

Effects of non-native Asian earthworm invasion on temperate forest and prairie soils in the Midwestern US

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Abstract Effects of invasive European earthworms in North America have been well documented, but less is known about ecological consequences of exotic Asian earthworm invasion, in particular Asian jumping worms (*Amyntas*) that are increasingly reported. Most earthworm invasion research has focused on forests; some *Amyntas* spp. are native to Asian grasslands and may thrive in prairies with unknown effects. We conducted an earthworm-addition mesocosm experiment with before–after control-impact (BACI) design and a complementary field study in southern Wisconsin, USA, in 2014 to investigate effects of a newly discovered invasion of two Asian jumping worms (*Amyntas agrestis* and *Amyntas tokioensis*) on forest and prairie litter and soil nutrient pools. In both studies, *A. agrestis* and *A. tokioensis* substantially reduced surface litter (84–95 % decline in foliage litter mass) and increased total carbon, total nitrogen, and available phosphorus in the upper 0–5 cm of soils over the 4-month period from July through October. Soil inorganic nitrogen (ammonium– and nitrate–N) concentration increased across soil depths of 0–25 cm, with greater effects on nitrate–

N. Dissolved organic carbon concentration also increased, e.g., 71–108 % increase in the mesocosm experiment. Effects were observed in both forest and prairie soils, with stronger effects in forests. Effects were most pronounced late in the growing season when earthworm biomass likely peaked. Depletion of the litter layer and rapid mineralization of nutrients by non-native Asian jumping worms may make ecosystems more susceptible to nutrient losses, and effects may cascade to understory herbs and other soil biota.

Keywords *Amyntas agrestis* · *Amyntas tokioensis* · Asian jumping worm · Ecosystem change · Nutrient cycling · Wisconsin

Introduction

Invasion of non-native earthworms profoundly alters biodiversity and ecosystem function (Bohlen et al. 2004c; Lavelle et al. 2006; Hendrix et al. 2008; Blouin et al. 2013). Such invasions are particularly problematic in glaciated regions of North America that were historically devoid of native earthworms prior to European settlement (James 1995). Prior studies have revealed that earthworm invasions can accelerate litter loss and reduce soil horizon thickness, alter nutrient pools and dynamics (e.g., carbon, nitrogen and phosphorus), deplete soil cations (e.g., calcium, magnesium and potassium), increase soil microbial biomass and activities (e.g., in forest soils), shift

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understory plant community composition and diversity, and decrease other soil invertebrates (Bohlen et al. 2004c; Groffman et al. 2004; McLean et al. 2006; Blouin et al. 2013; Resner et al. 2015). However, such effects have largely been based on research on European earthworms (family Lumbricidae), and earthworms originating in other regions are less well studied. Of particular concern are non-native Asian earthworms in the genus *Amyntas* (family Megascolecidae), which have successfully invaded North America and other tropical/subtropical regions (Gates 1982; Burtelow et al. 1998; Greiner et al. 2012). Warming temperatures and longer growing seasons may facilitate northward expansion of some *Amyntas* species that currently have a more southerly distribution (Bohlen et al. 2004a). Moreover, most earthworm invasion research has focused on forests, but some Asian earthworms native to sedge and grassland communities (Masamichi et al. 2011; Ishizuka and Minagoshi 2014) may colonize other ecosystems besides forests with unknown effects. Hence, understanding the extent to which *Amyntas* spp. can alter soils and ecosystems is increasingly urgent.

Asian jumping worms (also known as ‘Asian crazy worms’ or ‘Alabama jumpers’) normally refer to a subgroup of epigeic (i.e., litter and surface dwellers) *Amyntas* spp. such as *Amyntas agrestis* and *Amyntas tokioensis* (Ishizuka 2001; Ikeda et al. 2015), that are noted for their rapid, snake-like movements and jumping behavior when disturbed. Their dispersal mechanisms are not well understood, but these earthworms can be unknowingly transported in mulch and nursery plants as adults or cocoons (Bellitürk et al. 2015). Unlike many invasive European earthworms in temperate North America that grow more slowly and can live for multiple years, these jumping worms have an annual life cycle. Juveniles emerge in spring, grow rapidly to sexual maturity (within 60–90 days of hatching), then reproduce and die in the fall (Burtelow et al. 1998; Callahan et al. 2003; Greiner et al. 2012; Ikeda et al. 2015). The population persists overwinter via cocoons from which earthworms hatch the following spring. In addition, compared to many other epigeic European species that live in low densities, the Asian jumping worms can live at much higher densities (~10 times of European invaders) (Hale 2007), have notably larger body size

