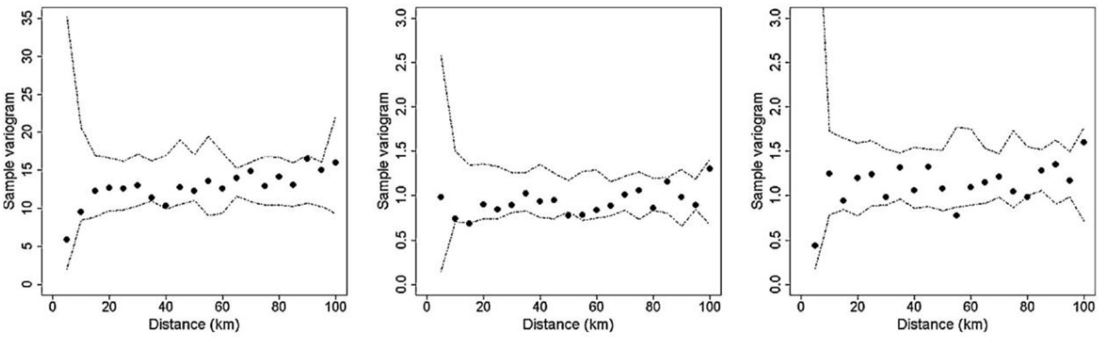

Fig. 4. Sample variogram and envelope of Monte Carlo variogram simulations for N_{canopy} (left), $\delta^{15}N_{open}$ (middle), and $\delta^{15}N_{canopy}$ (right).

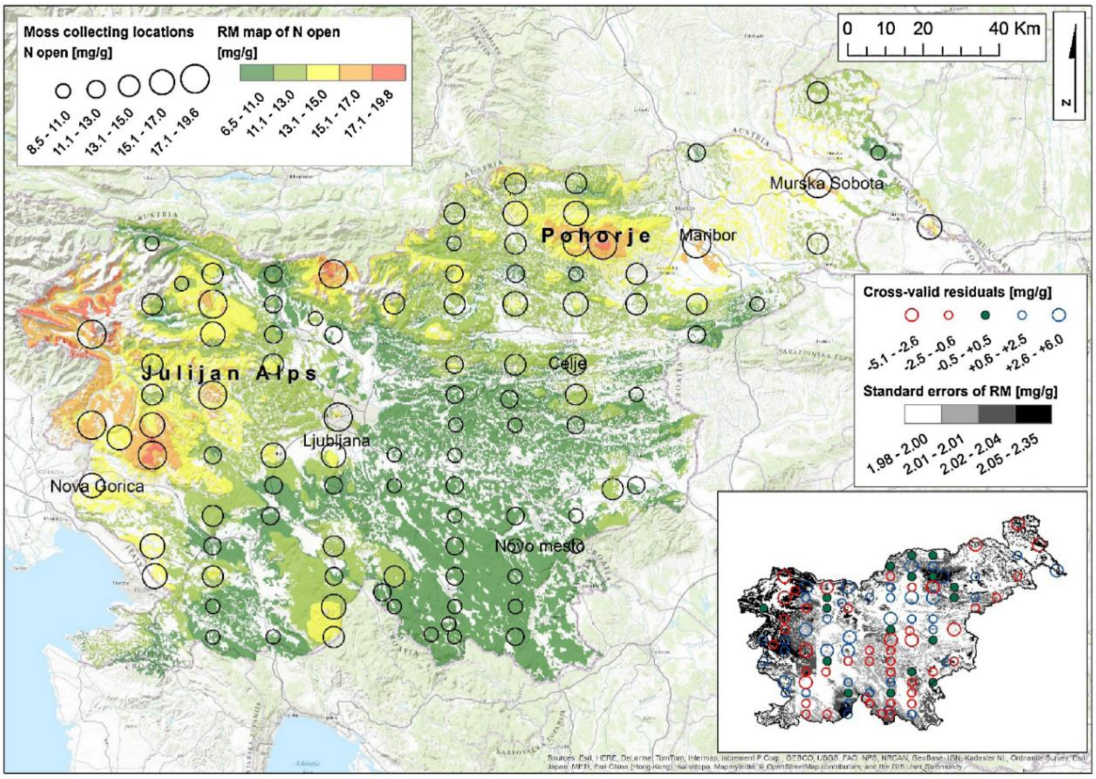

Fig. 5. Map of locations of measured N_{open} in mosses and spatial interpolation of N_{open} using regression model. Map in the right lower corner shows standard errors of the model predictions and the residuals from cross-validation.

was applied: the spatial interpolation was calculated as a sum of the regression predictions and the inverse distance weighted interpolations of the regression residuals. With the inverse distance weighted method, the predicted values were calculated as a linear combination of the values that were measured on the neighboring locations. The weights of the linear combination depended on some power of the inverse distance between the predicted and the measured locations. In the present study, the power of two was used which means that the measured data are inversely weighted as the square of distance. In the literature, this method is also called Inverse Distance Squared (Isaaks and Srivastava, 1989). The inverse distance weighted method did not provide standard errors. The difference between the techniques is that in ordinary kriging, the predictions (interpolated values) are also linear combination of measured values; here, however, the weights also depend on spatial correlation, not only on the distance between locations.

A cross-validation that was based on leave-one-out was used to evaluate the results of the spatial interpolations. With cross-validation, we compare the observed values with the values predicted by spatial interpolation. In leave-one-out cross-validation, the data locations are removed from the data set one at

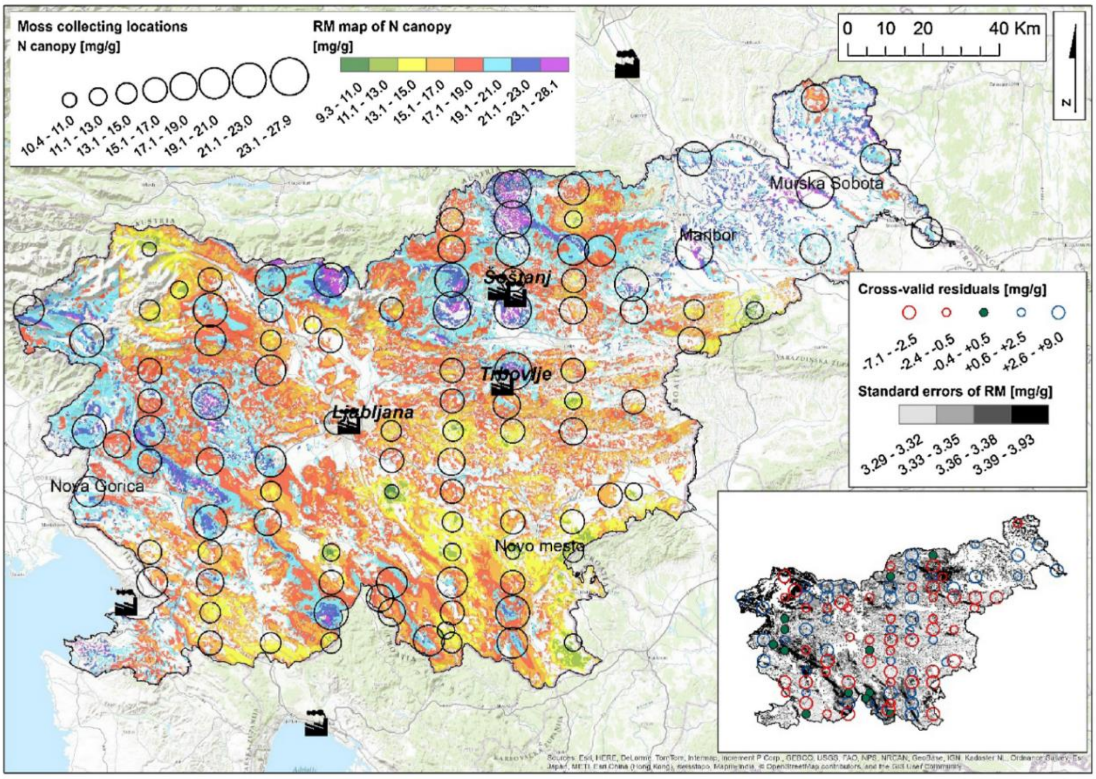

Fig. 6. Map of locations of measured N_{canopy} in mosses and spatial interpolation of N_{canopy} using regression model. Map in the right lower corner shows standard errors of the model predictions and the residuals of cross-validation.

a time. For each such removal step, the variable at this location is predicted with the given spatial model using the remaining locations (Geisser, 1975; Stone, 1974). As a result of cross-validation, the variance of the cross-validation residuals (*Var(cv.res)*) is compared with the variance of the spatial variable (*Var(y)*). The percentage of variance explained is calculated as (*Var(y) – Var(cv.res))/Var(y)*.

The maps of the explanatory variables used for the spatial interpolation (Table 1) and the final maps (Figs. 1, 3, 5–7) were created with the software ArcGIS 10.0 (ESRI, 2011). The statistical and geostatistical analyses were performed using the R 3.0 statistical software package (R Development Core Team, 2014) with the packages "*raster*" (Hijmans and van Etten, 2012), "*gstat*" (Pebesma, 2004), "*geoR*" (Diggle and Ribeiro, 2007) and "spatstat" (Baddeley and Turner, 2005).

3. Results and discussion

3.1. Spatial exploratory data analysis of the N and the $\delta^{15}N$ in mosses

3.1.1. Environmental characteristics that explained the data variation in the N concentrations and the $\delta^{15}N$ values in the mosses

The dependence of N concentration and $\delta^{15}N$ value on the environmental variables is presented in Table 2. The regressors of the models were ordered based on the percentage of the explained variance – from more to less important. Based on the regression models, for all of the data, except for the $\delta^{15}N_{canopy}$ values (the $\delta^{15}N_{canopy}$ values were not included in further discussions), the factors that mirrored the atmospheric N deposition, such as the type of land use that surrounded the moss sample location or the EMEP modeled deposition (Fig. 1 and Figs. S1 and S2), played a significant role in explaining the variation of N in the moss tissue (Table 2). Similar to our results, Schröder et al. (2010) also showed a strong dependence of the N concentration in mosses on the EMEP modeled depositions and the type of surrounding land use, especially urban and agricultural land use. For N_{canopy} and $\delta^{15}N_{open}$, the site characteristics such as the altitude, precipitation and in particular, the forest characteristics at the sample location were more important in explaining the variation in the moss data (see the relative importance of the regressors in the linear model in Table 2) than the factors that were connected to the atmospheric N deposition, as was observed for the N_{open}. Numerous reasons for the transformation of atmospheric N before deposition on the forest floor have been discussed in the literature. These reasons include the canopy drip effect (De Schrijver et al., 2008), orographic precipitation (Kalina et al., 2002; Miller et al., 1993), and the atmospheric transport of N (Asman et al., 1998), among others. According to Skudnik et al. (2015), for the data variation in the N_{canopy}, canopy closure at the moss sample location was particularly important. When the information on canopy closure was added to the model, the variation of the data that was explained increased significantly ($R^2 = 37\%$, $p < 0.001$; data not shown). However, in this paper, those

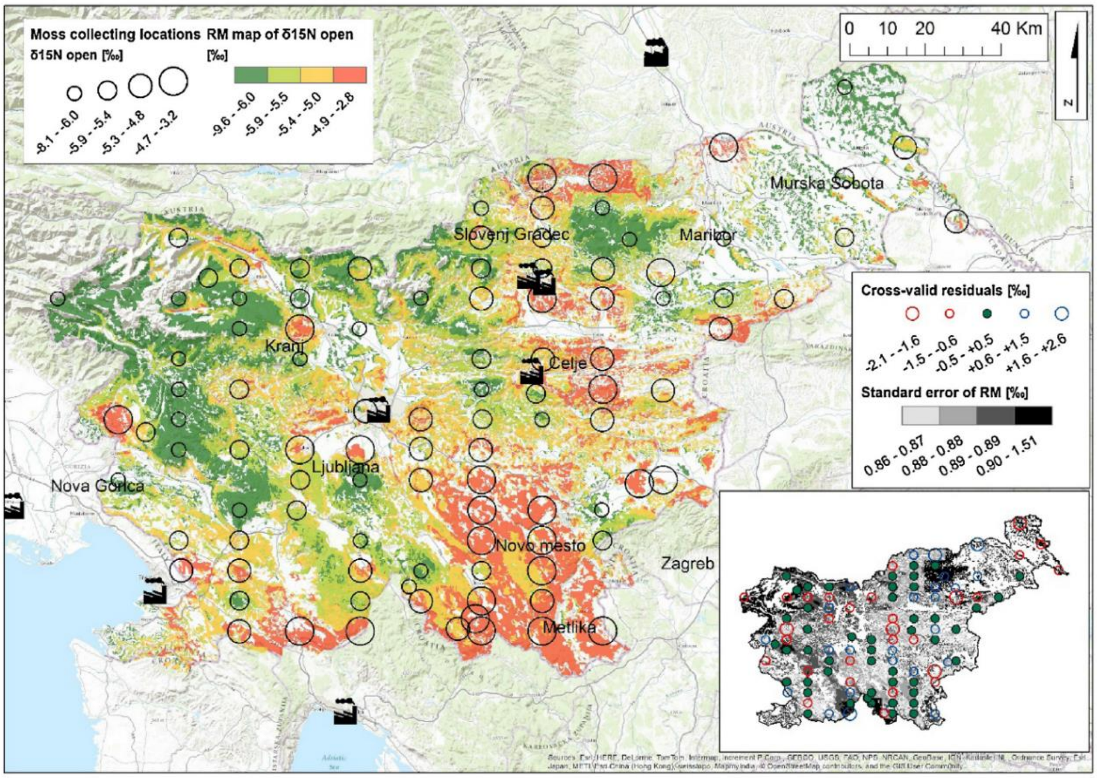

Fig. 7. Map of locations of measured $\delta^{15}N_{open}$ in mosses and spatial interpolation of $\delta^{15}N_{open}$ using regression model. Map in the right lower corner shows standard errors of the model predictions and the residuals of cross-validation.

results were not presented because the canopy closure estimations were available only for the moss sample sites (estimated within NFI), and a map of canopy closure for the entire country, which could be used for spatial interpolation of the data with regression prediction (see Section 3.2), was not available at the time of the preparation of this manuscript. With remote sensing techniques, the production of forest canopy cover maps is possible (Stojanova et al., 2010), and the addition of these maps would lead to better spatial interpolation of the results for the N_{canopy}.

The results show that exploration of the dependence of N concentrations and $\delta^{15}N$ values in moss on environmental factors is important for two reasons: first, to ask whether the element values in mosses reflect the environmental factors connected to potential atmospheric pollutants (Schröder et al., 2010); second, to use information on the environmental factors to increase the quality of spatial interpolation (see Section 3.2).

3.1.2. Spatial correlation

For N_{open}, N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$, a general spatial trend, as assessed by the degree of dependency of observations on the geographic coordinates (*X* and *Y* coordinates), was not found. If a general spatial trend occurred, then the observations would show some pattern based on the geographic coordinates.

The spatial correlation structure for the N concentrations and the $\delta^{15}N$ values is presented as sample variograms with the Monte Carlo envelope in Figs. 2 and 4. With spatial correlation, we assess the degree of dependency among observations in a geographic space. For N_{open} only, some spatial correlation among the measured N concentrations in moss tissue was found up to approximately 50 km, but the situation was not clear for short distances (i.e., distances less than 16 km) (Fig. 2 – left). For N_{open}, after fitting different variogram models and comparing the cross-validation results, the spherical variogram model was selected and was fitted by the maximum likelihood estimation method. According to the sample design, the estimation of the nugget effect that presented a combination of spatial variation on the microscale and the measurement error could only be performed by extrapolation.

For the N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ values, no spatial correlation was found. Fig. 4 shows that for all values, the N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$, sample variograms lie inside the Monte Carlo envelope and, consequently, show no statistically significant spatial correlations between the moss samples. Pesch et al. (2007) also explored the spatial correlation for N_{canopy} and, in contrast to our results, found a weak but significant spatial correlation for measured N concentrations in mosses collected under forest canopies. However, in our previous work (Skudnik et al., 2015) we found that for the N_{canopy} concentrations, the N emission sources could be obscured by the characteristics of forest at the moss sample location. Moreover, if the region of the moss survey had more structurally diverse forests, which is the case for Slovenia, which is located between the Mediterranean, Alpine and Pannonia regions, the spatial correlation between the sampling sites would be lost.

Varela et al. (2013) found no spatial structure for N_{open} and only weak spatial structure for $\delta^{15}N_{open}$ for mosses collected in Galicia. Because of such results, the authors recommended the evaluation of the $\delta^{15}N$ value in terrestrial mosses to investigate atmospheric N deposition, instead of measuring only the N concentrations in the mosses. The advantages of the $\delta^{15}N$ values are that the values are not as strongly affected by metabolic regulation as the N concentration (Liu et al., 2008) and that no evidence of saturation has been found in mosses as has been found for total N (Harmens et al., 2014). The disadvantage of the $\delta^{15}N$ values in mosses could be the high cation-exchange capacity of mosses (Bates, 1992) and, consequently, a higher uptake of NH_4 than of NO_3 (Forsum et al., 2006; Nordin et al., 2006).

However, the primary purpose of using mosses as biomonitors was to explore the patterns of atmospheric N deposition (Harmens et al., 2015), and, in our opinion, only the combination of the N concentration and the $\delta^{15}N$ signature provided holistic information on the amount of N deposition and also on the N source. With different environmental characteristics, the parameters (N or $\delta^{15}N$) should be explored to determine the parameter showing better performance in spatial correlation and, consequently, furnishing better results for mapping.

3.2. Spatial interpolation

3.2.1. Ordinary kriging

For the N_{open} concentrations, ordinary kriging was used for the interpolation of the moss data, and the results are presented in Fig. 3. The cross-validation showed that 25% of the N_{open} variance was explained with the model, and almost all the higher measured values of N_{open} were strongly underestimated using the cross-validation method.

3.2.2. Spatial interpolation with the regression model

Because spatial correlation was not detected for the other parameters (N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$), nongeostatistical spatial interpolation was applied; with the sum of regression predictions and the inverse distance weighted interpolations of regression residuals (Table 2 and Figs. 5–7). The same technique was applied also for N_{open}, with the aim of comparing the ordinary kriging results with the regression prediction results. The accuracy of regression predictions depends on the chosen regression model and on the quality of the input data for explanatory variables. The maps of the regression predictions are presented in Figs. 5–7. The cross-validation result for the N_{open} map created with the regression prediction was better (44% of explained variance) than that for the map created with ordinary kriging (25% of explained variance) (Table 3), showing that the role of explanatory variables is important and that the spatial correlation of N_{open} is weak. The cross-validation residuals are also presented in Figs. 5–7, showing a prediction bias for all locations. In Section 3.3.2 we provide further details on the comparison of Figs. 3 and 5.