(Greiner et al. 2010), exhibit greater dietary flexibility, and can outcompete other soil organisms such as millipedes (Snyder et al. 2011, 2013). Further, some Asian jumping worms are native to grasslands (Masamichi et al. 2011; Ishizuka and Minagoshi 2014) and may thrive in U.S. prairies. These trait differences suggest that Asian jumping worms may have stronger or different effects than European earthworms depending on the ecological context.

A population of Asian jumping worms was discovered at the University of Wisconsin–Madison Arboretum in October 2013. This was the first recorded presence of *Amyntas* spp. in Wisconsin, which had listed these earthworms as restricted species under Wisconsin’s Invasive Species Rule (Wisconsin Administrative Code Chapter NR 40). Genetic analyses conducted at Colgate University and Johns Hopkins University in 2015 determined that *A. agrestis* and *A. tokioensis* were both present (Brad Herrick, University of Wisconsin Arboretum, *personal communication*). While these two species vary somewhat in body length (*A. agrestis*: ~8 to 20 cm; *A. tokioensis*: ~6 to 15 cm), both are epigeic and similar in life history, feeding strategy, reproduction, and movement behavior (Uchida 2004; Uchida et al. 2004; Ishizuka and Minagoshi 2014). Despite their wide occurrence (e.g., *A. agrestis* has invaded at least 17 states across the eastern and southeastern U.S. since the first reported case in 1939; Gates 1982; Reynolds and Wetzel 2012; Ikeda et al. 2015), studies of these species are relatively infrequent. Most research to date has addressed their physiological tolerance (Richardson et al. 2009), dietary flexibility (Zhang et al. 2010), management and control strategies (Ikeda et al. 2015), and showed declining litter horizon and intensifying food resource competition due to their invasions (Snyder et al. 2013), yet their ecological consequences are not fully known.

We conducted an earthworm-addition mesocosm experiment and a complementary field study in 2014 to investigate effects of the incipient invasion of these two co-occurring Asian jumping worms. We asked: (1) What are the effects of *A. agrestis* and *A. tokioensis* invasion on litter quantity and soil nutrient pools (including organic matter, total carbon and nitrogen, available phosphorus, inorganic nitrogen, and dissolved organic carbon)? (2) Do effects on soil nutrients differ with depth (i.e., 0–5, 5–10, and 10–25 cm from the surface)? (3) How do effects on

litter and soil nutrient pools differ between forest and prairie ecosystems? We hypothesized that the invasion of *A. agrestis* and *A. tokioensis* would increase foliage litter loss because of their high population density and associated feeding activities, and further lead to a transient enrichment of total nutrient pools (e.g., organic matter, total C and N) in the upper soil layers through mixing and incorporation of litter nutrients into soils (Table 1). We also hypothesized that invasion of *A. agrestis* and *A. tokioensis* would increase soil inorganic nitrogen and dissolved organic carbon through enhanced mineralization in earthworm guts, secretion of nutrients in the form of earthworm casts and mucus, and changes in soil physical structure that facilitate nutrients mobilization across soil profiles. Because *A. agrestis* and *A. tokioensis* are epigeic, we expected stronger effects on nutrient pools in the O horizon and topsoils. We also expected greater effects on forest litter than prairie, as deciduous leaf litter is normally lower in C:N ratio, easier to decompose (decomposed leaf litter is preferred food source for *A. agrestis*; Snyder et al. 2013), and likely more digestible relative to prairie graminoid litter with its higher C:N ratio and lignin content. However, we still expected to find significant effects in prairies because *A. agrestis* were found to stimulate ligninolytic enzyme activities (Bellitürk et al. 2015), which could promote the consumption and decomposition of prairie graminoid litter.

Methods

Mesocosm experimental design

We conducted a paired and replicated mesocosm experiment with before-after control-impact (BACI) design in an open field facility at the University of Wisconsin-Madison Arboretum from July to October 2014. We used a BACI design because it can detect treatment effects while controlling for differences in initial conditions among sites (Underwood 1994). Intact soil cores ($N = 99$) with in situ leaf litter were collected from six representative uninvaded sites across southern Wisconsin (Fig. 1a). At each site, one forest and one adjoining prairie with the same soil type were selected, except for one site where prairie soils were too compacted to excavate (see online Appendix A for study area and site description). Within each forest and prairie, a 50-m transect was laid out in a random direction. At 0, 25, and 50-m marker along transect, a set of three intact soil cores (20-cm diameter \times 25-cm depth) was collected within a 1-m radius.

All soil cores were returned to the Arboretum field facility for incubation (Fig. 1b). Prior to adding earthworms for the mesocosm experiment, one soil core from each set of three was randomly chosen and destructively harvested to measure initial litter and soil conditions (Table 2). The remaining two were

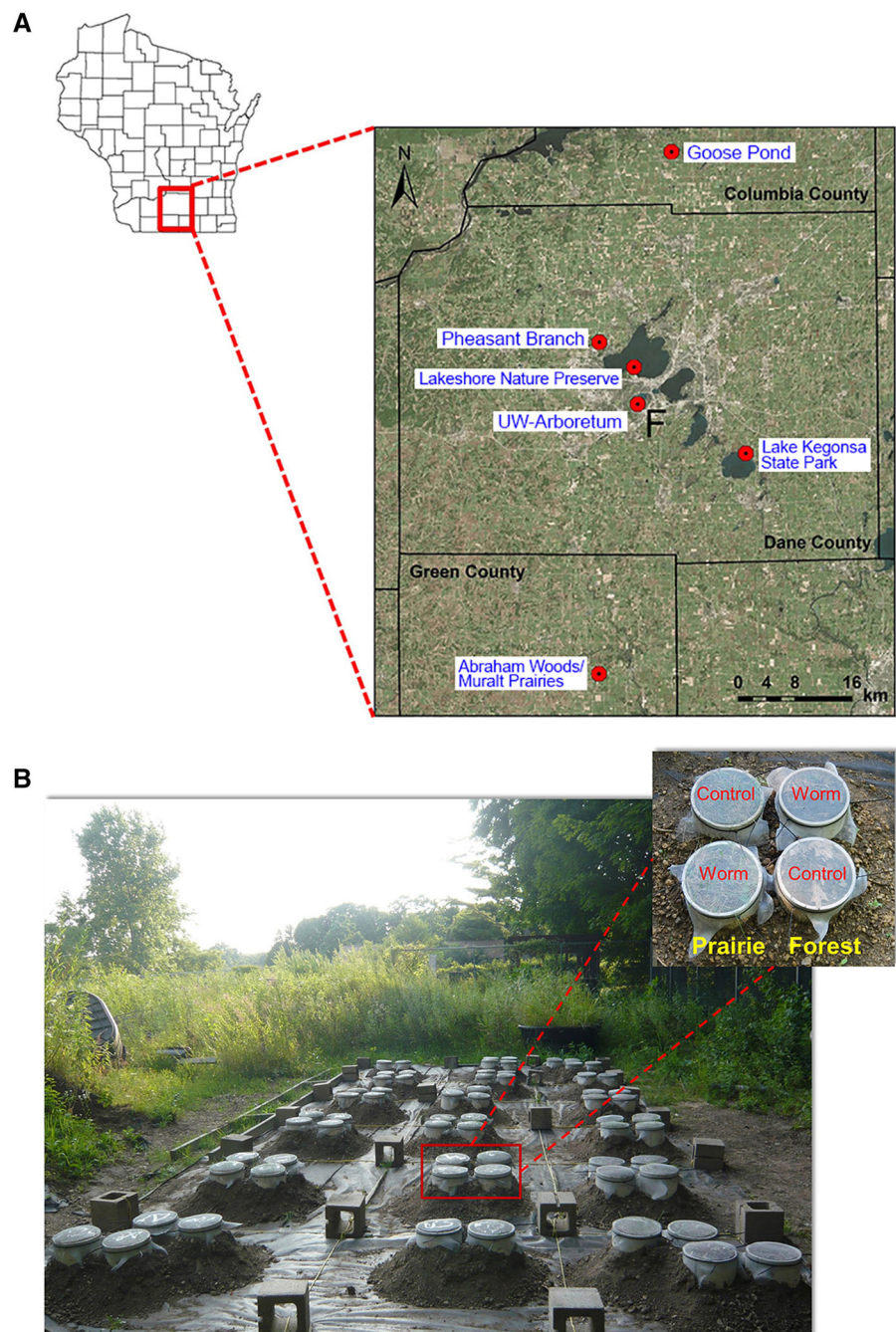
Table 1 Leaf litter and soil nutrient properties included in the study and the corresponding hypotheses regarding the effects of invasive Asian earthworms