3.3. Spatial distribution and the differences between N_{open}, N_{canopy} and $\delta^{15}N_{open}$ in mosses: the case study in Slovenia

3.3.1. Ordinary kriging

For Slovenia, the results of ordinary kriging for N_{open} ranged between 9.2 and 18.1 mg/g, with an estimated standard error between 1.6 and 2.9 mg/g (Table 3 and Fig. 3). The range of the interpolated values was smaller than the range of the measured N_{open} values (8.5 and 19.6 mg/g). The map shows high N_{open} values in the western and the northeastern parts of the country; in particular, the area on the border with Italy had elevated N_{open} values. We assumed that those high values reflected the intensive agricultural land use in northern Italy (the Friuli and Veneto regions). This assumption is confirmed also by the resulting $\delta^{15}N_{open}$ map, showing more negative values in the western part of the country, indicating that here the N is primarily derived from agricultural sources (Fig. 7). From these regions, the atmospheric N is transported by the prevailing westerly winds (Rakovec et al., 2009) into the mountainous areas of Slovenia. Compared with the modeled contributions of N from the transborder sources, which are based on source-receptor calculations with the unified EMEP/MSC-W model using meteorological and emission data for the year 2008 (Nyúri et al., 2010), our results were also consistent. The kriged map (Fig. 3) shows that high N_{open} concentrations were also located in the northeastern part of the country, with particularly elevated N_{open} in the Pohorje Mountains and in the areas around the cities of Maribor, Celje and Murska Sobota. In these locations, the sources of N could be the combustion processes in the cities, which are known for their industrial history and the heavy transit traffic via the highway that connects the eastern and western parts of the country. Another reason for the elevated N_{open} in this area is the fact that this part of the country is the beginning of the Pannonia flatlands with traditionally more intensive agricultural land use. In general, the map of the ordinary kriging (Fig. 3) was in good agreement with the EMEP that was modeled on the N depositions for the years 2008–10 (Fig. 1). The similarity was even better with the EMEP map of the modeled NH_y deposition (Fig. S1) than the with the EMEP map of the modeled NO_x deposition (Fig. S2). Based on N-uptake experiments (Forsum et al., 2006; Nordin et al., 2006; Skudnik et al., 2014) it was namely found that the uptake of NH_y in mosses was greater than that of NO_x. Because Slovenia has only one EMEP station, located in the south of the country (Fig. 1), the lack of correlation between EMEP and moss data could also be explained by the potential uncertainty of the EMEP modeled data.

3.3.2. Spatial interpolation with the regression model

The map that was created using interpolation with the regression model for N_{open} showed a similar N pattern but with slightly lower predicted values (6.5 and 19.8 mg/g) (Table 3 and Fig. 5). In this case, a map of the standard errors showed better predictions for the central part of the country but poorer predictions for the western and the eastern parts. The primary reasons for this disagreement might be found in the higher variability of the environmental characteristics in the western part (alpine region) and the lower sampling density for the eastern part (Pannonia flatland with low forest coverage) than for other the parts of the country.

The significant linear correlation exist between N_{open} and N_{canopy}, and the N concentrations in the moss were, on average, 41% higher within the forest stand than they were at least 3 m away from the nearest canopy (Skudnik et al., 2014). The interpolated N_{canopy} values ranged between 9.3 and 28.1 mg/g, and the range for the N_{canopy} data was larger than that for N_{open}, which was also reflected in the maps (Table 3 and Fig. 6). For an easier comparison between the N_{open} and the N_{canopy} classes in the map, the legend has equal intervals and colors (Fig. 6). The standard errors were much higher for the N_{canopy} than those of the N_{open} (3.3–3.9 mg/g) (Table 3). In general, all three maps showed similar N patterns with high values for the western and the northeastern parts of the county and low values in the southeast. However, some differences also occurred among the maps. In large part, the pattern for the higher N_{canopy} values was matched by the wind velocity map, with elevated areas around the urbanized areas and notably, in this map, some local emitters of NO_x were apparent, which were not apparent on the N_{open} map (for example, the locations around the power plants in Šoštanj and Trbovlje). The deposition rates of NO_x were low close to the source (Hertel et al., 2011), but the neighboring forests with the canopy crowns could be efficient sinks for the atmospheric gasses and particles (De Schrijver et al., 2008). However, the

Table 3
Range of measured values for N_{open}, N_{canopy}, $\delta^{15}N_{open}$ and $\delta^{15}N_{canopy}$ in mosses and the ranges of resulting interpolated values with cross-validation results. The maps were evaluated with cross-validation with leave-one-out.

Used spatial interpolation technique	Measurements	Range of measured values	Range of interpolated values	Range of interpolated standard error [mg/g]	Cross-validation results (%)
Ordinary kriging	N_{open}	8.5 to 19.6 mg/g	9.2 to 18.1 mg/g	1.57–2.93 mg/g	25
Regression predictions with inverse distance weighted interpolations of regression residuals	N_{open}	8.5 to 19.6 mg/g	6.5 to 19.8 mg/g	1.98–2.53 mg/g	44
	N_{canopy}	10.4 to 27.9 mg/g	9.3 to 28.1 mg/g	3.29–3.93 mg/g	15
	$\delta^{15}N_{open}$	−8.1 to −3.2‰	−9.6 to −2.8‰	0.86–1.51‰	22
	$\delta^{15}N_{canopy}$	−8.8 to −2.7‰	−8.8 to −2.6‰	1.02–1.16‰	11

cross-validation showed that only 15% of the observed variability was explained by spatial interpolation, and for each predicted value of N_{canopy}, the minimum standard error was estimated as 3.3 mg/g (Table 3).

The variation in the data for the spatial interpolation of $\delta^{15}N_{open}$ (using the regression prediction) in mosses ranged from −9.6‰ to −2.8‰, whereas the $\delta^{15}N_{open}$ values ranged between −9.1‰ and −3.2‰ (Table 3). A map showed that in the areas in which the N_{open} was high the interpolated $\delta^{15}N$ values were more negative, which indicated that in those areas the N was primarily derived from agricultural sources (the western mountainous part and the eastern Pannonian part of the country). In contrast to the areas located in the southeast of the country for which the N_{open} was the lowest, the $\delta^{15}N$ values were less negative, which indicated that the N was primarily derived from combustion processes. The map also showed less negative values of $\delta^{15}N_{open}$ in the areas that surrounded all the larger cities that were not surrounded with a high percentage of agricultural land (i.e., Celje, Kranj, Nova Gorica, Novo mesto, and Metlika), in the areas that surrounded the thermal power plants, and in the areas that surrounded some of the more heavily used transit roads such as the highways Ljubljana-Zagreb and Koper-Ljubljana-Celje and the regional road between Slovenj Gradec and Maribor (Fig. 7).

4. Conclusions

The cross-validation results and the maps of standard errors showed the limitations of the particular maps; therefore, we suggest that the interpretation of the maps should always include the estimated errors of the interpolation. To enhance the quality of the spatial interpolation for the N_{canopy} and for the $\delta^{15}N_{open}$, we suggest that the moss-sampling grid be denser and stratified for the different forest types. Because site characteristics in Slovenia are heterogeneous and change over short distances, a denser sampling grid would also provide more accurate results for the spatial interpolation of the N_{open}.

The study showed that numerous parameters influenced the maps of N concentrations and $\delta^{15}N$ values that were created with the spatial interpolation techniques. Thus, the potential users of the maps should always be informed about the procedure used to create the map, the level of accuracy and the limitations of the map. We suggest that the following steps or questions should be taken into consideration before maps of moss biomonitoring surveys will be published in the manuscripts or reports:

(i) Is there a spatial trend and a spatial correlation among the data, and if so, the variogram should be presented and described (nugget, sill and range)?
(ii) Which environmental factors are important in explaining the variation of the element in moss? What is the variability of the data explained by the models, and does spatial correlation occur between the residuals of the model?
(iii) What is the estimated bias of the interpolated map using the cross-validation methods?
(iv) What are the standard errors of the interpolated map?
(v) Is the resulting map consistent with expert knowledge of the spatial distribution of atmospheric deposition?

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Appendix A. Supplementary data

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2.2 DRUGO POVEZOVALNO BESEDILO

2.2.1 Odvisnost osutosti dreves, pokrovnosti lišajev in foliarnih koncentracij N od nekaterih značilnosti okolja ter njihova povezava z vsebnostjo N in vrednostjo δ^{15}N v mahu štorovo sedje (*Hypnum cupressiforme* Hedw.)

Dependence of tree defoliation, lichen cover and foliar N concentrations on selected environmental characteristics and their relation with N concentrations and δ^{15}N values in moss *Hypnum cupressiforme* Hedw.

Mitja Skudnik, Franc Batič, Zvonka Jeran, Primož Simončič in Damijana Kastelec
Članek je poslan v recenzijo.

Namen študije je raziskati, ali so foliarne analize, osutost drevja in lišajska obrast odvisne od izbranih značilnosti okolja ter od katerih. Rezultati kažejo, da značilnosti gozda pomembno vplivajo na rezultate bioindikacije; predvsem izbor vzorčnih drevesnih vrst in njihovo mešanost ter sestojni sklep okoliških gozdov. Za okoljske dejavnike, povezane z meteorologijo, rezultati kažejo, da so klimatološki trendi (dolgoletna povprečja) bolj pomembni kot letna nihanja meteoroloških razmer. Pri vsaki od preizkušenih tehnik se je kot pomemben dejavnik pokazala tudi vrsta rabe tal v okolici, še posebej odstotek pozidanih in kmetijskih zemljišč. Modelirane usedline celokupnega reaktivnega dušika so bile pomembne pri pojasnjevanju variabilnosti osutosti dreves in pojavljanju listnatih lišajev. Analiza korelacij je pokazala odvisnosti med koncentracijo dušika v mahovih in i) foliarnim dušikom v listih, ii) osutostjo v gozdovih z normalnim sklepom ter iii) obrastjo s skorjastimi lišaji v čistih sestojih ter korelacijo med vrednostjo δ^{15}N v mahovih in osutostjo v listnatih in mešanih sestojih. Med drugimi oblikami biomonitoringa/bioindikacije ni bilo statistično značilnih povezav.

Title:

Dependence of tree defoliation, lichen cover and foliar N concentrations on selected environmental characteristics and their relationships with N concentrations and δ^{15}N values of the moss *Hypnum cupressiforme* Hedw.

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Abstract

The aim of this study was to explore the extent to which foliar nitrogen (N) concentration, tree defoliation and lichen cover could be used to evaluate N deposition in Slovenia. The results showed that forest characteristics, especially the selection of tree species and the surrounding forest mixture and density, have an important influence on the bioindication results. For each of the tested techniques, the type of surrounding land use was also important, especially the percent of urban and agricultural land. The modelled total reactive N deposition was significant only for tree defoliation. With respect to the meteorological conditions, the results showed that longer trends were more important than annual meteorological conditions. Secondly, the relationships between these monitoring techniques and a moss biomonitoring technique were explored. The correlations between moss and leaf N concentration, tree defoliation and crustose lichen cover, and the moss δ^{15}N value and defoliation were significant.

Keywords: Biomonitoring, Bioindication, Forest health, Environmental factors, Generalized linear models, Mixed effect models, Nitrogen, N isotope

Capsule: A detailed analysis of the environmental factors that influenced the selected bioindication technique should be considered together with the interpretation of the results.

1 INTRODUCTION

Since the industrial revolution, humans convert an annual amount of approximately 120 million tons of atmospheric N_2 into reactive N, which is mostly used as fertilizer for food production (Sutton et al., 2011). Much of this reactive N ends up in various types of ecosystems and often leads to their eutrophication and acidification (Erisman et al., 2007).

To control air pollution and set emission reduction targets at a regional level, the United Nations Economic Commission for Europe (UNECE) has negotiated the Convention on Long-Range Transboundary Air Pollution (CLRTAP) (UNECE, 1988), which is implemented in Europe by the European Monitoring and Evaluation Programme (EMEP). Additionally, with the aim to understand the harmful effect of air pollution on recipients, six International Cooperative Programmes (ICPs) were established under the CLRTAP (UNECE, 1979). Among these is ICP Forest, which monitors forest conditions in Europe at two monitoring intensity levels: Level I, to obtain information on dense spatial and temporal variations in forest conditions and Level II, to study cause-effect relationships between pollutants and the condition of forest ecosystems (de Vries et al., 2003).

Within the framework of the European monitoring systems, the data on atmospheric N deposition are collected at all EMEP stations (Simpson et al., 2012) and in selected Level II plots of ICP Forest (Waldner et al., 2014). Nevertheless, in some European regions, the number of these stations is low due to the high costs associated with establishing and maintaining permanent stations and continuous sampling and analysis. In Slovenia, which is located in the southern part of central Europe, there is only one EMEP station (Nyíri et al., 2010) and in the last year, bulk and throughfall collectors were only established in four Level II plots (Simončič et al., 2015).

To gain additional spatial information on deposited N, different biomonitoring and bioindication methods were established at the EU level. Bioindicators are organisms or group of organisms that contain information on the quality of the environment, while biomonitors are organisms that contain quantitative information pertaining to the quality of the environment (Markert et al., 2003). The advantage of biomonitoring and bioindication methods, compared with technical measurements of air constituents and precipitation, is that the methodology is usually simpler and, in the case of biomonitors, sampling and chemical analysis are repeated every n-years, which makes the methodology less expensive and consequently allows greater sampling density.

One of the widely used biomonitoring techniques in Europe is the moss biomonitoring survey, which was developed during the last few years inside the framework of ICP Vegetation (Harmens et al., 2015). Initially, mosses were mostly used to explore atmospheric heavy metal deposition, but in the last few years, they have also been analyzed to determine their N concentration and δ^{15}N value. The latter is used to determine the source of N in atmospheric deposition (Bragazza et al., 2005; Kendall et al., 2008; Pearson et al., 2000; Skudnik et al., 2014). The δ^{15}N value, i.e., the ratio between stable N isotopes ($^{15}N/^{14}N$), differs between the reduced and oxidized forms of N; less negative values indicate that the primary source of N is NO_x from combustion processes, while more negative values indicate that the primary source of N is NH_y from agricultural activities (Heaton, 1986; Kendall et al., 2008).

Similar to the concept of the biomonitoring techniques, such as biomonitoring using mosses, is the analysis of the N concentration in tree leaves or needles (Pitcairn et al., 1998). Within ICP Forest, foliar analyses are regularly (every two years) conducted on selected Level II plots with the aim to monitor the nutritional state of the trees (Rautio et al., 2010).

In contrast to biomonitoring, the bioindication technique is based on the visual assessment of the response of selected organisms to changes in environmental conditions or qualities. Crown condition, assessed as defoliation, is one of the most important bioindication techniques developed within ICP Forest and aims to show the response of a forest ecosystem to anthropogenic and natural stress factors and air pollution (Vitale et al., 2014). Other widely used organisms for the assessment of air pollution are epiphytic lichens, which could be used as bioindicators (indicator lichen species, lichen cover, etc.) (Batič et al., 2011; Gadsdon et al., 2010) and biomonitors if the lichen tissue is analyzed for certain elements or substances (Boltersdorf et al., 2014; Jeran et al., 2007).

The measurement of pollutants in air and precipitation is the most reliable way to obtain information on the composition and amount of deposited atmospheric pollutants. However, there are biomonitoring or bioindication techniques that have advantages over former techniques (Boltersdorf et al., 2014; Ordonez et al., 2009; Sardans et al., 2015) as well as some drawbacks (de Vries et al., 2014; Mayer et al., 2009; Staszewski et al., 2012).

Therefore, our main aim was to compare the different existing biomonitoring and bioindication techniques to evaluate the condition of forests. Specifically, in the present study we aim to:

i) present the dependence of foliar N concentration, tree defoliation and lichen cover on the selected environmental characteristics, which describe the characteristics of the monitoring location as well as the potential anthropogenic N emitters (surrounding land use type and modeled N depositions); and

ii) explore the correlations between the above monitoring techniques and the results from the moss biomonitoring technique (N concentrations and δ^{15}N values).

For the second aim, the N concentration and δ^{15}N value in moss collected at each location from two different sites (outside and inside the area of the canopy drip line) were used (Skudnik et al., 2014). The two different sites provided information on N deposited on the forest overstory or in clearings, as well as information on the N deposited on the forest floor, i.e., underneath the tree canopies (Skudnik et al., 2015b). We included the moss biomonitoring data because the suitability of using mosses for exploring N pollution has been shown in previous studies (Harmens et al., 2014 and the references therein; Skudnik et al., 2014).

2 MATERIALS AND METHODS

2.1 Foliar N concentration

Analyses of foliar N concentration were conducted in the period from 2005–2010 on 10 ICP Forest Level II plots in Slovenia (Fig. 1). The number of collected samples varies among years and plots. A total of 231 samples were collected and analyzed for N. The samples were collected from Norway spruce (*Picea abies* (L.) Karst.), silver fir (*Abies alba* Mill.), pines (*Pinus* sp.), common beech (*Fagus sylvatica* L.) and oak trees (*Quercus* sp.). The sampling and analysis were conducted in accordance with the ICP Forest manual on sampling and analysis of needles and leaves (Rautio et al., 2010).

2.2 Defoliation

In 2007, defoliation was assessed on all living trees in the FI plots (91 plots, 2424 trees) and on trees in the Slovenian ICP Forest Level II research plots (10 plots, 779 trees) (Fig. 1). Tree defoliation is an indicator that is based on a visual estimate of the loss of needles or leaves in the accessible crown compared with a fully leafed reference tree. Defoliation is assessed in 5% classes from 0% (fully leafed tree) to 100% (dead tree) (Eichhorn et al., 2010). A tree was included in the sample if its social class, compared with the neighboring trees, was dominant, co-dominant or subdominant. Consequently, we partially excluded the influence of a shadowing effect on defoliation. Crown defoliation was assessed in accordance with the ICP Forest tree crown condition manual (Eichhorn et al., 2010). Within the 16-km x 8-km grid, defoliation was assessed on all trees growing within the concentric FI plot and in two M6 plots (six trees nearest the plot center), and in the case of the 16-km x 16-km grid, defoliation was assessed in four M6 plots. For a more detailed description of the sampling methodology, see Kovač et al. (2014a). In the case of the Slovenian Level II plots, all trees within a plot area of 0.25 ha were included (Eichhorn et al., 2010; Skudnik et al., 2011).