	Response	Hypothesis
Leaf litter	Litter cover (%)	–
	Foliage litter mass (g/m^2)	–
	Litter depth (mm)	–
Soil nutrient pools	Soil organic matter (%)	+
	Total C (%)	+
	Total N (%)	+
	Total C:N ratio	+
	Available P (ppm)	+
	Nitrate (NO_3^-) ($\text{mg N kg}_{\text{soil}}^{-1}$)	+
	Ammonium (NH_4^+) ($\text{mg N kg}_{\text{soil}}^{-1}$)	+
Dissolved organic C ($\text{mg C kg}_{\text{soil}}^{-1}$)	+	

Soil responses were measured at three depths from the surface (i.e., 0–5, 5–10 and 10–25 cm)

In the hypothesis column, “+” indicates expected positive and “–” negative effects of invasive earthworms

Fig. 1 a Non-invaded sites across southern Wisconsin where intact soil cores were collected for the mesocosm experiment. At each site, 20-cm diameter \times 25-cm depth soil cores were excavated from a pair of adjoining forest and prairie ecosystems with similar soil type, except for the site of Goose Pond where only forest soil cores were collected. **b** Setup of the mesocosm experiment with random blocks in the field facility at the University of Wisconsin–Madison Arboretum (Photo credit: J. Qiu), and a close-up of a block with forest and prairie soil cores. Each earthworm treatment core was paired with a control. Letter “F” (also annotated ‘UW-Arboretum’) in **A** where the complementary field study was conducted



paired—one for introducing earthworms and the other as the control—and were placed into completely randomized blocks that accounted for microclimatic variation across the field facility (Fig. 1b). We installed shade cloth above the mesocosm and piled soils around all PVC cores to mimic field conditions. We then introduced six adult earthworms into a

randomly selected core of each pair (Fig. 1b); this number of earthworms approximated the maximum density of Asian jumping worms ($167/\text{m}^2$) measured in the adjacent invaded forest using mustard pours (in $30\text{-cm} \times 30\text{-cm}$ quadrats, $n = 15$) and is consistent with other studies of the similar species (e.g., Greiner et al. 2012; Richardson et al. 2016). Because of dry

Table 2 Initial conditions (i.e., prior to introducing earthworms) of leaf litter and soil nutrient pools, from the mesocosm experiment: means values and standard error (SE)

Leaf litter	Forest			Prairie		
	0–5 cm	5–10 cm	10–25 cm	0–5 cm	5–10 cm	10–25 cm
Litter cover (%)		97.2 ± 0.8			99.8 ± 0.2	
Foliage litter mass (g/m ²)		236.0 ± 31.2			439.8 ± 48.9	
Litter depth (mm)		26.6 ± 1.3			32.1 ± 5.2	
Soil nutrient pools	0–5 cm	5–10 cm	10–25 cm	0–5 cm	5–10 cm	10–25 cm
Soil organic matter (%)	6.25 ± 0.46	4.50 ± 0.37	3.16 ± 0.47	4.57 ± 0.27	3.45 ± 0.26	2.48 ± 0.20
Total C (%)	4.67 ± 0.34	3.12 ± 0.34	1.85 ± 0.27	3.15 ± 0.19	2.25 ± 0.18	1.57 ± 0.13
Total N (%)	0.35 ± 0.02	0.26 ± 0.02	0.17 ± 0.02	0.26 ± 0.01	0.21 ± 0.02	0.16 ± 0.01
Total C:N ratio	13.25 ± 0.30	11.65 ± 0.31	11.00 ± 0.42	11.96 ± 0.45	10.53 ± 0.38	9.77 ± 0.35
Available P (ppm)	37.72 ± 3.09	31.64 ± 3.75	25.90 ± 3.27	29.53 ± 7.85	23.25 ± 6.55	16.17 ± 4.15
Nitrate (NO ₃ ⁻) (mg N kg _{soil} ⁻¹)	27.20 ± 1.68	12.30 ± 0.79	6.17 ± 0.56	2.20 ± 0.64	1.97 ± 0.67	1.08 ± 0.36
Ammonium (NH ₄ ⁺) (mg N kg _{soil} ⁻¹)	2.72 ± 0.43	1.90 ± 0.24	1.92 ± 0.24	3.39 ± 0.58	3.14 ± 0.70	2.38 ± 0.32
Total inorganic nitrogen (mg N kg _{soil} ⁻¹)	29.92 ± 1.81	14.20 ± 0.87	8.09 ± 0.65	5.59 ± 0.70	5.11 ± 0.88	3.46 ± 0.47
Dissolved organic C (mg C kg _{soil} ⁻¹)	90.25 ± 5.88	59.26 ± 4.18	48.76 ± 2.95	61.31 ± 2.26	51.06 ± 7.38	37.56 ± 2.35

Sample size $N = 18$ for forest and $N = 15$ for prairie soil cores

conditions during summer 2014 and concern that death of the earthworms would cause the experiment to fail, we added two more earthworms in August. All earthworms were collected from the adjacent forest where the invasion was first discovered and earthworms had been collected for identification (Brad Herrick, University of Wisconsin Arboretum, *personal communication*). Density of *Amyntas* spp. at the end of the experiment averaged five per core (159/m²), indicating that the densities achieved in the experiment were consistent with the desired densities. At the end of the experiment, we only found three of the 66 cores with non-*Amyntas* earthworms (two in controls and one in earthworm treatment); each of those cores had a single one *Lumbricus terrestris*.

The top of each core was covered with a mesh layer to prevent earthworms from escaping, and the bottom was covered with a permeable fabric layer to allow vertical flow of water. Mesocosms (i.e., soil cores) were incubated under ambient conditions from July through October 2014. Throughout the experiment,

mesocosms were monitored weekly for soil temperature and moisture, and deionized water was added consistently to all cores (with and without earthworms) as needed to maintain soil moisture, because earthworms in the cores could not move to more favorable sites and we wanted to minimize the likelihood that earthworms would desiccate and die. Cores were also inspected weekly for indications of earthworm activity and changes in surface soil and litter conditions. At the end of experiment, soil and litter samples were collected to measure the same properties as for the initial conditions (Table 1); all soil cores were then harvested to tally the earthworms remaining in each core.

Field study design

The mesocosm experiment tested for effects under controlled conditions but could not detect changes over time in situ. Hence, we also sampled litter and soil monthly in invaded and nearby uninvaded forests with

similar soil conditions and stand structure at the University of Wisconsin-Madison Arboretum (Fig. 1). Both stands were dominated by sugar maple (*Acer saccharum*), interspersed with red maple (*Acer rubrum*), red oak (*Quercus rubra*), and American beech (*Fagus grandifolia*). At each invaded and uninvaded site, three 25-m² plots were established at random locations separated by >50 m. Monthly sampling occurred the same day of each month from July through October 2014. For each sampling effort, litter and soil samples were collected at each plot from three randomly placed 30-cm × 30-cm quadrats, and the same properties were measured as for the mesocosm experiment (Table 1). Detailed sampling protocols are described below.