2.3 Lichens

Within the same year and in the same grid where defoliation was assessed, the coverage of three main lichen growth forms (crustose, foliose and fructicose) was assessed on six tree trunks per plot. The coverage of lichens was assessed on tree trunks within a net sampling area of 10 dm^2 (20 cm x 50 cm), which was located at a height of one meter from the tree base and positioned where the coverage of lichens was the highest. If possible, all selected trees on which the lichens were assessed were of the same species. The tree species selected had to fulfil the following criteria: the species is autochthonous and the main representative of the stand, old-growth trees with undamaged trunks, and moss coverage is less than 20%. If six trees of the same species were not present within a selected plot, two or more different tree species were used. The selected trees were grouped according to tree bark and crown characteristics as follows: 1. beech group (*Fagus sylvatica* L., *Carpinus betulus* L.); 2. spruce group (*Picea abies* Karst., *Larix decidua* Mill., *Pinus* sp.); 3. oak group (*Quercus* sp., *Castanea sativa* Mill.); 4. maple group (*Acer* sp., *Tilia* sp., *Fraxinus* sp., *Ostrya carpinifolia* Scop., *Salix* sp.); and 5. fir group (*Abies alba* Mill., *Alnus* sp., *Prunus* sp.). The methodology used is described in detail by Batič et al. (2011).

2.4 Environmental factors

Data on environmental characteristics were collected directly in the field or later using geographic information system (GIS) tools (Table 1). Variables describing the characteristics of the forest were recorded in the FI plots according to the national forest monitoring guidelines (Kovač et al., 2014b). Information on the types of bedrock was acquired from the

FI for the year 2000 (Kovač et al., 2000). Climate data were obtained from the Slovenian Environment Agency (ARSO) - the maps are freely available online (ARSO, 2006). Daily precipitation data for 2007 were acquired from the ARSO, which collects information from 241 metrological stations across the country. Monthly raster maps with a 100-m resolution were created, and information on precipitation was then added to all locations.

Table 1: Predictors used to investigate the influence of environmental factors on foliar N concentrations, tree defoliation and lichen cover. Categorical variables are presented in bold font.

Environmental factor - source		Environmental factors – explanatory variables	Data type	Data provider
Site characteristics		x and y coordinates, altitude, mean tree diameter, basal area, **canopy closure, tree species mixture, development phase,** number of trees per hectare, **type of bedrock**	Site-specific	SFI
Meteorological environmental factors	Annual precipitation data	Average precipitation in 2007, sum of precipitation in growing season in 2007 (1. March – 1. September), sum of precipitation in spring months in 2007 (1. April – 1. July), max monthly precipitation from 1. January – 1. September 2007, min monthly precipitation from 1. January – 1. September 2007	Raster map – resolution 100x100 m	ARSO
	Climate data	Average yearly precipitation (1971-2000), number of days with more than 30 mm of rain (1971-2000), average air temperatures (1971-2000), sunshine duration (1971-2000), average snow cover duration (1971-2000)	Raster map – resolution 100x100 m	ARSO
Anthropogenic environmental factors	Land use – local scale	Forest land, cropland, urban land (0.5, 1, 5, 8 km radius)	Shape map	MKGP
	Land use – regional scale	Forest land, cropland, urban land (40, 80, 100 km radius)	Raster map – resolution 100x100 m	EEA
	Modeled air deposition data	Total deposition of reduced and oxidized nitrogen for the years 2005 – 2010	Raster map – resolution 50 x 50 km	EMEP MSC-W

To understand the possible sources of N emissions, the proportions of land use were derived from a national land-use map (MKGP, 2010) and from the Corine land cover map (EEA, 2006). The national land-use map was used for the local-scale analysis (radii of 0.5, 1, 5 and 8 km); the Corine land cover map was used for the regional-scale analysis (radii of 40, 80 and 100 km). The radii were selected based on the knowledge of atmospheric N transport (Asman et al., 1998; Hertel et al., 2011). The modeled total reduced and oxidized N (years 2005-2010), reported by the EMEP/MSC–W model (Simpson et al., 2012), were also included in the analyses (Table 1) (EMEP, 2004).

For this study, all of the numerical variables were centered; the original value was lowered by the mean. Centered variables are more convenient for the interpretation of the models presented in the article.

All GIS analyses were conducted using ArcGIS 10.2.1 software (ESRI, 2014).

2.5 Moss survey

Samples of cypress-leaved plait-moss (*Hypnum cupressiforme* Hedw.) were collected at 103 locations in forests of Slovenia (Fig. 1) in summer 2010. At each location, moss samples were collected from two types of sites: a) under the tree canopies (N_{canopy} and $\delta^{15}N_{canopy}$, inside the area of the canopy drip line) and b) in adjacent (< 1 km) forest openings/clearings (N_{open} and $\delta^{15}N_{open}$). Skudnik et al. (2015a; 2015b; 2014) presented a detailed description of the sampling methodology, chemical analysis and quality control results.

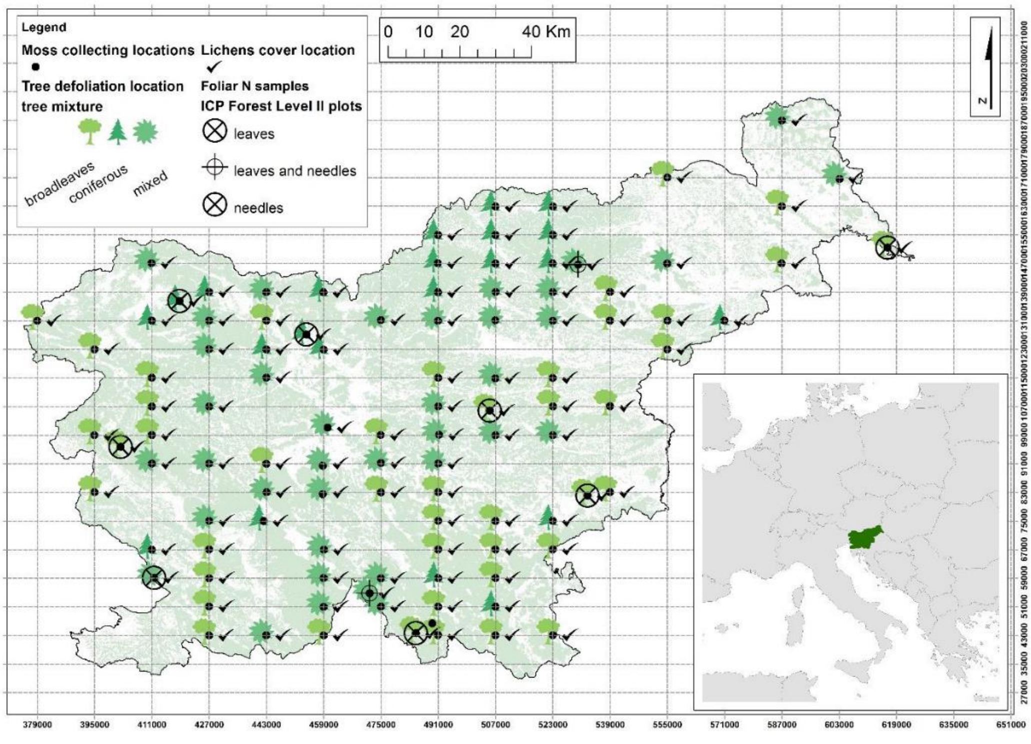

Figure 1: Locations for the foliar N concentration measurements, tree defoliation assessment, lichen cover assessment and moss survey.

2.6 Statistical Analysis

2.6.1 Foliar N concentration

For each year within each plot, the average foliar N concentration was calculated separately for coniferous and broadleaf species. Based on the density plot, a normal distribution of the data was assumed (Fig. 2 - left). In general, there was no significant time trend for the foliar N concentrations in the selected plots across years (Fig. 3). We calculated mean values for each year and for each plot. The dependence of the foliar N concentrations on selected environmental characteristics (Table 1) was explored using a linear mixed effect model (LMEM) with year and plot as crossed random effects. The lmer (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2015) package in R was used.

2.6.2 Tree defoliation

A single mean for the main tree species groups per plot was calculated. The plot was included in the model if at least three trees from the same tree species group were present in the plot. The defoliation data were not normally distributed (Fig. 2 - middle); consequently, generalized linear modeling (gamma GLM with log-link) (Zuur et al., 2009) was applied to explore the dependence of defoliation on the selected environmental characteristics (Table 1).

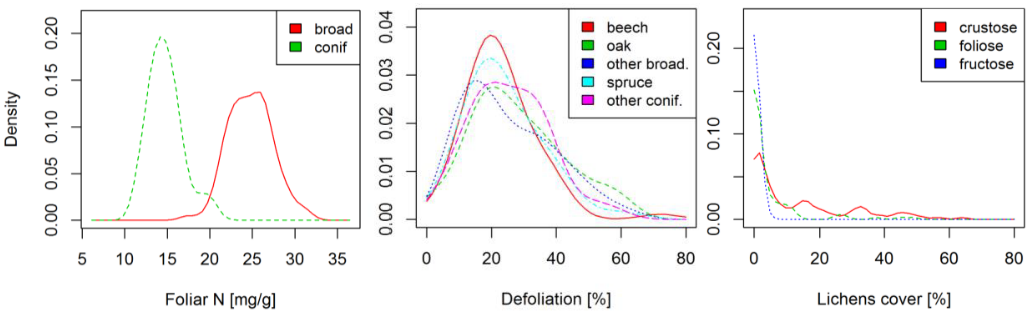

Figure 2: Density plot for foliar N concentration (left), tree defoliation (middle) and lichen cover (right).

2.6.3 Lichen cover

For each plot, the average lichen cover for each tree species group was calculated if at least three trees from the same species group were available per plot. Consequently, there were only sufficient data for three tree species groups (beech, spruce and oak) to perform a consistent statistical analysis. For some plots, two means were calculated for two different species groups. The lichen data were strongly positively skewed, with a high number of zero lichen cover (Fig. 1 - right) - especially in the case of foliose and fructicose lichens. For the latter, the number of zeros was so high that the data were not used for further analysis. Because of zero inflation, gamma hurdle models were used to explore the dependence of lichen cover on the environmental variables (Table 1). The hurdle model consists of two parts: a binomial GLM to predict the probability of non-zero values (zero lichen cover vs. non-zero lichen cover) and a gamma GLM with a log link to predict the probability of non-zero values, such that only trees with lichen cover were retained.

2.6.4 Correlation analysis

The Pearson correlation coefficient was calculated to assess the relationships between the N concentrations and δ^{15}N values in the mosses, foliar N concentration, defoliation and lichen cover. The moss survey values were centered - the original value was lowered by the mean. All statistical analyses presented within this manuscript were performed using the R 3.0 statistical software package (R Development Core Team, 2014).

3 RESULTS

3.1 *Foliar N concentration*

Between 2005 and 2010, the average leaf N concentration was 24.8 ± 0.26 mg/g and the average needle N concentration was 14.8 ± 0.17 mg/g (Table 2).

Table 2: Descriptive statistics for foliar N concentrations.

Tree species groups	No. of plots	No. of sampled trees	Mean [mg/g]	1st Q [mg/g]	Median [mg/g]	3rd Q [mg/g]	Min [mg/g]	Max [mg/g]
Broadleaves	6	96	24.8	23.0	24.7	26.4	17.3	31.4
Conifers	5	135	14.8	13.5	14.5	15.8	11.2	20.8

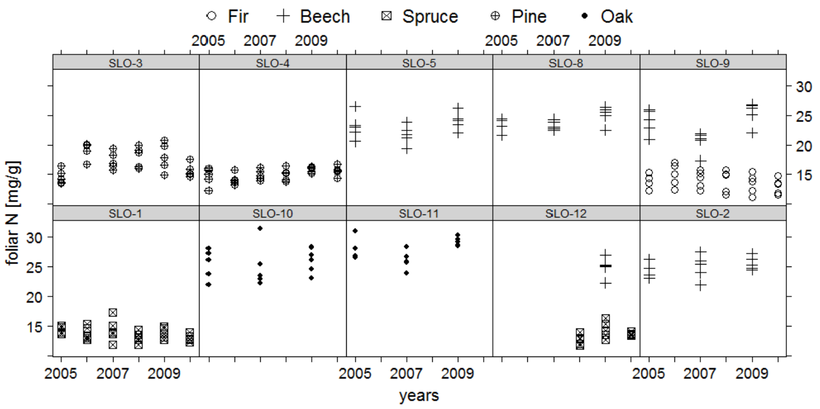

Figure 3: Foliar N concentration of different tree species in selected ICP Forest Level II plots in Slovenia over the period 2005 – 2010.

The LMEM shows that there was a statistically significant dependence of foliar N concentration on tree group, snow cover duration and percentage of urban land within a 100-km radius. At average values of the numerical environmental characteristics, the estimated mean leaf N concentration was 24.3 mg/g (with 95% confidence interval (CI) between 23.5 mg/g and 25.0 mg/g). The estimated mean coniferous needle N concentration was 15.0 mg/g (CI between 14.4 mg/g and 15.7 mg/g) (Table 3). If the snow cover duration extended for 10 days, then the estimated mean foliar N concentration decreased by 0.3 mg/g at a constant value of the other environmental characteristics. With a 1% increase in the percentage of urban land within a 100-km radius, the foliar N concentration increased by 1.0 mg/g (Table 3).

Table 3: Linear mixed effect model results for the dependence of foliar N concentration on selected environmental variables.

Explanatory variables	Estimate	Lower 95%	Upper 95%	p-value
Estimated mean for **Broadleaves**	24.3	23.5	25.0	
Estimated mean for **Conifers**	15.0	14.4	15.7	
Average snow cover duration (1971-2000) (10 days)	-0.3	-0.4	-0.2	0.002**
% of urban land within 100 km radius (%)	1.0	0.4	1.5	0.021*

3.2 Tree defoliation

The descriptive statistics for selected tree species groups are presented in Table 4. Regarding the tree species, oaks had the highest crown defoliation with a mean value of 27.7%. By contrast, beech had the lowest value (22.9%) (Table 4).

Table 4: Descriptive statistics for tree defoliation (Q – quartile).

Tree species groups	No. of plots	No. of assessed trees	Mean [%]	1st Q [%]	Median [%]	3rd Q [%]	Min [%]	Max [%]
Spruce	60	791	23.9	16.5	21.6	29.5	4.0	62.9
Other coniferous species	34	482	25.5	16.7	24.6	33.5	6.0	56.3
Beech	72	1146	22.9	16.6	21.2	27.6	3.8	72.2
Oak	25	177	27.7	17.9	25.6	36.3	0.0	58.1
Other broadleaves	46	567	25.2	13.4	21.8	34.9	7.9	61.7

The dependence of defoliation on the selected tree species groups was not statistically significant. The model shows that forest stand characteristics (tree mixture and canopy closure) are statistically significant in explaining the variation in tree defoliation (Table 5). Using average values of three numerical environmental characteristics (precipitation, snow cover and urban land use), the estimated tree defoliation for trees growing within fragmented mixed or broadleaved forests was higher compared with other types of forests (28% with CI between 24.7% and 32.0%). At average values of other environmental characteristics, the lowest estimated mean defoliation was observed for trees growing within coniferous stands with normal canopy closure (20% with CI between 17.2% and 24.1%) (Table 5).

Plots with a higher amount of annual precipitation had significantly higher tree defoliation, i.e., an additional 10 mm (10 litres/m^2) of precipitation increased defoliation by 2%. In contrast to precipitation, snow cover duration had a positive effect on tree defoliation: with an additional 10 days of snow cover duration, the defoliation decreased by 4%, at average values of other environmental characteristics. The EMEP MSC-W-modeled total reduced N deposition was statistically significant in explaining the variation in the defoliation data: with 1 kg/ha additional N per year, the defoliation decreased by 49% compared with the estimated mean defoliation (Table 5).

Table 5: Gamma GLM (log link) results for the dependence of tree defoliation on other environmental variables. Description of variables: broadleaved forests (at least 75% broadleaves), mixed forests, coniferous forests (at least 75% coniferous species), normal canopy closure (forests with no gaps or only small gaps), fragmented canopy closure (gaps large enough to insert single or multiple crowns).

Explanatory variables	Estimate	Lower 95%	Upper 95%	p-value
Estimated mean for:				
Mixed and broadleaved forests with normal canopy closure	23.8	22.0	25.8	
Mixed and broadleaved forests with a fragmented canopy	28.1	24.7	32.0	
Coniferous forests with normal canopy closure	20.4	17.2	24.1	
Coniferous forests with a fragmented canopy	24.1	20.2	28.7	
Average yearly precipitation (1971-2000) (10 mm)	1.02	1.00	1.04	0.062.
Average snow cover duration (1971-2000) (10 days)	0.96	0.93	0.99	0.007**
EMEP reduced N (2005-2010) (kg/ha year)	0.51	0.29	0.89	0.019*

3.3 Lichen cover

The highest median cover of crustose lichens was found on the beech trees (21.1%); only one plot in this category did not have lichens. For oak trees, the median cover was lower (4.7%); however, crustose lichens were present in all of the oak plots (Table 6). The cover of foliose and especially fructicose types of lichens was significantly lower. The latter were consequently excluded from further analysis. The highest median cover of the foliose type of lichens was observed on the spruce tree group (4.2%) (Table 6).

Table 6: Number of plots without lichen cover and number of plots with lichen cover and their descriptive statistics.

Lichen type	Tree group		Beech	Spruce	Oak
Crustose lichen type	No lichen cover - number of plots		1	4	0
	With lichen cover	number of plots	42	34	17
		1st Q [%]	7.6	0.6	0.5
		Median [%]	21.1	2.5	4.7
		3rd Q [%]	37.6	5.4	13.2
Foliose lichen type	No lichen cover - number of plots		14	15	9
	With lichen cover	number of plots	29	23	8
		1st Q [%]	0.3	0.4	1.3
		Median [%]	1.5	4.2	2.3
		3rd Q [%]	4.1	10.3	7.1
Fructicose lichen type	No lichen cover - number of plots		41	35	16
	With lichen cover	number of plots	2	3	1
		Median [%]	0.1	1.7	0.7

The logistic regression indicated that the mean probability of observing crustose lichens in a selected location was 95% (CI between 89% and 98%). None of the selected environmental characteristics explained the presence or absence of the crustose lichens. At average values of the other numerical environmental characteristics included in the model, the estimated mean crustose lichen cover on beech trees within mixed forest stands was 36.5% with CI between 22.4% and 62.1% (Table 7). By contrast, at average values of the other environmental characteristics, the lowest estimated mean of crustose lichen cover was observed for spruce and oak trees within pure forests (4.0%). Another important forest stand characteristic was the number of trees per hectare. If the number of trees increased by 100 at average values of the other environmental characteristics, the estimated mean crustose lichen cover decreased by 11% (CI between 5% and 17%). Land use characteristics also explained the variation in crustose lichen cover – if the percentage of cropland increased by 1%, the estimated mean crustose lichen cover increased by 5%. In contrast to cropland, urban land had a negative impact on the coverage of crustose lichens - if the cover of urban land increased by 1%, the estimated mean coverage decreased by 53% (Table 7).