Sample collection and laboratory analyses

Protocols for litter and soil sampling and subsequent laboratory analyses were identical for the mesocosm experiment and field study. In brief, for each soil core (mesocosm experiment) or quadrat (field study), litter depth was recorded at three locations and averaged, and percent litter cover was visually estimated. All fresh litter was removed from each core or quadrat, and separated into foliage (e.g., dead leaves) and woody litter (e.g., twigs, fragments). Percent cover of earthworm castings in each mesocosm was visually estimated as an indication of earthworm activity. Litter was oven-dried at 70 °C for 48 h or until constant mass.

Ten soil subsamples were collected evenly across each mesocosm or quadrat using a 2.5-cm diameter soil probe sampler; soil samples were divided at incremental depths of 0–5, 5–10 and 10–25 cm, and pooled by depth. Soils were then homogenized and sieved (2-mm mesh) to remove fine roots, coarse fragments or gravel, and placed in a plastic bag and kept cool for transport to the laboratory.

For laboratory analyses, gravimetric soil moisture was first determined by wet and dry weights (oven-drying at 105 °C for 48 h). Subsamples were then oven-dried (70 °C for 48 h) and ground with a ball mill to pass through a 100 mesh (0.15 mm) sieve to determine total soil C and N. Total C and N were analyzed by high-temperature catalytic combustion using a Carlo-Erba Model NA 1500 C and N analyzer (CE Instruments, Milan, Italy). Other subsamples were air dried and sent to the Soils and Plant Analysis Lab at the University of Wisconsin-Madison for

analyzing soil organic matter and available phosphorus. Soil organic matter was determined by dry combustion of a 5-g subsample using Tekmar-Dohrman 183 TOC Boat Sampler DC-190 (Tekmar-Dohrman, Mason OH), and available phosphorus was analyzed using Bray P1 extraction procedure (Bray and Kurtz 1945).

Inorganic N (NH₄⁺-N and NO₃⁻-N) was extracted within 24 h of soil sampling. We placed 8 ± 0.1 g of sieved (2-mm mesh) fresh subsamples of soil into centrifuge tubes with 40 ml of 2 M KCl. After capping and shaking the tubes for 1 h on an orbit shaker at 200 rpm, the samples were centrifuged (3000 rpm) for 10 min (Wiltshire and Laubscher 1989; Robertson et al. 1999). Supernatants were then extracted and filtered through 0.7 µm Whatman GF/F glass microfiber filters, and frozen (−18 °C) until analysis. Concentrations of NH₄⁺-N and NO₃⁻-N were analyzed on a Technicon segmented flow autoanalyzer (Tarrytown, New York, USA). Dissolved organic carbon (DOC) was extracted following the same protocol as extractions for inorganic N, except for using 0.5 M K₂SO₄ solution (Balser and Firestone 2005). Extracts were frozen pending analysis. DOC concentrations were analyzed using a Shimadzu TOC-V carbon analyzer.

Statistical analyses

For the mesocosm experiment, we calculated changes between end and initial measurement of soil and litter properties. Effects of earthworms were tested using a general linear-mixed effect model in which the end measurement was the response variable and the initial condition was a covariate (Vickers 2001). Linear-mixed effect models were used to account for unbalanced design and inherent variation from random effects. Random effects included site (six locations where mesocosm soil cores were collected) and block (a factor nested within site that allows for comparison between the earthworm treatment and control in each pair). Earthworm presence, ecosystem type, soil depth, and their interactions were considered as fixed effects.

To evaluate earthworm effects from the field study, we used linear-mixed effect models to handle repeated measures and subsampling. To avoid pseudoreplication, samples taken within each plot were considered

as subsamples, and monthly sampling efforts were treated as repeated measurements. Random effects included subsampling (nested within plot), and plot (as repeated statement), and fixed effects included earthworm presence, month, soil depth and their interactions. We interpreted a significant interaction ($P < 0.05$) between earthworm presence and month (a test for the rate of change) for a given soil depth as evidence of earthworm effects.