Table 7: Gamma hurdle model for the dependence of lichen cover on selected environmental factors. Description of variables: pure forests (at least 75% broadleaves or coniferous species), mixed forests (less than 75%).

Lich. type	Model part	Explanatory variables	Estimate	Lowe r 95%	Upper 95%	p-value
Crustose type of lichens	Binomial	~ 1	0.95	0.89	0.98	< 0.001***
	Gamma	Estimated mean for				
		Beech tree group within mixed forest	36.5	22.4	62.1	
		Beech tree group within pure forest	19.1	13.7	27.6	
		Spruce + oak tree group within mixed forest	7.6	5.2	11.6	
		Spruce + oak tree group within pure forest	4.0	2.7	6.0	
		Number of trees per hectare (hundreds)	0.89	0.83	0.95	< 0.001***
		% cropland within 80-km radius	1.05	1.01	1.09	0.035*
		% urban land within 100-km radius	0.47	0.28	0.82	0.022*
Foliose type of lichens	Binomial	Estimated mean (intercept)	0.66	0.54	0.76	0.009**
		Number of trees per hectare (hundreds)	0.85	0.75	0.96	0.010*
		% forest land within 5-km radius	1.06	1.03	1.10	<0.001***
	Gamma	Estimated mean (intercept)	4.4	3.2	6.1	<0.001***
		Altitude (10 m)	1.04	1.03	1.06	<0.001***
		Average yearly precipitation (1971-2000) (10 mm)	0.89	0.81	0.98	0.023*
		Sunshine duration (1971-2000) (10 h)	1.01	1.00	1.02	0.006**
		% urban land within 8-km radius	1.24	1.05	1.46	0.015*

The results for foliose lichen cover show that at the average value of the other significant environmental characteristics, the mean probability of lichen occurrence at a selected location was 66% (CI between 54% and 76%) (Table 7). The appearance of foliose lichens was dependent on two environmental characteristics – the density of the forest and the percentage of forestland within a 5-km radius. If the number of trees increased by 100 at the average value of the other variables, the estimated probability of foliose lichen appearance decreased by 15%, and if the percent of forests increased by 1%, the estimated probability of appearance increased by 6% (CI between 3% and 10%) (Table 7). When the foliose lichens were present in the plot, the characteristics of the surrounding forest were not as important for the coverage of this lichen type, in contrast to the case of the crustose lichens. In this case, other environmental factors were important. At higher altitude, sunshine duration and percentage of urban land use, the foliose lichen cover increased. By contrast, a higher average yearly precipitation decreased the estimated mean foliose lichen cover (Table 7).

3.4 Correlations between moss biomonitoring results, foliar N concentrations, tree defoliation and lichen cover

The models showed (Table 3, Table 5 and Table 7) that some of the forest characteristics significantly influenced the variation in the bioindication results. Only significant differences between groups were taken into account in the correlation analysis. No significant correlation was found between foliar N concentration, tree defoliation and lichen cover. However, significant correlations were found between (Table 8):

i) Moss N_{open} and leaf N concentration (Fig. 4 - left),

ii) Moss N_{open} and crustose lichen cover in plots within pure coniferous or broadleaved forests (Fig. 4 - right),

iii) Moss N_{canopy} and defoliation of trees in plots with normal canopy closure (Fig. 5 - left),

iv) Moss $\delta^{15}N_{canopy}$ and defoliation of trees in mixed and broadleaved plots (Fig. 5 - right).

Table 8: Results of Pearson correlation statistics between different combinations of moss N value (centered) and foliar N, defoliation and lichen cover. The defoliation and lichen cover data were log transformed.

Variable 1	Variable 2	Pearson corr. coef.	Min CI	Max CI	p-value
N_{open} cent.	Foliar N in leaves	0.67	0.31	0.86	0.002**
	Crustose lichen cover – pure stands	-0.47	-0.65	-0.23	<0.001***
N_{canopy} cent.	Defoliation - stands with normal canopy closure	-0.24	-0.45	-0.01	0.043*
$\delta^{15}N_{canopy}$ cent.	Defoliation - mixed and broadleaved stands	0.32	0.10	0.51	0.005**

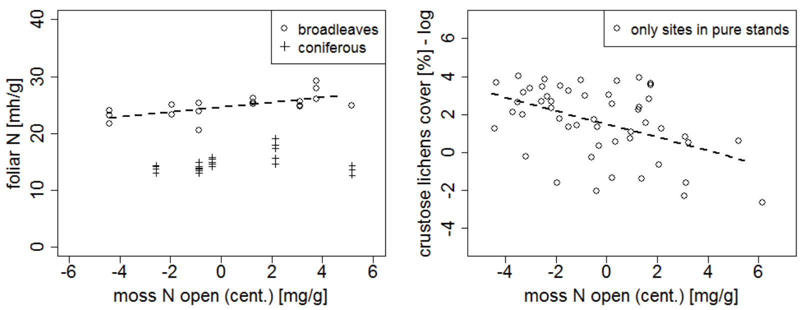

Figure 4: Correlation between centered N_{open} and foliar N (yearly averages) (left) and correlation between centered N_{canopy} and crustose lichen cover (right).

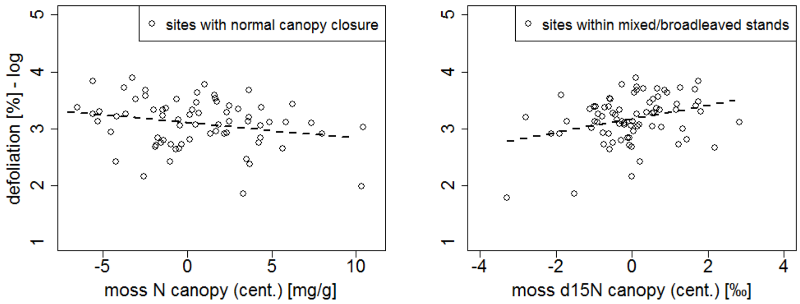

Figure 5: Correlation between centered N_{canopy} and defoliation (left) and between centered $\delta^{15}N_{canopy}$ and tree defoliation (right).

4 DISCUSSION

4.1 Foliar N concentration

4.1.1 Site characteristics and meteorological explanatory factors

Leaves have significantly higher N concentrations then needles (Table 3). There is a compositional difference between leaf and needle structure: needles are sclerophyllous, with greater leaf mass and a thicker cuticle, and contain high amounts of carbon-rich components that are used for efficient water usage (Larcher, 1995; Smith and Smith, 2001). Consequently, the amount of N in the total needle mass is lower in comparison to leaves (Sardans et al., 2011). Numerous studies indicate that climate characteristics can influence the foliar N concentration - under lower precipitation and temperature, the foliar N concentration decreases (Kerkhoff et al., 2005; Reich et al., 2004; Sardans et al., 2011). Precipitation can affect foliar N concentration directly through photosynthesis or indirectly through the mineralization and decomposition of soil (Patrick et al., 2007; Urbancic et al., 2009). In addition, the foliar N concentration decreases at high altitude (Reich et al., 2004) and under low soil fertility (Ordonez et al., 2009). Altitude and temperature are often correlated variables –the temperature decreases as altitude increases. The above-mentioned characteristics are also correlated with snow cover duration, which proved to be one of the significant environmental characteristics influencing the foliar N concentration in Slovenia (Table 3).

4.1.2 Anthropogenic environmental factors

As evident from our study, foliar N concentrations only depended significantly on the percentage of urban land within a 100-km radius (Table 3). Urban land includes households, industries and traffic infrastructure that all emit mainly NO_x-N from combustion processes (Sutton et al., 2011). Incorporating the modeled total reduced or oxidized N deposition into the model did not significantly increase the explained variability of the foliar N concentrations. The outcome of our analysis, i.e., that an anthropogenic factor (urban land use) is correlated with foliar N concentrations, is in agreement with other studies that showed foliar N concentration can be used as an indirect method to identify areas with high atmospheric N deposition (McGroddy et al., 2004; Ordonez et al., 2009; Reich et al., 2004; Sardans et al., 2015). However, the number of locations in our study was very low and consequently, the power of the analysis is limited.

4.2 Tree defoliation

4.2.1 <u>Site characteristics and meteorological explanatory factors</u>

Tree defoliation depended significantly on certain forest stand characteristics. The highest estimated mean defoliation was found for trees growing within mixed and broadleaved stands with fragmented canopy closure, while the lowest estimated mean was observed for trees growing within pure coniferous stands with normal canopy closure (Table 5). The report on Forest condition for Europe also shows that in general, broadleaf trees have greater defoliation than coniferous trees (Seidling et al., 2015). Canopy closure reflects the age of the stand. Older trees consume more space and old-growth forests are usually fragmented before the final felling. The age of the trees, as the strongest predictor of tree defoliation, was reported by Klap et al. (2000) and by Vitale et al. (2014) for fir and pine species. Canopy closure also has a direct influence on the amount of light reaching the leaves in the canopy. The quantity of canopy light intercepted in combination with leaf mass per area (LMA) is directly correlated with the photosynthetic capacity of trees, their respiration and foliar N concentration (Wright et al., 2004). Studies on sugar maple trees have shown that LMA, leaf thickness, leaf density and diffuse non-interception (DIFN) (the fraction of radiation that is transmitted through the canopy), differ between trees with closed canopies and those with exposed canopies (Coble and Cavaleri, 2014). LMA, leaf thickness and leaf density were higher in an exposed canopy; however, the DIFN was also higher (Coble and Cavaleri, 2014). This indicates that in more exposed canopies, the total number of leaves on a tree may be lower, but the remaining leaves are more efficient with respect to photosynthesis and respiration.

Lower number of developed leaves could explain the higher defoliation of trees growing in stands with a fragmented canopy.

In contrast to some literature data (Ferretti et al., 2014; Vilhar et al., 2014), we found that data on annual precipitation were not important for explaining the variation in tree defoliation; however, climatological data (averages from 1971-2000) on yearly precipitation and the duration of snow cover were important. Opposite to expectations, the estimated tree defoliation increased for forests with higher average yearly precipitation (Table 5). There are numerous studies correlating drought with tree defoliation (de Vries et al., 2014; Ferretti et al., 2014; Klap et al., 2000; Seletković et al., 2009; Vitale et al., 2014). Until now, drought has apparently not been problematic in our research plots. We assume that the negative influence of precipitation on tree defoliation could be attributed to humid conditions, which promote the development of different pathogenic agents (Waller, 2013). In contrast to the average yearly precipitation, the snow cover duration has a positive effect on tree defoliation. The significance (p-value) of this climatological factor was higher than that for precipitation (Table 5). Snow cover is strongly correlated with elevation and can also have a negative effect on tree defoliation (Klap et al., 2000). In some regions, soil water reservoirs are refilled in the spring when the snow melts (Zierl, 2001), and this fresh water supply at the beginning of the growing season could decrease the crown defoliation.

4.2.2 Anthropogenic environmental factors

The lack of or a weak dependence of crown defoliation on air pollutant deposition has been reported in the literature (de Vries et al., 2014; de Vries et al., 2000; Klap et al., 2000; Staszewski et al., 2012). However, our results showed a statistically significant dependence of tree defoliation on modeled total reduced N deposition. The N deposition had a positive effect on tree defoliation (Table 5), indicating that in most of the Slovenian forests, N deposition does not exceed the critical level (12–14 kg N/ha per year) and has a positive effect as a nutrient additive. The above is also in line with the results of the Coordination Centre for Effects status report for year 2011 (Eler et al., 2011), in which relatively small areas where the critical levels were exceeded were reported for Slovenia. Numerous studies have shown that N deposition could lead to an increase in tree growth (de Vries et al., 2009; Solberg et al., 2009; Spiecker et al., 1996) and improve the vitality of the trees.

4.3 Lichen cover

4.3.1 Site characteristics and meteorological explanatory factors

None of the tested environmental characteristics explained the appearance of crustose lichens. In the cases where the crustose lichens were found, it was more likely that they grew on the trunks of beech trees than on the trunks of spruce or oak trees (see description of tree groups in section 2.3) (Table 7). The dependence of lichen cover on substrate factors has already been discussed in the literature (Giordani, 2006; Spier et al., 2010), where it has been shown that the pH of the bark, in particular, could be the driving factor of lichen coverage (Frahm et al., 2009). As shown in our study, the estimated mean crustose lichen cover was higher in the mixed forests than in the pure stands. We assumed that the species in the mixed forests could be associated with local humidity and light conditions - most of the lichen species, especially those of the foliose and fructicose type, are photophilic (Nash, 1996). However, the relative crown projection area and relative crown ground cover is higher in mixed than in pure stands (Pretzsch, 2014); consequently, the dependence remains unexplained. The photophilic characteristics of the lichens were in agreement with the dependence of crustose lichen coverage on the number of trees per hectare (Table 7). The coverage of crustose lichens was lower in denser stands (less light). The number of lichens species is correlated with canopy openness – the number and coverage of lichens increases with higher canopy openness (Marmor et al., 2012; Pearson, 1969). This site characteristic was also significant for the appearance of foliose lichens - the probability was higher if the number of trees per hectare was lower (Table 7). When foliose lichens were present on a selected tree trunk, the estimated mean depended on altitude, the amount of yearly precipitation and on the sum of yearly sunshine duration. The positive influence of sunlight has already been discussed. The macrolichen diversity could decrease with altitude, but the total coverage of lichens may generally increase (Giordani et al., 2014).

4.3.2 Anthropogenic environmental factors

Compared with some standard protocols such as VDI (VDI, 1995), the lichen bioindication technique used in this study was simplified (Poličnik et al., 2008). The suggested technique could be used with limited knowledge on lichen species; consequently, non-specialized personnel could conduct the field assessment. Giordani et al. (2014) showed that at the European level, the occurrence of macrolichens correlated more strongly with N deposition than with lichen diversity. Our results showed that type of land use is reflected in both, i.e., crustose and foliose lichen cover (Table 7). Urban land use that emits mainly NO_x had a strong negative effect on the cover of crustose lichens, while croplands, which emit mainly NH_y had a small but significant positive effect. Gadsdon et al. (2010) reported a negative effect of NO_2 on total lichen cover in oak forests and no effect of NH_3. Our results showed that foliose lichen cover also showed a positive response to potential N pollution. Lichens are sensitive to atmospheric reactive N deposition, but some of them are oligotrophic and some are nitrophytic (Frahm et al., 2009; Munzi et al., 2014). Studies have shown that N addition could lead to an increase in foliose lichen thallus growth, but only if the concentrations are low; this could also occur in our case. Thallus growth was reduced under high N concentrations (Welch et al., 2006). The disadvantage of our method is that it does not enable discrimination between acidophytic and nitrophytic lichens.

4.4 Correlations between moss biomonitoring results, foliar N concentration, tree defoliation and lichen cover

Moss N_{open}, which provides information on the amount of atmospheric N deposited on the forest overstory (Skudnik et al., 2015a), was correlated with the leaf N concentration and crustose lichen cover in pure stands. Some studies reported a significant correlation between throughfall N deposition and needle N concentration, but no correlation between throughfall N and leaf N concentration (Kristensen et al., 2004), which was explained by higher translocation of N in broadleaves. However, some authors found correlations for both conifers and broadleaves (Boggs et al., 2007; Eichhorn et al., 2001; Thimonier et al., 2010). Further, for broadleaves, stronger correlations have been found between NH_4^+-N total deposition and leaf N concentration (Eichhorn et al., 2001). Higher uptake of NH_4^+-N has been reported for mosses, presumably due to their high cation-exchange capacity (Bates, 1992). This could also be the explanation for the stronger correlation between the moss and leaf N concentration in our study.

We assume that the negative correlation between N_{open} and crustose lichen cover in pure stands is associated with stemflow (Hauck et al., 2002). Higher N concentrations in atmospheric deposition produce higher N concentrations in stemflow (Levia and Germer, 2015) and consequently, lower the pH of the tree bark, which may contribute to a lower number of lichen species (Fritz et al., 2009) and less lichen cover.

N_{canopy}, which provides information on the N deposited on the forest floor (Skudnik et al., 2015a), i.e., below the tree canopies, was negatively correlated with the defoliation of trees in pure stands. Studies have shown that climatic factors are usually more important drivers of tree defoliation than air pollution (de Vries et al., 2014; Ferretti et al., 2014). However, some studies have showed a positive relationship between defoliation and foliar N concentration of coniferous species (Thimonier et al., 2010), and others have shown a negative relationship between defoliation and the foliar N:P ratio (Veresoglou et al., 2014). In areas where N deposition does not exceed the critical level, N could positively influence tree vitality and growth (Pretzsch et al., 2014)

A positive correlation between $\delta^{15}N_{canopy}$ and tree defoliation in mixed and broadleaved stands was evident at lower levels of defoliation in cases where the main source of N was derived from NH_y (more negative $\delta^{15}N$ values in mosses). This is in agreement with the models presented in section 3.2, where we showed that defoliation was lower in areas with higher modeled reduced N deposition. Apparently, deposited N is an important nutrient source in Slovenian forests.

However, despite the fact that we took into account all specific significant site characteristics that influenced the results of a certain biomonitoring or bioindication technique, no correlation was found between the foliar N concentration, tree defoliation and lichen cover.

5 Conclusions

In this paper, the presented natural environmental factors that significantly influenced the variability of the biomonitoring and bioindication results were all in accordance with the latest knowledge on ecophysiological plant characteristics. The results show that the characteristics of the sites where the bioindication method was applied have an important influence on the bioindication end results. This was observed especially for those factors that were directly associated with the type of organism selected, water and light availability, and temperature, etc. However, all of the tested techniques also reflected the presence of human activities.

Different bioindication techniques, used in the same area, should in principal produce similar end results. However, the correlation analysis only indicated a statistically significant relationship for the moss N concentration and $\delta^{15}N$ value with the foliar N concentrations, lichen cover and tree defoliation. No correlations were found among the other biomonitoring and bioindication techniques.

It should also be stressed that defoliation of forest trees and epiphytic lichen cover are not as directly connected to N deposition as the two other biomonitoring methods (moss N concentration and δ^{15}N value, and foliar N concentration). However, these two methods are based on relatively limited field observations, and some additional information on the condition of forest ecosystems across larger areas is required.

We can conclude that the result obtained from foliar N analysis, tree defoliation and lichen cover are not directly associated with atmospheric N deposition. However, based on the knowledge of the influence of natural environmental factors on the bioindication results, it is possible to estimate how human disturbances have influenced the selected biomonitoring or bioindication technique. Further, this knowledge can enable more concise planning of sampling designs in future surveys, where the study area is clustered based on known influential environmental characteristics, such that the dependence of results on natural environmental characteristics could be partly avoided.

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3 RAZPRAVA IN SKLEPI

3.1 RAZPRAVA

3.1.1 Izmerjene vsebnosti N in vrednosti δ^{15}N v mahovih v Sloveniji

Po industrijski revoluciji so se emisije N povečale zaradi dejavnosti, kot so intenzivnejša predelava hrane (Rockstrom in sod., 2009) in proizvodnja energije (Galloway in sod., 2008). Velik del tega novega N_r se v obliki mokrih in suhih usedlin vrne na tla, kar lahko vodi do evtrofikacije in zakisanja različnih ekosistemov (Erisman in sod., 2007). Za spremljanje časovnih in prostorskih informacij o količinah atmosferskih usedlin N-spojin so bile razvite številne analitične (fizikalno-kemijske) in posredne oblike monitoringa. Med slednje spada tudi biomonitoring z mahovi (Harmens in sod., 2015).

Glede na koncentracije N v mahovih, nabranih v gozdnih vrzelih (v nadaljevanju N_{open}) lahko večino lokacij v Sloveniji uvrstimo med podeželske ali lokacije ozadja. Rezultati so v skladu s programom ICP Vegetation, katerega cilj je raziskati onesnaženost zraka na velikih razdaljah prek državnih meja (ICP Vegetation Coordination Centre, 2010). Izmerjena srednja vrednost N_{open} je bila 13,1 mg/g (min = 8,5 mg/g in max = 19,9 mg/g) in je bila primerljiva z vrednostmi v sosednji Avstriji (povprečje = 12,1 mg/g, min = 7,6 mg/g, max = 19,9 mg/g) (Zechmeister in sod., 2008) ter večja v primerjavi z nekaterimi severnoevropskimi državami; na primer na Finskem (Poikolainen in sod., 2009), kjer je onesnaženje z N med najmanjšimi v Evropi (Harmens in sod., 2014). Izmerjena povprečna koncentracija N v mahovih, nabranih pod drevesnimi krošnjami (N_{canopy}) je znašala 17,5 mg/g (min = 10,4 mg/g in max = 27,9 mg/g) in je značilno večja od N_{open}. Največje razlike med N_{open} in N_{canopy} smo opazili v jugovzhodnih in severovzhodnih predelih države, v bližini dveh termoelektrarn (TE Šoštanj in TE Trbovlje) ter ob nekaterih avtocestnih odsekih.

V Sloveniji je bila povprečna vrednost $\delta^{15}N$ v mahovih, nabranih v gozdnih vrzelih ($\delta^{15}N_{open}$) -5.4 ‰ (min = -8,1 ‰ in max = -3,2 ‰), povprečna vrednost $\delta^{15}N$ v mahovih, nabranih pod drevesnimi krošnjami ($\delta^{15}N_{canopy}$) pa -5.5 ‰ (min = -8,8 ‰ in max = -2,7 ‰). Vrednosti so primerljive z rezultati za Španijo (povprečje = -5.6 ‰, min = -7.9 ‰, in max = -3.3 ‰), so pa manj negativne kot tiste za Francijo (povprečje = -6,0 ‰, min = -8,0 ‰, in max = - 3.3 ‰), Švico (povprečje = -6,9 ‰, min = -10,8 ‰, in max = -3,3 ‰) (Foan in sod., 2014) in sosednjo Avstrijo (povprečje = -6,0 ‰, min = -10,0 ‰, in max = -2,5 ‰) (Zechmeister in sod., 2008). Rezultati kažejo, da so, v primerjavi s sosednjimi državami, za Slovenijo glavni vir atmosferskih usedlin N procesi zgorevanja (NO_x) in ne proizvodnja hrane oz. kmetijstvo (NH_y) (Beyn in sod., 2014). Vendar pa so razlike med državami v tem delu Evrope še vedno zanemarljive. Na primer, ARSO, ki je odgovoren za nacionalni program spremljanja kakovosti padavin, je za leto 2012 poročal o večjih vrednostih NH_y kot NO_x, s povprečnimi suhimi usedlinami 0.80 µg/m^3 za (NH_3 + NH_4^+)-N in 0,26 mg/m^3 za (HNO_3 + NO_3^-)-N in povprečnimi mokrimi usedlinami 3,8 kg/ha letno za NH_4^+-N in 3,0 kg/ha letno za NO_3^-–N (Bolte in sod., 2013).

3.1.2 Odvisnost N v tkivih mahov od atmosferskega vnosa N-spojin

V zadnjih letih je bilo pogosto izpostavljeno raziskovalno vprašanje, ali so vsebnosti N v mahovih odvisne od količin atmosferskih usedlin N (Harmens in sod., 2011). Medtem ko so nekatere študije pokazale, da je mogoče na podlagi biomonitoringa z mahovi samo bolj ali manj uspešno identificirati onesnažena območja (Pitcairn in sod., 2006; Zechmeister in sod., 2008), so drugi raziskovalci predstavili modele, s katerimi se lahko oceni količine atmosferskih usedlin N na podlagi vsebnosti N v mahovih (Harmens in sod., 2011; Solga in sod., 2005). Takšni modeli so običajno izdelani na podlagi spremljanja odvisnosti vsebnosti N v mahovih od količin N v atmosferskih usedlinah. Tovrstna odvisnost je bila dokazana tudi v naši raziskavi, ki potrjuje, da N_{open} v mahu vrste štorovo sedje (*Hypnum cupressiforme* Hedw.), ki je pogost v naravnem okolju v južnem delu srednje Evrope, odraža v atmosferskih usedlinah izmerjene količine N za to območje. S tem smo potrdili prvo hipotezo disertacije. Pred našo raziskavo so bile podobne odvisnosti dokazane tudi za pet drugih plevrokarpnih vrst mahu (*Hylocomium splendens, Pleurozium schreberi* (Brid.) Mitt., *Scleropodium purum, Rhytidiadelphus squarrosus* (Hedw.) Warnst. in *Thuidium tamariscinum* (Hedw.) Schimp., 1852) (Hicks in sod., 2000; Leith in sod., 2005; Mohr, 1999; Raymond in sod., 2010; Solga in sod., 2005). Podobno kot nekatere druge raziskave so tudi naši rezultati pokazali relativno šibko odvisnost. Nekateri viri navajajo, da so odvisnosti močnejše, če so v analizo vključeni tudi podatki o totalnih količinah suhih usedlin N (»*dry N deposition*«) (Raymond in sod., 2010; Skinner in sod., 2006), ki pa jih v času pisanja disertacije še ni bilo na voljo za v naši raziskavi uporabljene lokacije. V raziskavi smo namreč uporabili podatke o atmosferskih usedlinah, zbranih v odprtih zbiralnikih (»*bulk deposition*«), ki zbirajo skupne mokre in del suhih atmosferskih usedlin. Pri interpretaciji odvisnosti moramo upoštevati tudi dejstvo, da smo imeli možnost ugotavljati odvisnost med N v mahovih od N v atmosferskih usedlinah na relativno majhnem številu merilnih mest (N = 18). Odvisnosti smo testirali z uporabo triletnih povprečnih količin atmosferskih usedlin N in tudi za krajše časovne nize od enega do dvanajstih mesecev. Rezultati kažejo, da je odvisnost N_{open} v zelenem delu mahu vrste *H. cupressiforme* večja, če uporabimo podatke o atmosferskih usedlinah N za tekoče leto (tj. 2010), kot pa če uporabimo podatke večletnih povprečij (npr. 2007–2010). O razlikah v vsebnosti N v različno starih tkivih mahov so razpravljali tudi Liu in sod. (2008a), ki so za vrsto *Haplocladium microphyllum* (Hedw.) Broth ugotovili, da imajo starejša tkiva manjše vsebnosti N. Te ugotovitve kažejo, da mora biti nabiranje mahu na terenu opravljeno v kratkem časovnem obdobju – v nekaj mesecih, če želimo N_{open} na lokacijah nabiranja mahu primerjati med seboj.

Odvisnost N_{open} od atmosferskih usedlin N-spojin je statistično močnejša, če upoštevamo samo količine NH_4^+-N v usedlinah. Boljšo vezavo NH_4^+-N od NO_3^--N v tkiva mahu so na eksperimentalni ravni že dokazali (Forsum in sod., 2006; Nordin in sod., 2006), in sicer naj bi ta bila posledica večje kationske izmenjevalne sposobnosti mahu (Bates, 1992). Rezultati kažejo, da se odstotek pojasnjene variabilnosti N v mahovih in atmosferskih usedlinah NO_3-N poveča, če so bile v analizo vključene izključno lokacije z več kot 1000 mm letnih padavin. V naši raziskavi so imele lokacije z majhnimi količinami padavin izmerjene tudi manjše količine atmosferskih usedlin NO_3-N. Ugotovitev bi lahko pojasnili s tem, da sta NO in NO_2^- slabo topna v vodi, vendar pa je rezultat njune reakcije, HNO_3, zelo topen. V predelih z večjimi količinami padavin se NO_3^- sproti izpira iz atmosfere, medtem ko v območjih z manjšimi količinami padavin HNO_3 reagira z NH_3 in se spremeni v NH_4^+ (Asman in sod., 1998). Vpliv količine padavin na odvisnost $\delta^{15}N$ v mahu od razmerja $NH_4^+ : NO_3^-$ v atmosferskih usedlinah je bila še izrazitejša. Model je bil statistično značilen samo pod pogojem, da smo izključili vse lokacije z manj kot 1000 mm padavin. Do podobnih zaključkov so prišli tudi Zechmeister in sod. (2008). V prihodnosti bodo potrebne dodatne raziskave, ki bi pojasnile odvisnost N v mahovih od N v atmosferskih usedlinah na bolj sušnih območjih, tj. območjih z manj kot 1000 mm letnih padavin.

V nasprotju z rezultati, ki so jih predstavili Leblond in sod. (2009), v naši raziskavi nismo odkrili statistično značilne odvisnosti N_{canopy} od količine N v sestojnih usedlinah (»*throughfall*«). Na vseh 14-ih lokacijah, je bila N_{canopy} večja kot N_{open}, količina N v sestojnih usedlinah pa je bila večja od količine N v atmosferskih usedlinah na odprtem le v 7 od 14-ih lokacij. Vpliv drevesnih krošenj na vsebnosti elementov v sestojnih usedlinah se razlikuje tako med iglavci in listavci kot tudi med posameznimi drevesnimi vrstami (Moreno in sod., 2001; Nieminen in sod., 1999). Naši rezultati nakazujejo, da je odvisnost med N_{canopy} in vsebnostjo N v sestojnih usedlinah odvisna od zgradbe sestoja (iglavci, listavci). Rezultati, ki so jih predstavili Leblond in sod. (2009), se nanašajo na 23 lokacij v Franciji, od katerih jih je bilo kar 18 v iglastih sestojih. V našem primeru smo imeli več lokacij v listnatih sestojih, kar bi lahko pojasnilo razlike v rezultatih obeh raziskav. Sestojne usedline vsebujejo tako organske (aminokisline, sečnina itd.) kot anorganske oblike N (Forsum in sod., 2006) in druge v vodi raztopljene snovi (Gundersen in sod., 1998), vendar ne vsebujejo N, izpranega iz razkrojenega opada, ki je padel na gozdna tla (Boxman in sod., 2008). Dodatne količine N iz opada bi lahko v listnatih sestojih značilno vplivale na odvisnost N_{canopy} od količine N v sestojnih usedlinah, vpliv pa ne bi bil tako izrazit v iglastih sestojih, kjer je količina N iz opada običajno manjša (Reich in sod., 2005).

Eden izmed pogojev, da je organizem primeren za uporabo kot biomonitor je, da je variabilnost izmerjenih elementov znotraj lokacije nabiranja oz. vzorčenja manjša kot med lokacijami. V našem primeru je bila variabilnost N_{open} in tudi vrednosti $\delta^{15}N_{open}$ v mahovih, nabranih na petih podvzorcih v gozdnih vrzelih, manjša od variabilnosti med lokacijami. V vzorec je bilo vključenih 26 lokacij. Ugotovitev potrjuje, da v tem delu Evrope štorovo sedje (*H. cupressiforme)* izpolnjuje zahtevane pogoje biomonitoringa, ki so jih predstavili Wolterbeek in sod. (1996). Je pa bila variabilnost N_{open} med podvzorci znotraj lokacije večja kot smo pričakovali. Predpostavljamo, da so k večji variabilnosti podatkov znotraj lokacije vodile predvsem težave pri iskanju mahu, ki je dovolj oddaljen od najbližje krošnje (manj kot 3 m) in s tem ni več pod vplivom skozi krošnje prepuščenih padavin.

3.1.3 Vpliv krošnje na vsebnost N in vrednosti δ^{15}N v tkivih mahov

V raziskavi smo pokazali, da N_{canopy} vključuje dodatne vnose N (poleg direktnega vnosa suhih in mokrih depozitov iz zraka tudi spiranje iz krošenj); N_{canopy} je bil v povprečju za 41 % večji, kot so bile vsebnosti N v mahovih, nabranih vsaj tri metre stran od najbližje projekcije krošnje. S tem je bila potrjena druga hipoteza disertacije, da je obremenjenost z N spojinami pod drevesnimi krošnjami v gozdu večja kot v gozdnih vrzelih ali jasah. Rezultati so tudi v skladu z ugotovitvami, da krošnje dreves predstavljajo učinkovit ponor atmosferskih plinov in delcev, ki se kasneje izperejo iz krošenj dreves na gozdna tla (De Schrijver in sod., 2008). Struktura gozdov in značilnosti podnebja pomembno vplivajo na razmerje med N v usedlinah na odprtem in v sestojnih usedlinah (De Schrijver in sod., 2007), vendar znotraj gozda opad vedno predstavlja še dodaten vir N, ki se usede na gozdna tla (Reich in sod., 2005). Skozi krošnje prepuščene padavine in opad so lahko vzrok za večje vsebnosti N, ki so bile ugotovljene za južno in vzhodno Evropo (Harmens in sod., 2011), kjer je mah štorovo sedje najpogosteje uporabljen biomonitor (Harmens in sod., 2008; Sabovljević in sod., 2009). O vplivu skozi krošnje prepuščenih padavin na vsebnost elementov v mahovih so poročali tudi nekateri drugi avtorji (Kluge in sod., 2013; Pesch in sod., 2007; Samecka-Cymerman in sod., 2010).

Na podlagi podatkov disertacije in drugih raziskav lahko sklepamo, da ni primerno primerjati rezultatov biomonitoringa z mahovi med državami ali znotraj države, če mahovi niso sistematično nabrani na odprtem – zunaj vpliva skozi krošnje prepuščenih padavin. Vendar pa šibka odvisnost vsebnosti N v mahovih, ki so bili nabrani pod drevesnimi krošnjami, z vsebnostmi NH_4^+-N v atmosferskih usedlinah na odprtem kaže, da se v primeru, da nabiranje mahov na odprtem ni mogoče, te lahko nabiramo tudi v sestoju pod drevesnimi krošnjami. Zavedati pa se je treba, da je zaradi vpliva bližnjih krošenj variabilnost teh podatkov večja, hkrati pa je odvisnost med N_{canopy} in količinami N v usedlinah na odprtem drugačna, zaradi česar je potrebno za preračun količin N v atmosferskih usedlinah, na podlagi N_{canopy}, uporabiti drugačne modele.

Z našo raziskavo nismo mogli potrditi ugotovitve Liu in sod. (2007), da nekatere značilnosti krošnje potencialno regulirajo tudi vrednosti $\delta^{15}N$ v mahovih. Naši rezultati namreč kažejo, da razlike med $\delta^{15}N_{canopy}$ in $\delta^{15}N_{open}$ niso bile statistično značilne. Kljub statistični neznačilnosti tudi naši rezultati nakazujejo, da so v povprečju vrednosti $\delta^{15}N_{canopy}$ bolj negativne. V nasprotju z našimi ugotovitvami in ugotovitvami Liu in sod. (2007) so Heaton in sod. (1997) pokazali, da se suhe usedline in prašni delci, sprani iz drevesnih krošenj, odražajo v obliki manj negativnih vrednosti $\delta^{15}N$ v sestojnih usedlinah. Opozoriti je treba, da je bila omenjena študija izvedena v iglastih gozdovih, ki imajo večje potenciale usedanja (De Schrijver in sod., 2008) od listnatih gozdov. Nekatere druge študije so pokazale, da so vrednosti $\delta^{15}N$ v mahu odvisne tudi od nadmorske višine ter padavinskega režima (Zechmeister in sod., 2008), $\delta^{15}N$ pa je odvisna tudi od vrste mahu (Liu in sod., 2010). Omenjeni dejavniki so lahko vzrok za nekatere razlike v rezultatih in nadaljnje raziskave bi bile potrebne za boljše razumevanje teh procesov.