All statistical analyses were performed using R statistical software (R Development Core Team 2009). Models were fit using restricted maximum likelihood (REML), and the significance of differences in earthworm effects between levels of ‘soil depth’ and ‘ecosystem type’ was tested using Tukey’s multiple comparison with the *glht* function in the “*multcomp*” package (Hothorn et al. 2008). Residual plots were visually assessed for assumptions of normality and homogeneity of variance; no violations were detected. Linear-mixed effect models were analyzed using the “*lme4*” package (Bates et al. 2015), and significance of fixed effects was evaluated using the Satterthwaite’s approximation for degrees of freedom in the “*lmerTest*” package (Kuznetsova et al. 2014).

Results

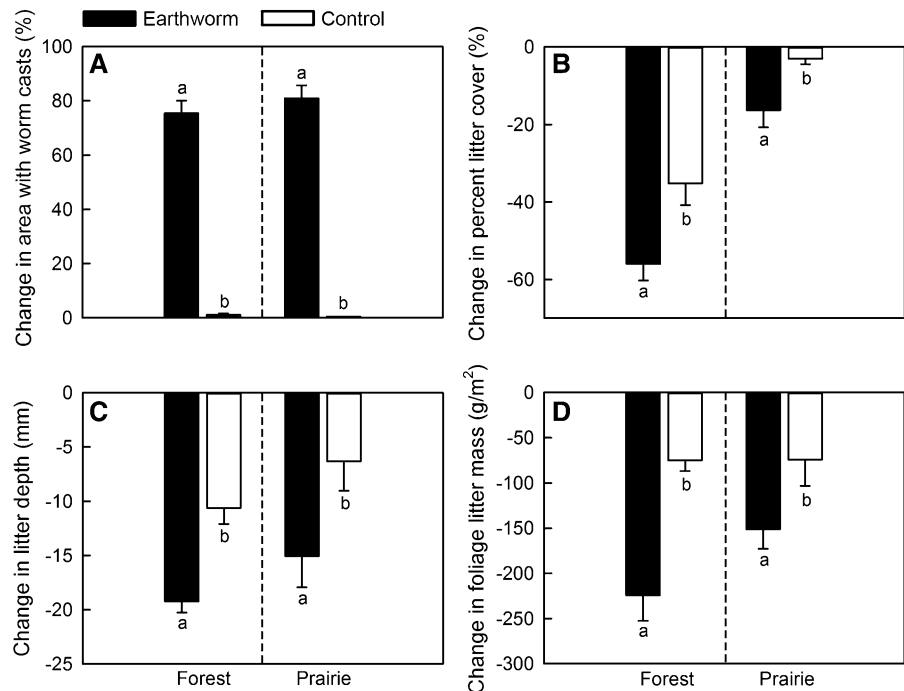
Mesocosm experiment

Earthworm activities and leaf litter

Most *A. agrestis* and *A. tokioensis* survived in mesocosms for the incubation period, with an average of 5.0 ± 0.48 Asian jumping worms recovered. By the end of the experiment, earthworm casts covered $>75\%$ of the surface area of soil cores that had earthworms introduced but were absent in controls (Fig. 2a).

As expected, *A. agrestis* and *A. tokioensis* substantially reduced the litter layer in forest and prairie mesocosms (Fig. 2). Litter cover, litter depth and foliage litter mass declined more in earthworm treatments than in controls (all $P < 0.001$). While declines in litter cover and depth were comparable across ecosystems (Fig. 2b, c), declines in foliage litter mass were much greater in forest than prairie mesocosms (95 vs. 34 % loss respectively, relative to initial litter mass, Fig. 2d; $P < 0.05$, ANOVA model results in online Appendix B Table B1).

Fig. 2 Effects of *A. agrestis* and *A. tokioensis* on: **a** percent area with earthworm casts (%); **b** litter cover (%); **c** litter depth (mm); **d** foliage litter mass (g/m^2) from the mesocosm experiment. Bars represent mean changes as compared to initial conditions for the earthworm treatment (black) and control (white), and error bars are standard errors (SE). Bars with different lowercase letters indicate a significant difference ($\alpha = 0.05$) between the earthworm treatment and control from Tukey’s multiple comparison test