3.1.4 Vpliv značilnosti okolja na vsebnost N in vrednosti $\delta^{15}N$ v mahovih

Rezultati kažejo, da se okoljski dejavniki, ki vplivajo na N_{open}, razlikujejo od tistih, ki vplivajo na N_{canopy}. Za N_{open} je bila, z vidika deleža pojasnjene variance, najpomembnejša okoljska spremenljivka odstotek (%) urbanih površin znotraj 80-kilometrskega radija, kar kaže na to, da potencialni viri emisij N iz urbanega okolja pojasnijo največji delež variabilnosti podatkov. Nasprotno, so pri N_{canopy} glavni vir emisij N zasenčili okoljski dejavniki, povezani z značilnostjo gozda na lokaciji nabiranja mahu (sestojni sklep in mešanost). N_{canopy} je bil večji v gozdovih z bolj tesnim sestojnim sklepom in z večjim odstotkom iglavcev. Ugotovitev je skladna z drugimi študijami, ki so pokazale, da tla v iglastih gozdovih prek atmosferskih usedlin prejmejo večje količine N kot tla v listnatih gozdovih v podobnih podnebnih razmerah (De Schrijver in sod., 2008; van Ek in Draaijers, 1994; Wuyts in sod., 2008). Običajno imajo iglasti gozdovi večjo gostoto (število dreves na hektar), gostejše sklepe krošenj in večjo intercepcijsko površino (Cole in Rapp, 1981). Veter je eden izmed najpomembnejših dejavnikov pri transportu onesnažil po zraku, zlasti suhih usedlin in prašnih delcev, ki se lahko ujamejo v drevesne krošnje, tj. iglic, ki so bolj učinkovite pri zbiranju kapljic in prašnih delcev od listov (Erisman in Draaijers, 2003). Naša raziskava kaže, da so zlasti struktura gozda (sestojni sklep in mešanost drevesnih vrst) ter podnebne značilnosti (povprečna hitrost vetra) najpomembnejši dejavniki, ki vplivajo na dodatne vnose N pri N_{canopy}. De Schrijver in sod. (2007) opisujejo podobne okoljske dejavnike, ki pojasnjujejo razlike med usedlinami na odprtem in sestojnimi usedlinami.

Vpliv količine padavin v zadnjih štirih mesecih pred dnevom nabiranja mahu na N_{open} kaže, da je potrebno mah za namene biomonitoringa nabrati v suhem obdobju leta in s tem zmanjšati variabilnost podatkov med lokacijami. Odvisnost N_{open} od nadmorske višine je pozitivna, kar je v skladu z ugotovitvami, da količine atmosferskih usedlin N naraščajo z nadmorsko višino (Miller in sod., 1993). Po drugi strani se podatki v literaturi za odvisnost vsebnosti N v mahu od nadmorske višine razlikujejo. Nekatere raziskave so pokazale pozitivno odvisnost (Baddeley in sod., 1994; Zechmeister in sod., 2008), medtem ko druge negativno (Schröder in sod., 2010). Hicks in sod. (2000) so v svojem članku ugotovili linearno povečanje atmosferskih usedlin N z nadmorsko višino, vendar niso odkrili povezave z vsebnostmi N v mahu vrste *H. splendens*. Ti neskladni rezultati kažejo, da bi lahko bila odvisnost vrstno in reliefno specifična in da so o tem potrebne nadaljnje raziskave.

Značilnosti okoliške rabe tal, ki vplivajo na vsebnost N v mahovih, so v nasprotju z drugimi okoljskimi značilnostmi, ki smo jih raziskovali v tej študiji, tesno povezane z vprašanjem, ali je z analizo mahov na vsebnosti N mogoče ugotavljati glavne vire emisij N. Rezultati naših modelov so primerljivi z nekaterimi drugimi študijami, ki so prav tako izpostavile, da je raba prostora (delež pozidanih površin) tista spremenljivka, ki pomembno vpliva na pojasnitev antropogenega N v Evropi (Schröder in sod., 2010). Pozidana zemljišča so namreč povezana z emisijami NO_x, ki so rezultat procesa zgorevanja fosilnih goriv v prometu, industriji in proizvodnji električne energije. NO_x se v atmosfero večinoma sprošča kot NO in NO_2^-. Značilnost obeh spojin je majhen delež suhega useda in posledično imajo NO_x spojine majhen vpliv blizu vira sproščanja (Hertel in sod., 2011). Majhne količine NO_x usedlin v bližini njegovega vira so skladne z našimi modelnimi izračuni, ki kažejo, da so pri spremenljivki pozidane površine pomembnejši večji radiji (80 km za N_{open} in 40 km za N_{canopy}). Po drugi strani pa ima NH_y, predvsem NH_3, zaradi velikih količin suhega useda, velik vpliv v bližini vira (Asman in sod., 1998; Dungait in sod., 2012). Naš model za N_{open} je pokazal podobne rezultate; spremenljivka odstotek kmetijskih površin je bila pomembna v manjših (5-kilometrskh) radijih, medtem ko spremenljivka delež kmetijskih površin znotraj večjih radijev, kot so 40, 80 ali 100 km, ni bil več pomembna. Za N_{canopy} je bil pomemben tudi delež gozdnatosti – odvisnost je bila negativna. Večji delež gozda v okolici nabiranja mahu pomeni manjši odstotek kmetijskih in pozidanih zemljišč ter posledično manj onesnaževanja z N. Dodatno, večji delež gozda ustreza manjšim vplivom gozdnega roba, na katerem je lahko vnos N v gozdni ekosistem znatno povečan (Wuyts in sod., 2008).

Vpliv nadmorske višine na $\delta^{15}N$ je bil negativen, kar podpira ugotovitve pri nekaterih drugih vaskularnih rastlinah (Männel in sod., 2007) in ugotovitve, da se z naraščajočo nadmorsko višino povečuje tudi količina padavin. Ker se NH_4^+ bolj učinkovito izpira iz atmosfere kot NO_x (Asman in sod., 1998), se lahko delež reaktivnega N v mahu poveča. V primerjavi z drugimi vrstami gozda so bile vrednosti $\delta^{15}N_{canopy}$ v iglastih gozdovih bolj negativne, kar je skladno z eksperimentom v odprti komori, v kateri je bila hitrost odlaganja NH_3 večja pri smreki kot pri bukvi (Huber in sod., 2002). Negativna odvisnost med $\delta^{15}N$ in odstotkom kmetijskih zemljišč znotraj 5-kilometrskega radija od lokacije nabiranja mahu potrjuje, da so vrednosti $\delta^{15}N$ v mahu kljub vplivom nekaterih okoljskih dejavnikov še vedno pokazatelj vira N.

3.1.5 Okoljske značilnosti, ki pojasnjujejo razlike med N_{open} in N_{canopy} v mahovih

V okviru disertacije smo izpostavili težavo, da na nekaterih območjih v Evropi ni mogoče popolnoma slediti smernicam ICP Vegetation za nabiranje mahu (ICP Vegetation Coordination Centre, 2010), predvsem ni vedno mogoče najti izbrane vrste mahu, ki bi bila dovolj oddaljena od najbližje drevesne krošnje, da bi se s tem izognili vplivom skozi krošnje prepuščenih padavin, ki povzročajo večje vsebnosti N v tkivih mahu (Kluge in sod., 2013; Samecka-Cymerman in sod., 2010). Ker kaže N_{open} boljšo odvisnost od atmosferskih usedlin N v primerjavi z N_{canopy}, smo z namenom ocene N_{open} na podlagi N_{canopy} predstavili model, ki kaže, da na N_{open} poleg N_{canopy} vplivajo še naslednje okoljske spremenljivke: odstotek pozidanih zemljišč v radiju 80 km, vsota padavin v zadnjih štirih mesecih pred nabiranjem mahov, razdalja do najbližjega drevesa, nadmorska višina in odstotek kmetijskih površin v radiju 5 km. Podobne raziskave bi lahko uvedle korekcijske faktorje za bolj dosledne primerjave med lokacijami nabiranja mahu. Izpostaviti pa je treba, da model pojasni le 54 % variabilnosti podatka N_{open}.

Potencialni uporabniki predlaganega modela, se morajo zavedati intervalov zaupanja modeliranih rezultatov. Pomanjkljivost predstavljenega modela je, da so bili v naši raziskavi mahovi samo na 14 lokacijah nabrani vsaj 3 m od najbližje krošnje. Tako je lahko korekcija N_{open} na podlagi N_{canopy} podcenjena. Na primer, Kluge in sod. (2013) so pokazali, da so bile vrednosti N_{canopy} dvakrat večje kot N_{open} v primeru, da so bili mahovi nabrani vsaj 10 m od najbližje krošnje. Naš predlagani model bi bilo mogoče izboljšati, če bi bili na voljo podatki o vsebnosti N v mahu, nabranem na isti lokaciji, vendar na različnih razdaljah od projekcije drevesnih krošenj.

3.1.6 Prostorska interpolacija vsebnosti N in vrednosti $\delta^{15}N$ v mahovih v Sloveniji

Pri nobeni od izmerjenih vrednosti v mahovih (N_{open}, N_{canopy}, $\delta^{15}N_{open}$ in $\delta^{15}N_{canopy}$) nismo odkrili splošnega prostorskega trenda oz. odvisnosti med lokacijami nabiranja mahu glede na geografske koordinate (X in Y). Če bi ta obstajal, bi pomenilo, da se za Slovenijo kaže vzorec, ki temelji na geografskih koordinatah.

Uporaba prostorske interpolacije vsebnosti N v mahovih je temeljila na predpostavki, da je v prostoru atmosfersko usedanje N zvezni proces. Ob predpostavki, da so koncentracije N v mahovih odvisne od količin atmosferskih usedlin N, smo pri prostorski interpolaciji za vsebnost N v mahovih predpostavili, da so med seboj v prostoru odvisne tudi koncentracije N v mahovih. Strukturo prostorske korelacije med podatki za vsebnost N in vrednot δ^{15}N v mahovih smo ugotavljali s pomočjo vzorčnih variogramov z Monte Carlo ovojnico. Prostorska korelacija je pokazatelj povezanosti odvisnosti med slučajnimi deli izbrane spremenljivke, v našem primeru izmerjenimi vrednostmi v mahovih v geografskem prostoru. Prostorska korelacija je obstajala samo za N_{open}, in sicer do razdalje približno 50 km, ni pa bila jasno izražena pri kratkih razdaljah (manj kot 16 km). Po testiranju prilagoditve različnih variogramov in primerjavi rezultatov navzkrižnega preverjanja smo izbrali sferični variogram, katerega parametre smo ocenili po metodi največjega verjetja (»maximum likelihood estimation method«).

Za vrednosti N_{canopy}, $\delta^{15}N_{open}$ in $\delta^{15}N_{canopy}$ ni bilo prostorske korelacije med podatki. Pesch in sod. (2007) so prav tako raziskovali prostorsko korelacijo med podatki N_{canopy} in so, v nasprotju z našimi rezultati, odkrili šibko, a statistično značilno prostorsko povezavo. V poglavju 3.1.2 smo že razpravljali, da lahko na N_{canopy} namesto virov emisij N močneje vplivajo značilnosti gozda na lokaciji nabiranja mahu. Če je imelo območje nabiranja mahu strukturno bolj raznolike gozdove, kar je značilno za Slovenijo, ki se nahaja med sredozemsko, alpsko in panonsko regijo, je lahko prostorska korelacija med lokacijami nabiranja mahu neznatna. Varela in sod. (2013) so med vzorci mahov, nabranimi v Galiciji, odkrili zgolj šibko prostorsko korelacijo za $\delta^{15}N_{open}$, niso pa odkrili prostorske korelacije za N_{open}. Omenjeni avtorji zato predlagajo uporabo vrednosti δ^{15}N za ovrednotenje atmosferskih usedlin N namesto vsebnosti N v kopenskih mahovih. Prednost uporabe vrednosti δ^{15}N v mahovih pred določanjem vsebnosti N v mahovih je, da na izotopsko sestavo N v mahovih metabolizem mahu nima pomembnega vpliva (Liu in sod., 2008b), ne glede na vsebnost N v mahu. Raziskave so namreč pokazale, da pri večjih količinah atmosferskih usedlin N_{total} (večjih od 20 kg N/ha na leto) (Harmens in sod., 2014) pride do zasičenja z N v mahu. Pomanjkljivost spremljanja vrednosti δ^{15}N v mahovih pa je velika kationska izmenjevalna sposobnost mahov (Bates, 1992) in posledično boljša vezava NH_4^+-N kot pa NO_3^--N (Forsum in sod., 2006; Nordin in sod., 2006). Osnovni namen uporabe mahov kot biomonitorjev je raziskati izvore atmosferskih usedlin N-spojin (Harmens in sod., 2015). Po našem mnenju lahko le kombinacija obeh vrednosti v mahovih, vsebnost N in vrednost δ^{15}N, poda celostno informacijo o ocenjenih količinah usedlin N in tudi o glavnih virih emisij N. Z različnimi okoljskimi značilnostmi bi bilo treba proučiti parametre (N ali δ^{15}N) in izbrati tiste okoljske značilnosti, ki nudijo izboljšavo prostorske korelacije in posledično boljše rezultate pri prostorski interpolaciji podatkov.

Za prostorsko interpolacijo N_{open} smo uporabili osnovni kriging. Metoda navzkrižnega preverjanja je pokazala, da model pojasni 25 % variabilnosti N_{open}. Skoraj vse večje izmerjene vrednosti N_{open} so bile z metodo navzkrižnega preverjanja podcenjene. Za Slovenijo so bili rezultati osnovnega kriginga za N_{open} med 9,2 in 18,1 mg/g, z ocenjeno standardno napako med 1,6 in 2,9 mg/g. Razpon interpoliranih vrednosti je bil manjši od razpona izmerjenih vrednosti N_{open} (8,5 in 19,6 mg/g). Karta prikazuje večje vrednosti N_{open} v severovzhodnem in zahodnem delu države; vrednosti so velike še zlasti na območju, ki meji na Italijo. Predpostavljamo, da so te velike vrednosti posledica intenzivne rabe kmetijskih zemljišč v severni Italiji (Furlaniji in Benečiji). To domnevo potrjujejo tudi $\delta^{15}N_{open}$ vrednosti, ki so v tem zahodnem delu države bolj negativne, kar kaže, da je tu glavni vir N v mahovih kmetijstvo (NH_y). Ocenjujemo, da prevladujoči zahodni vetrovi prenašajo atmosferski N iz severne Italije v zahodna, hribovita območja Slovenije (Rakovec in sod., 2009). Izdelana karta N_{open} je v skladu s karto modeliranih čezmejnih usedlin N, ki temelji na izračunih modela EMEP MSC-W za leto 2008 (Nyíri in sod., 2010). Karta N_{open} izpostavlja tudi nekatere predele v severovzhodnem delu države, s povečanimi N_{open} na območju Pohorja in na območjih okoli mest Maribor, Celje in Murska Sobota. V mestih predstavljajo procesi zgorevanja pomemben vir emisij N. Omenjena mesta so znana po njihovi industrijski zgodovini ter gostemu avtocestnemu tranzitnemu prometu, ki povezuje vzhodni in zahodni del države. Drugi razlog za povečane vrednosti N_{open} na tem območju je dejstvo, da ta del države predstavlja začetek Panonske ravnine s tradicionalno bolj intenzivnimi kmetijskimi rabami zemljišč. Na splošno je karta osnovnega kriginga skladna s karto EMEP, ki smo jo pripravili glede na EMEP-podatke atmosferskih usedlin N v letih 2008–10. Ker ima Slovenija le eno postajo EMEP, ki je postavljena na jugu države (Iskrba), bi lahko nekatera odstopanja med kartami EMEP in mahovi pojasnili tudi s potencialno negotovostjo EMEP-modela.

Za N_{canopy}, $\delta^{15}N_{open}$ in $\delta^{15}N_{canopy}$ nismo odkrili prostorske korelacije med podatki, zato smo za prostorsko interpolacijo podatkov uporabili negeostatistično metodo – vsoto regresijske napovedi in interpoliranih ostankov regresijskega modela. Za interpolacijo ostankov regresijskega modela smo uporabili matematično metodo, kjer se interpolirana vrednost izračuna kot linearna kombinacija vrednosti v okolici. Koeficienti te linearne kombinacije so obratne vrednosti razdalje med lokacijami na izbrano potenco. Interpolirane vrednosti N_{canopy} se gibljejo med 9,3 in 28,1 mg/g. Na splošno obe karti (N_{open} in N_{canopy}) kažeta podobne prostorske vzorce vsebnosti N v mahovih z velikimi vrednostmi na zahodu in severovzhodu države in majhnimi na jugovzhodu, vendar pa se med obema kartama opazijo tudi nekatere razlike. Prostorski vzorec z večjimi vrednostmi N_{canopy} se močno ujema s karto o povprečnih hitrostih vetra, s povečanimi vrednostmi okoli urbaniziranih območij, predvsem pa so na tej karti izpostavljeni nekateri lokalni onesnaževalci z NO_x spojinami. Ti niso bili razvidni na karti N_{open} (na primer lokacije okoli elektrarne Šoštanj in Trbovlje). Količine odlaganja NO_x spojin so majhne blizu vira (Hertel in sod., 2011), vendar lahko bližnji gozdovi s svojimi krošnjami ustvarijo filter in s tem povečajo used atmosferskih plinov in delcev (De Schrijver in sod., 2008). Kakorkoli, navzkrižna validacija je pokazala, da s to prostorsko interpolacijo pojasnimo le 15 % variabilnosti podatka, za vsako napovedano vrednost N_{canopy} pa je bila minimalna standardna napaka ocenjena na 3,3 mg/g.

S prostorsko interpolacijo podatkov $\delta^{15}N_{open}$ v mahovih so bile, na podlagi vsote regresijske napovedi in interpoliranih ostankov regresijskega modela, vrednosti med -9.6 ‰ in -2.8 ‰, medtem ko so izmerjene vrednosti $\delta^{15}N_{open}$ v mahovih med -9,1 ‰ in -3.2 ‰. Karta kaže, da so na območjih, v katerih je N_{open} velik, interpolirane vrednosti $\delta^{15}N$ bolj negativne, iz česar lahko sklepamo, da je v teh območjih N predvsem iz kmetijskih virov (NH_y) (zahodni gorati del in vzhodni panonski del države). V nasprotju z območji, ki se nahajajo na jugovzhodu države, kjer je bil N_{open} najmanjši, so bile vrednosti $\delta^{15}N$ manj negativne, kar kaže, da je tukaj vir N predvsem proces zgorevanja (NO_x). Na karti so območja z manj negativnimi vrednostmi $\delta^{15}N_{open}$ v okolici vseh večjih mest, ki niso obdana z velikim deležem kmetijskih zemljišč (tj. Celje, Kranj, Nova Gorica, Novo mesto in Metlika), nekatera območja, ki obkrožajo termoelektrarne, in nekatera območja, ki obdajajo nekatere bolj obremenjene tranzitne ceste, kot so avtocesta Ljubljana–Zagreb, Koper–Ljubljana–Celje ter regionalna cesta med Slovenj Gradcem in Mariborom.

Na podlagi interpretacije izmerjenih in interpoliranih vsebnosti N in vrednosti $\delta^{15}N$ lahko potrdimo tretjo hipotezo disertacije, da so naravni ekosistemi v Sloveniji z N spojinami različno obremenjeni. Vsebnosti N v mahovih potrjujejo večje količine N-usedlin v zahodni in severovzhodni Sloveniji ter v bližini večjih mest, vrednosti $\delta^{15}N$ pa kažejo na daljinski transport NH_y iz kmetijsko bolj intenzivnih predelov Italije in v Prekmurju ter prevladujoči vpliv NO_x ob nekaterih bolj tranzitnih regionalnih cestah, avtocestah in ob nekaterih večjih mestih.

3.1.7 Odvisnost foliarnih analiz N, osutosti dreves in pokrovnosti lišajev od nekaterih okoljskih značilnosti in njihova povezava z vsebnostjo N in vrednostjo $\delta^{15}N$ v mahovih

Z namenom pridobivanja večjega števila prostorskih podatkov o onesnaženosti okolja z N so bile na ravni EU razvite in testirane različne oblike biomonitoringa in bioindikacije ter primerjane z meritvami količin atmosferskih usedlin N ali koncentracij N-spojin v zraku (Boltersdorf in sod., 2014; Munzi in sod., 2012; Sardans in sod., 2015a). Naši rezultati kažejo, da so izbrane tehnike biomonitoringa (foliarna analiza) in bioindikacije (osutost krošenj in obrast z različnimi rastnimi tipi lišajev) odvisne od okoljskih dejavnikov, ki opisujejo tako naravno okolje kot tudi vnos onesnažil zaradi človekove dejavnosti.

Rezultati kažejo, da imajo listavci statistično značilno večje koncentracije foliarnega N od iglavcev. Razlike nastanejo zaradi drugačne zgradbe asimilacijskih organov. Iglice so namreč sklerofilne, z več listne mase, debelejšo kutikulo, bogatejše so z ogljikom (Larcher, 1995; Smith in Smith, 2001) in posledično je količina N v skupni masi iglice manjša. Številne študije kažejo, da lahko tudi meteorološke značilnosti vplivajo na foliarni N – z manj padavinami in nižjimi temperaturami se foliarne koncentracije N zmanjšujejo (Kerkhoff in sod., 2005; Reich in sod., 2004; Sardans in sod., 2011). Padavine lahko vplivajo na foliarni N neposredno preko fotosinteze ali posredno preko koreninskega sistema zaradi mineralizacije in razgradnje tal (Patrick in sod., 2007). Nadmorska višina in temperatura sta pogosto korelirani spremenljivki – z višino temperatura pada. Kombinacija vseh omenjenih značilnosti je povezana tudi s trajanjem snežne odeje. Ta se je pokazala kot eden izmed pomembnih okoljskih dejavnikov, ki vplivajo na foliarni N v Sloveniji. Absorpcija N v listih zahteva energijo in zaradi tega je odvisna od dihanja, kar pojasnjuje, zakaj rastline v hladnih, zbitih tleh pogosto trpijo zaradi pomanjkanja N (Larcher, 1995). V zvezi z antropogenimi dejavniki, ki vplivajo na foliarni N, rezultati kažejo, da je značilno odvisen od deleža pozidanih zemljišč v radiju 100 km. Med pozidana zemljišča prištevamo gospodinjstva, industrijo in prometno infrastrukturo, vsem je skupno, da zaradi procesov zgorevanja v atmosfero tvorijo NO_x-N-spojine (Sutton in sod., 2011). Odvisnost foliarnega N od antropogenih izpustov N-spojin so potrdili tudi drugi avtorji (McNulty in sod., 1991; Ordonez in sod., 2009; Reich in sod., 2004; Sardans in sod., 2015b).

Osutost dreves se je razlikovala predvsem glede na strukturo gozda, v katerem je drevo rastlo. Značilno bolj osuta so bila drevesa, ki so rastla v listnatih ali mešanih gozdovih z redkim sklepom krošenj. Najmanjšo osutost pa so imela drevesa v iglastih gozdovih z normalnim sklepom. Sklep krošenj je močno povezan s starostjo dreves, s starostjo drevesa se namreč lahko osutost povečuje (Klap in sod., 2000). Sklep krošenj ima tudi neposreden vpliv na količino svetlobe, ki doseže asimilacijske organe drevesa. Za nekatere vrste javorja so raziskave pokazale, da so v bolje presvetljenih krošnjah listi debelejši in njihovo tkivo gostejše, posledično pa bolje opravljajo fotosintezo. Pri istih drevesih je bil večji tudi skupni delež svetlobnega sevanja, ki prodre skozi krošnje (Coble in Cavaleri, 2014), kar kaže, da je lahko pri bolj presvetljenih krošnjah skupno število listov manjše, s tem pa je lahko ocena osutosti večja. Osutost drevja je bila odvisna od povprečne letne količine padavin in trajanja snežne odeje. V nasprotju s pričakovanji je odvisnost od padavin pozitivna; če se je povprečna letna količina padavin povečala za 10 mm, se je, ob konstantni vrednosti drugih spremenljivk, osutost povečala za 2 %. Obstajajo številne študije, ki so poročale o odvisnosti med sušo in osutostjo dreves (de Vries in sod., 2014; Ferretti in sod., 2014; Klap in sod., 2000; Vitale in sod., 2014). Očitno pa suša še ni pomemben dejavnik v večini slovenskih gozdov. Predvidevamo, da je negativen vpliv padavin na osutost dreves v tem, da so bolj vlažne razmere pogosto ugodne za razvoj različnih povzročiteljev bolezni (Waller, 2013). V nasprotju s padavinami je odvisnost osutosti od trajanja snežne odeje pozitivna; dlje ko traja snežna odeja, manjša je osutost. V nekaterih regijah predstavlja sneg pomembno zalogo spomladanske vode, ki je pomembna za drevesa v začetku rastne sezone (Zierl, 2001). Večina raziskovalcev ni poročala o nobenih ali samo o šibkih odvisnostih osutosti dreves od onesnaženosti zraka (de Vries in sod., 2014; De Vries in sod., 2000; Klap in sod., 2000; Staszewski in sod., 2012). Naši rezultati kažejo, da obstaja statistično značilna odvisnost osutosti drevesa od celokupnih reduciranih oblik usedlin N (NH_y-N), ki pa vplivajo pozitivno na osutost drevja. V večini slovenskih gozdov atmosferske usedline N ne presežejo kritične ravni (Eler in sod., 2011) in je njihov učinek na rast lahko še vedno pozitiven.

Pokrovnost skorjastih lišajev je bila odvisna od drevesne vrste, na kateri so bili popisani lišaji. Za skorjaste lišaje je večja verjetnost, da se bodo pojavili na deblu bukve kot na smreki ali hrastu. Odvisnost pokrovnosti lišajev od substrata je bila že obravnavana v literaturi (Giordani, 2006; Spier in sod., 2010). Večina vrst lišajev, predvsem listnatih in grmičastih, je svetloljubnih (Nash, 1996), kar se ujema z našim rezultatom, da je pokrovnost skorjastih lišajev odvisna od števila dreves na hektar. V gostejših sestojih je pokrovnost skorjastih lišajev manjša. Ta značilnost je pomembna tudi pri pojavu listnatih lišajev. Pokrovnost listnatih lišajev je odvisna tudi od nadmorske višine, količine letnih padavin in vsote trajanja sončnega obsevanja. Z nadmorsko višino se lahko vrstna pestrost listnatih lišajev zmanjša, skupna pokrovnost pa se lahko poveča (Giordani in sod., 2014). Naši rezultati kažejo, da človekove dejavnosti vplivajo na pokrovnost skorjastih in listnatih lišajev. Pozidana zemljišča, ki so v glavnem vir spojin NO_x, imajo močan negativen vpliv na pokrovnost skorjastih lišajev, medtem ko ima kmetijska raba tal, ki je predvsem vir NH_y, majhen, a pozitiven učinek na pokrovnost. Učinek soli N-usedlin na drevesno skorjo bi lahko bil potencialna razlaga za povečanje nekaterih nitrofilnih vrst lišajev (Frahm in sod., 2009). Podobno imajo tudi pri listnatih lišajih nekatere rabe tal, povezane z večjimi vnosi N-spojin v okolje, pozitiven vpliv na lišajsko obrast.

Analiza korelacij je pokazala šibke povezanosti med: i) N_{open} in foliarnim N v listih, ii) N_{open} in obrastjo s skorjastimi lišaji v čistih sestojih, iii) N_{canopy} in osutostjo v gozdovih z normalnim sklepom ter iv) $\delta^{15}N_{canopy}$ in osutostjo v listnatih ter mešanih sestojih. Med drugimi oblikami biomonitoringa in bioindikacije ni bilo statistično značilnih povezav. S prvo točko smo potrdili prvi del četrte hipoteze, da so mahovi kot biomonitorji primerljivi z vsebnostjo N v foliarnih vzorcih, vendar je bila povezava statistično značilna samo pri listavcih. Za bolj podrobne analize bi potrebovali večje število ploskev. Povezave so bile statistično značilne tudi z drugimi oblikami bioindikacije, vendar samo ob upoštevanju določenih pogojev glede značilnosti gozdov. Rezultati kažejo, da lahko značilnosti gozda pomembno vplivajo na v disertaciji predstavljene odvisnosti. Naša študija je pokazala, da bi morali pri uporabi bioindikacije upoštevati dobljene povezanosti bioindikatorjev z okoljskimi dejavniki, da bi lahko bolje ocenili potencialno onesnaženost. Skladno z novim znanjem bi bilo treba pripraviti bolj podrobna navodila za terensko delo.

3.2 SKLEPI

Na podlagi rezultatov bi lahko podali naslednje ugotovitve:

- Za Slovenijo lahko večino lokacij, glede na izmerjene vrednosti N_{open} v mahovih, uvrstimo med podeželske ali lokacije ozadja. Izmerjena srednja vrednost je bila 13,1 mg/g in je primerljiva z vrednostmi v sosednji Avstriji, vendar velika v primerjavi z nekaterimi severnoevropskimi državami. Izmerjeno povprečje N_{canopy} je bilo 17,5 mg/g in je značilno večje od N_{open}.
- V Sloveniji je bila povprečna $\delta^{15}N_{open}$ -5.4 ‰ in $\delta^{15}N_{canopy}$ -5.5 ‰. Rezultati kažejo, da so, v primerjavi z okoliškimi državami, za Slovenijo glavni vir atmosferskih usedlin N procesi zgorevanja (NO_x-N) in ne proizvodnja hrane oz. kmetijstvo (NH_y-N).

- Odvisnost N_{open} od atmosferskih usedlin N je bila statistično značilna, a šibka. S tem smo delno potrdili trditev, da so mahovi primeren kazalnik (biomonitor) za identifikacijo območij, ki jih ogrožajo veliki vnosi atmosferskih usedlin N. Za natančnejšo oceno bi morali teste odvisnosti ponoviti na večjem številu lokacij. Odvisnost je bila bolj značilna za atmosferske usedline NH_4^+-N in slabša za NO_3^--N.
- N_{canopy} ni bil statistično značilno odvisen od vsebnosti N v sestojnih usedlinah, obstajala pa je mejno značilna odvisnost N_{canopy} od vsebnosti NH_4^+-N v atmosferskih usedlinah na odprtem.
- Vrednost $\delta^{15}N$ v mahovih je bila značilno odvisna od $NH_4^+ : NO_3^-$ razmerja v atmosferskih usedlinah na odprtem, vendar samo v primeru, da smo izključili bolj sušne lokacije, tj. tiste, ki so imele povprečne letne padavine manjše od 1000 mm.
- Obremenjenost z N-spojinami pod drevesnimi krošnjami v gozdu je večja kot v gozdnih vrzelih ali jasah. Vsebnosti N v mahovih, nabranih najmanj tri metre stran od najbližje projekcije drevesne krošnje, so bile v povprečju za 41 % manjše kot pod drevesnimi krošnjami.
- Razlike med vrednostmi $\delta^{15}N_{open}$ in $\delta^{15}N_{canopy}$ v mahovih, nabranih v gozdnih vrzeli, niso bile statistično značilne.
- Vsebnosti N v mahovih padajo z naraščajočo razdaljo med mestom nabiranja mahu in najbližjo projekcijo drevesne krošnje, vendar se interval zaupanja napovedanih vsebnosti N na razdalji 3 m od krošnje in tistih na razdalji 1 m od krošnje prekriva. Vsebnosti N v mahovih, nabranih na razdalji, manjši od 1 m, so statistično značilno večje kot tiste na razdalji vsaj 3 m.
- Okoljski dejavniki, ki vplivajo na N_{open} se razlikujejo od tistih, ki vplivajo na N_{canopy}. Za prve je bila, z vidika pojasnjenega deleža variance, najpomembnejša okoljska spremenljivka odstotek urbanih površin znotraj 80-kilometrskega radija. V nasprotju s tem, so pri N_{canopy} glavne vire emisij N zasenčili okoljski dejavniki, povezani z značilnostjo gozda na lokaciji nabiranja mahu (sestojni sklep in mešanost).
- Na podlagi podatka N_{canopy} z upoštevanjem nekaterih drugih značilnosti lokacije nabiranja mahu (odstotek pozidanih zemljišč v radiju 80 km, vsota padavin v zadnjih štirih mesecih, razdalja do najbližjega drevesa, nadmorska višina in odstotek kmetijskih površin v radiju 5 km) je mogoče pojasniti 54 % variabilnosti N_{open}.

- Prostorska korelacija med vsebnostjo N in vrednostjo $\delta^{15}N$ v mahovih je obstajala samo v primeru N_{open}.
- Karte prostorske interpolacije vsebnosti N v mahovih kažejo, da so naravni ekosistemi v Sloveniji z N-spojinami različno obremenjeni. Večje vsebnosti N so bile značilne za zahodno in severovzhodno Slovenijo, manjše za južno Slovenijo.
- Karte prostorske interpolacije vrednosti $\delta^{15}N$ v mahovih kažejo, da so deleži NO_x večji na lokacijah v bližini večjih urbanih središč, prometnic, večjih industrijskih ter termoenergetskih objektov, večje količine NH_y pa v naravnih ekosistemih, ki so v bližini površin, na katerih se izvaja intenzivno kmetijstvo.
- Vsebnost foliarnega N v listih in iglicah, delež osutosti in delež pokrovnosti skorjastih ter listnatih lišajev so odvisni od različnih okoljskih dejavnikov, predvsem od značilnosti okoliškega gozda. Izbrani bioindikatorji so delno odvisni tudi od odstotka pozidanih zemljišč v različnih radijih okoli lokacij testiranja metod bioindikacije. Osutost pa tudi od modeliranega atmosferskega useda reaktivnega N.
- Rezultati kažejo, da obstaja šibka korelacija med N_{open} in foliarnim N v listih. N_{canopy} je bila v nekaterih primerih povezana z osutostjo dreves in pokrovnostjo skorjastih lišajev. Vrednost $\delta^{15}N$ v mahovih pa je bila povezana z osutostjo. V drugih primerih ni bilo statistično značilnih povezav.

4 POVZETEK/SUMMARY

4.1 POVZETEK

Po industrijski revoluciji so se bistveno povečale količine v ozračje izpuščenega N antropogenega izvora. Dušik, ki se useda na zemeljsko površje, ima lahko vrsto škodljivih učinkov. Med njimi so najpomembnejši zakisanje in evtrofikacija kopnih ali vodnih ekosistemov. Da bi lahko spremljali količine atmosferskih usedlin N in identificirali bolj obremenjene lokacije oz. ekosisteme, so bile razvite ter implementirane številne analitske (fizikalno-kemijske) in posredne oblike spremljanja oz. monitoringa N-spojin. Med slednje spada tudi biomonitoring z mahovi, ki ga na evropski ravni koordinira ICP-Vegetation in kjer posredno prek vsebnosti N in vrednosti $\delta^{15}N$ v tkivih mahu sklepamo o količinah in vrsti atmosferskih N-usedlin. Prednost mahov kot organizmov za spremljanje atmosferskih usedlin je v tem, da so mahovi ektohidrični, nimajo torej razvite kutikule in imajo slabše razvit koreninski sistem – rizoide. Posledično lahko sklepamo, da v večjem delu privzemajo N neposredno iz ozračja prek atmosferskih usedlin. Glavna vira antropogeno proizvedenega N sta reducirana (NH_y) in oksidirana (NO_x) oblika N-spojin. Te imajo različno izotopsko sestavo (vrednost $\delta^{15}N$). Majhne vrednosti $\delta^{15}N$ (bolj negativne) v tkivu mahu kažejo, da je glavni vir N v atmosferskih usedlinah NH_y-N, medtem ko večje vrednosti $\delta^{15}N$ (manj negativne) pomenijo več NO_x-N. Na podlagi teh informacij je mogoče razlikovati med območji, kjer je glavni vir antropogenega N intenzivno kmetijstvo (uporaba gnojil) (NH_y) in območji, kjer je glavni vir zgorevanje fosilnih goriv (NO_x).

Cilj disertacije je bil ugotoviti, ali je mah štorovo sedje (*Hypnum cupressiforme* Hedw.) primeren kot biomonitor za zračne usedline N za Slovenijo, kateri okoljski dejavniki pojasnjujejo vsebnost N in vrednost $\delta^{15}N$ v mahovih ter na podlagi mahov identificirati območja v Sloveniji, ki so bolj obremenjena z N-spojinami. S tem namenom smo v letu 2010 nabrali vzorce mahu vrste štorovo sedje na 103 lokacijah v Sloveniji in dodatno še na sedmih lokacijah v sosednjih državah blizu slovenske meje (Avstrija, Italija in Hrvaška). Na vsaki lokaciji so bili mahovi nabrani na dveh mestih v gozdovih: pod drevesnimi krošnjami in v bližnji gozdni vrzeli ter analizirani za vsebnost N in vrednost $\delta^{15}N$.

Rezultati kažejo, da mah štorovo sedje, nabran v gozdnih vrzelih, odraža atmosferske usedline N na odprtem. S tem smo potrdili prvo hipotezo, vendar pa nismo odkrili odvisnosti vsebnosti N v mahovih, ki so bili nabrani pod drevesnimi krošnjami od količine N v sestojnih padavinah. Delež pojasnjene variabilnosti N v mahovih je bil večji v primeru upoštevanja izključno NH_4^+-N v atmosferskih usedlinah in manjši ob upoštevanju izključno NO_3^--N. V primeru, da pri testiranju odvisnosti nismo upoštevali bolj sušnih lokacij (vsaj 1000 mm letnih padavin), se je delež pojasnjene variabilnosti vsebnosti N v mahovih s strani vsebnosti N v atmosferskih usedlinah povečal. Odvisnost vrednosti δ^{15}N v mahovih od razmerja NH_4^+ : NO_3^- v usedlinah na odprtem ni bila statistično značilna, razen če smo izključili bolj sušne lokacije, tj. tiste, ki so imele povprečne letne padavine manjše od 1000 mm.

Zaradi razlik med količinami N v sestojnih usedlinah in usedlinah na odprtem protokol ICP Vegetation za uporabo mahov kot biomonitorjev predlaga, da mora biti minimalna razdalja med mestom nabiranja mahu in horizontalno projekcijo najbližje drevesne krošnje vsaj tri metre. Za določene vrste mahu in v določenih tipih gozdov je tej omejitvi težko zadostiti. Rastišče mahu štorovo sedje je npr. pogosto vezano na zračno vlažna območja, torej tudi na senco dreves. Hkrati je v Sloveniji gospodarjenje z večino gozdov sonaravno (pomlajevanje poteka po naravni poti v manjših vrzelih), kar še dodatno otežuje iskanje primerno velikih nezastrtih lokacij za nabiranje mahu. V doktorskem delu smo potrdili in kvantificirali vpliv krošnje na vsebnost N v mahovih in s tem potrdili drugo hipotezo disertacije. Vsebnosti N v mahovih pod drevesnimi krošnjami so bile v povprečju za 41 % večje kot na razdalji, večji od treh metrov od najbližje drevesne krošnje. Vsebnost N v mahovih pada z naraščajočo razdaljo med mestom nabiranja mahu in najbližjo projekcijo drevesne krošnje, vendar se intervali zaupanja napovedanih vsebnosti N na razdalji 3 m od krošnje in tistih na razdalji 1 m od krošnje prekrivajo. Pri vrednostih δ^{15}N med mahovi, nabranimi pod zastori dreves, in tistimi nabranimi v gozdnih vrzelih, ni bilo statistično značilnih razlik. Rezultati kažejo, da sta N v mahovih, nabranih v gozdnih vrzelih, in N v mahovih, nabranih pod zastorom krošenj, statistično značilno povezana. Ob upoštevanju podatkov o vsebnosti N v mahovih pod zastorom, odstotku pozidanih zemljišč v radiju 80 km, količini padavin v zadnjih štirih mesecih pred nabiranjem mahov, nadmorski višini in odstotku kmetijskih površin v radiju 5 km je mogoče pojasniti 54 % variabilnosti vsebnosti N v mahovih, nabranih v gozdni vrzeli.

V študiji smo prikazali, katere značilnosti lokacije nabiranja mahu vplivajo na vsebnost N in vrednosti $\delta^{15}N$ v mahovih. Za mah, nabran na isti lokaciji, vendar na dveh različnih mestih (pod drevesnimi krošnjami in v bližnji gozdni vrzeli), se vsebnosti N in vrednosti $\delta^{15}N$ skladajo s trenutnimi znanstvenimi dognanji, ki temeljijo na spremljanju atmosferskih usedlin in reakcij N v atmosferi. Kot vplivne so se pokazale naslednje značilnosti: količina padavin, nadmorska višina, vetrovnost, bližina drevesne krošnje, sklep krošnje, mešanost gozda in različne rabe tal v okolici v različnih radijih. Glede na vplivnost posamezne značilnosti okolja obstajajo razlike med vsebnostjo N v mahovih, nabranih v gozdni vrzeli, in v mahovih, nabranih pod zastorom dreves. Pri pojasnjevanju variabilnosti vsebnosti N v mahovih, nabranih v gozdni vrzeli, so bolj pomembne značilnosti rabe okoliških tal, predvsem delež pozidanih in kmetijskih zemljišč, posledično pa še glavni viri emisij N. Pri vsebnosti N v mahovih, nabranih pod drevesnimi krošnjami, so glavni viri emisij zasenčeni z značilnostjo gozda na lokaciji nabiranja mahu. V tem primeru imata večji vpliv na pojasnjevanje variabilnosti vsebnosti N vrsta sklepa krošenj in mešanost gozdov. V bolj gostih in pretežno iglastih gozdovih so bile vsebnosti N večje. Vplivi okoljskih dejavnikov na vrednosti $\delta^{15}N$ v mahovih se ne razlikujejo toliko glede na mesto vzorčenja (v vrzeli/pod zastorom). Okoljski dejavniki, ki značilno vplivajo na delež variabilnosti $\delta^{15}N$ v mahovih, so nadmorska višina (pri večji nadmorski višini so vrednosti $\delta^{15}N$ bolj negativne), mešanost gozdov (v iglastih gozdovih so vrednosti $\delta^{15}N$ bolj negativne) in odstotku kmetijskih površin v bližini lokacije vzorčenja (pri večjem odstotku so vrednosti $\delta^{15}N$ bolj negativne).

Ker se horizontalna in vertikalna struktura gozdov med evropskimi državami razlikuje in posledično na nekaterih predelih ni mogoče v celoti upoštevati navodil protokola ICP Vegetation, zlasti oddaljenosti lokacije vzorčenja do najbližje krošnje dreves, v disertaciji predlagamo, da se pripravi podroben metapodatkovni protokol, ki opisuje značilnosti lokacije nabiranja mahu. Te metapodatke je potrebno upoštevati pri interpretaciji izmerjenih vrednosti v tkivu mahu in s tem povezati podatke o vsebnosti elementov v mahovih z značilnostmi lokacije nabiranja mahu. Poleg tega bi lahko s podobnimi raziskavami uvedli korekcijske faktorje za mahove, nabrane zunaj predpisanih lokacij.

Končni rezultat študij s področja onesnaženosti okolja je pogosto karta onesnaženosti. Karte so za uporabnike običajno laže berljive in predstavljive, saj je podatek o interesni spojini ali elementu zvezno prikazan za celotno analizirano območje in ne samo za izbrane lokacije. Naši rezultati kažejo, da je prostorska korelacija med vsebnostjo N in vrednosti δ^{15}N v mahovih obstajala samo v primeru vsebnosti N v mahovih, nabranih v gozdnih vrzelih. Za prostorsko interpolacijo teh podatkov smo uporabili geostatistično metodo osnovni kriging. Nasprotno, za N v mahovih, ki so bili nabrani pod krošnjami, prostorska korelacija ni bila odkrita, prav tako ne za vrednosti δ^{15}N nabranih na obeh vzorčevalnih mestih (v gozdni vrzeli ali pod krošnjami). V tem primeru je bila prostorska interpolacija podatkov narejena kot vsota regresijske napovedi in utežne inverzne razdalje ostankov regresijskega modela. Karti vsebnosti N v mahovih, nabranih v gozdni vrzeli, in tistimi, nabranimi pod drevesnimi krošnjami, sta se prostorsko gledano relativno dobro ujemali, razlika je ostajala v ocenjenih velikostih vsebnosti N, ki so bile v primeru mahu, nabranem pod drevesnimi krošnjami, značilno večje. Na obeh kartah sta kot območji z velikimi N-vsebnostmi označeni zahodna in severovzhodna Slovenija. Predpostavljamo, da so velike vrednosti v zahodni Sloveniji posledica intenzivne rabe kmetijskih zemljišč v severni Italiji (Furlaniji in Benečiji). To domnevo potrjujejo tudi vrednosti δ^{15}N, ki so v tem zahodnem delu države bolj negativne, kar kaže, da je tu glavni vir N v mahovih predvsem kmetijstvo, tj. NH_y. V severovzhodnem delu države so bile povečane vsebnosti N na območju Pohorja in na območjih okoli mest Maribor, Celje in Murska Sobota. V mestih predstavljajo procesi zgorevanja fosilnih goriv pomemben vir emisij N, kar so potrdile tudi manj negativne vrednosti δ^{15}N v mahovih. Drugi razlog za povečane vrednosti v tem delu države je dejstvo, da ta del države predstavlja začetek Panonske ravnine s tradicionalno bolj intenzivnimi kmetijskimi rabami zemljišč. S tem smo potrdili tretjo hipotezo, da so naravni ekosistemi v Sloveniji z N-spojinami različno obremenjeni in da je o potencialnih virih N-spojin mogoče sklepati na podlagi izotopskih metod. Rezultati navzkrižne validacije prostorske interpolacije in karte standardnih napak so izpostavile omejitve pri interpretaciji posameznih izdelanih kart. Študija je pokazala, da številni parametri vplivajo na prostorsko interpolacijo vsebnosti N in vrednosti δ^{15}N in posledično na končne karte. Predlagamo, da naj bo potencialni uporabnik kart vedno obveščen o izbrani tehniki za prostorsko interpolacijo podatkov, o natančnosti končne karte in o njenih omejitvah.

Namen študije je bil tudi raziskati, ali so rezultati, pridobljeni z različnimi tehnikami biomonitoringa in bioindikacije, med seboj primerljivi. Analiza korelacij je pokazala značilne odvisnosti med koncentracijo dušika v mahovih in i) foliarnim dušikom v listih, ii) osutostjo v gozdovih z normalnim sklepom in iii) obrastjo s skorjastimi lišaji v čistih sestojih ter korelacijo med vrednostjo δ^{15}N v mahovih in osutostjo v listnatih in mešanih sestojih. Med drugimi oblikami biomonitoringa in bioindikacije ni bilo statistično značilnih povezav.

4.2 SUMMARY

After the industrial revolution the amount of anthropogenic N released into the atmosphere has increased significantly. Nitrogen deposited on the Earth surface can have numerous negative effects. Especially acidification and eutrophication of terrestrial and aquatic ecosystems are most important. In order to monitor the quantities of atmospheric N deposition and to identify more polluted areas or ecosystems, a number of different analytical and indirect monitoring methods have been developed and implemented. The latter also includes biomonitoring with mosses, which is coordinated by ICP Vegetation at the European level and which indirectly, through the N concentrations and δ^{15}N values in the moss tissue, estimates the quantities and the types of atmospheric N deposition. The advantage of mosses as organisms for monitoring of atmospheric depositions is that the mosses are ectohydric what means that cuticle and root system are not jet fully developed. Consequently, we can conclude that moss uptakes N directly from the atmosphere via atmospheric deposition. The main source of anthropologically produced N are reduced (NH_y) and oxidized (NO_x) forms of N compounds. Those two forms have a different isotopic composition (δ^{15}N value). Smaller δ^{15}N value (more negative) in the moss tissue suggests that the main source of N in atmospheric deposition is NH_y-N, while higher δ^{15}N value (less negative) means more NO_x-N. Based on this information it is possible to distinguish between areas where the main source of the anthropogenic N is intensive agriculture (use of fertilizer) (NH_y), and areas where the main source of N is combustion of fossil fuels (NO_x).

The aim of the dissertation was to determine, for Slovenia as a case study, whether cypress-leaved moss (*Hypnum cupressiforme* Hedw.) is suitable to be used as a biomonitor for atmospheric N depositions. Additionally, the aim was to explore which environmental factors explain the N concentration and δ^{15}N value in mosses and to identify areas with higher atmospheric N depositions in Slovenia on the basis of mosses. To answer those questions in 2010, the cypress-leaved moss samples were collected at 103 locations in Slovenia and additionally on seven locations in Slovenia's neighboring countries (Austria, Italy and Croatia). At each location mosses were collected from two sites within forest: under the canopy of trees and in nearby forest clearings. All samples were cleaned and analyzed for N concentration and δ^{15}N value.

The results show that moss *Hypnum cupressiforme* Hedw., collected in forest clearings, reflects the atmospheric deposition of N in the open. This confirmed the first hypothesis. However, we have not detected the dependence of N concentration in mosses collected under the tree canopies on the amount of N in throughfall precipitation. The amount of explained variability of N concentration in mosses was higher, if only NH_4^+-N in deposition was included into the model, and weaker, if only NO_3^--N was used. If locations with less than 1000 mm of annual precipitation were excluded from the model, the proportion of explained variability of N concentration in mosses by N content in atmospheric depositions increased. Dependence of δ^{15}N value in mosses on NH_4^+: NO_3^- ratio in open deposition was not statistically significant, except in the cases where locations with average annual precipitation less than 1000 mm were excluded.

Due to differences between the content of N in depositions sampled in open area and depositions sampled under the tree canopies, ICP Vegetation protocol for moss biomonitoring survey suggests that the minimum distance between the moss sampling location and the nearest horizontal crown projection should be at least three meters. For certain moss species and within certain forest types this limitation is hard to follow. The favorable habitat of cypress-leaved moss is often connected with tree shade and higher air moisture. Additionally in Slovenia, the majority of forests are managed sustainable and close to nature, what means that rejuvenation takes place naturally in small gaps, which further complicates the search for suitable large clearings for moss collecting. In dissertation, we have confirmed and quantified the influence of the tree canopy on N concentrations in mosses and thus confirmed the second hypothesis of the dissertation. Nitrogen concentrations in mosses collected under the tree canopy were on the average 41% higher than at a distance greater than three meters from the nearest canopy. N concentrations in mosses decreased with increasing distance between the moss collecting locations and the nearest tree canopy projection, but the confidence intervals of model prediction for N concentration in mosses collected at least three meters away from the canopy and those collected at least one meter from the canopy overlapped. For δ^{15}N values there was no statistically significant difference between mosses collected under the canopy of trees and in the nearby forest clearing. The results show that N concentrations in mosses collected within forest clearings and N in mosses collected under the tree canopy correlate. With information on N concentration in mosses collected under the canopy, the percentage of settlements within radii of 80 km, amount of rainfall in the last four months before moss collecting, altitude and percentage of agricultural land within a radius of 5 km it is possible to explain 54% of the variability of N concentration in mosses collected within forest clearings.

Our study shows which environmental characteristics of the moss collecting location affect N concentration and δ^{15}N value in moss. For mosses collected in the same location, but in two different sites (under a tree canopy and in the nearby forest clearings), the N concentration and δ^{15}N values in moss agree with current scientific knowledge, based on monitoring of atmospheric N deposition and their reactions in the atmosphere. An important role in explaining of the variation of N concentrations in mosses were: amount of precipitation, altitude, wind velocities, distance to the nearest tree crown projection, canopy closure, tree species mixture and a variety of surrounding land use types in different radii. Based on the amount of explained variability by a certain environmental factor there are differences between the N concentrations in mosses collected within forest clearings and mosses collected under the canopy of trees. In explaining the variability of the N concentration in mosses collected within forest clearings, characteristics of the surrounding land use, particularly urban and agricultural land use type, are of importance and, consequently, represent the main potential sources of N emissions. For N concentration in mosses collected under the tree canopy, however, the main sources of emissions are obscured by the characteristics of the forests at the moss collecting location. In this case, the types of canopy closure and tree species mixture were more important for explaining the variability of N concentrations in mosses. In denser and predominantly coniferous forests N concentrations were higher. There were no significant differences between environmental characteristics, important for explaining the variability of δ^{15}N value in mosses collected under the canopy and in forest clearings. Environmental factors that significantly explain the variability δ^{15}N value in mosses were altitude (at higher altitudes the δ^{15}N values were more negative), tree mixture (in coniferous forests δ^{15}N values were more negative) and percentage of agricultural land in the vicinity of the moss collecting location (at larger percentage δ^{15}N values were more negative).

Since the horizontal and vertical structure of forests varies between European countries, in some areas it is consequently not possible to follow the instructions of the ICP Vegetation protocol; we propose to prepare detailed meta-data protocol describing the characteristics of the moss collecting location in particular for the distance between moss collecting location and the nearest tree canopy projection. These meta-data should be considered when interpreting the measured values in the moss tissue and thereby integrate the information on the moss content with the characteristics of the moss collecting location. Moreover, similar research could introduce correction factors for mosses that were not collected within forest clearings at least three meters away from the nearest tree canopy projection.

Pollution maps are often the result of similar environmental pollution studies. For potential users the maps are usually easier to read and interpret, since the information on the selected compound or element is continuously displayed through the entire analyzed area and not only for the selected location. Our results show that for N concentrations and δ^{15}N values in mosses the spatial correlation existed only in the case of N concentration in mosses collected within forest clearings. For spatial interpolation of these data geostatistical technique

Ordinary kriging was used. In contrast, the spatial correlation was not found for N in mosses that were collected under the canopy, nor for $\delta^{15}N$ values in mosses collected on both sampling sites (within clearings and below the canopy). In this case, the spatial interpolation of the data was calculated as the sum of the regression prediction and inverse distance weighted interpolation of regression residuals. Maps of N concentration in mosses collected within forest gaps and those collected under the canopies are, spatially speaking, relatively well-matched. The differences remain in the estimated size of N concentration, which was significantly higher in the case of mosses collected under the tree canopies. Both maps show that the areas with higher N concentrations are situated in western and northeastern part of Slovenia. We assume that high values in western Slovenia are result of the intensive agricultural land use in northern Italy (Friuli and Veneto). This assumption is confirmed by $\delta^{15}N$ values which are more negative in the western part of the country. These indicate that the major source of N in mosses is mainly from agriculture use (NH_y). In the northeastern part of the country, the increased N concentrations were observed in the Pohorje mountain area and in the locations around the cities of Maribor, Celje, and Murska Sobota. In these areas, combustion processes represent an important source of N, which was also confirmed by less negative $\delta^{15}N$ values. Another reason for the increased N concentrations in this part of the country is also the fact that this part of the country represents the beginning of the Pannonian plain with the traditionally more intensive agricultural land use. Those results confirmed the third hypothesis saying that the natural ecosystems in Slovenia are variously loaded with N compounds and that the potential sources of N compounds could be determined with isotopic methods. The results of cross-validation and maps of standard errors present the limitations in the interpretation of the individual resulting maps. The study showed that many environmental factors affect the spatial interpolation of N concentrations and $\delta^{15}N$ values and, consequently, the resulting maps. We suggest that the potential user of the pollution maps should always be informed about the geostatistical technique used for the spatial interpolation of the data, the accuracy of the final map and its limitations.

The aim of this study was to investigate whether the results obtained by diverse biomonitoring (moss and foliar analysis) and bioindication techniques (tree defoliation and lichens cover) are comparable. The correlation analysis showed statistically significant dependence between the nitrogen concentration in mosses and i) foliar nitrogen in leaves, ii) defoliation of trees in forests with normal canopy closure and iii) crustose lichens cover in pure stands and correlation between the isotope $\delta^{15}N$ values in mosses and defoliation in deciduous and mixed stands. There was no statistically significant correlation among other biomonitoring and bioindication techniques.

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