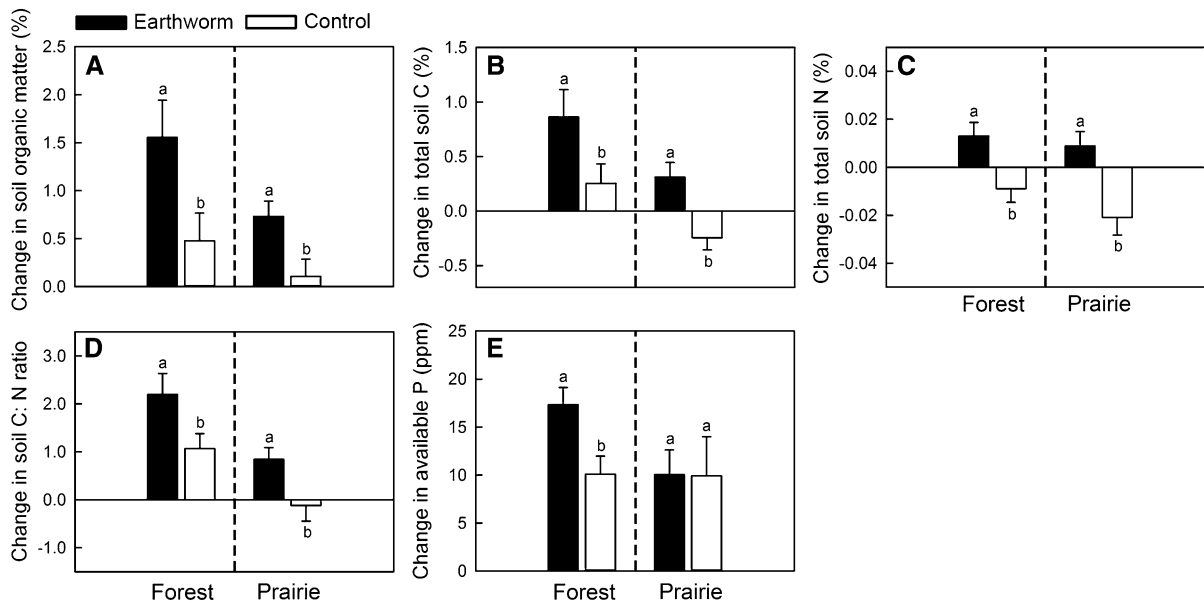


Fig. 3 Effects of *A. agrestis* and *A. tokioensis* on 0–5 cm soil: **a** organic matter (%); **b** total C (%); **c** total N (%); **d** total C:N ratio; **e** available P (ppm) from the mesocosm experiment. Bars represent mean changes relative to initial conditions for the earthworm treatment (black) and control (white), and error bars

are standard errors (SE). Bars with different lowercase letters indicate a significant difference ($\alpha = 0.05$) between the earthworm treatment and control from Tukey's multiple comparison test

Soil nutrient pools

Soil nutrient pools were enriched in the earthworm treatment for both forest and prairie soils (Figs. 3, 4). Specifically, at 0–5 cm soil depths, percent organic matter, total C and total N content substantially increased in both forest and prairie soils with introduced *A. agrestis* and *A. tokioensis* (Fig. 3a, b, c). Changes in C and N content resulted in net increases of 2.2 and 0.8 in the C:N ratio of affected forest and prairie soils, respectively (Fig. 3d). The magnitude of effects on percent organic matter, total C and total N content did not differ between forest and prairie soils (online Appendix B Table B2). For example, soil organic matter increased 25 % relative to initial conditions in the earthworm treatment for 0–5 cm forest soils (vs. 16 % increase for 0–5 cm prairie soils). Available P increased (46 %) at 0–5 cm depth in forest soils, but no effects were found for prairie soils (Fig. 3e). These nutrients were not affected by the earthworms at greater depths (i.e., 5–10, 10–25 cm; online Appendix B Fig. B1).

As predicted, soil inorganic N and DOC concentrations were very responsive to the presence of *A. agrestis* and *A. tokioensis* (Fig. 4). Nitrate–N concentrations largely

increased across all depths of forest soils in the earthworm treatment, but either declined or showed minimal increases in no-earthworm controls (Fig. 4a). The effects on nitrate–N differed by ecosystem (earthworm \times ecosystem interaction, $P < 0.001$; online Appendix B Table B2), with greater effects in forest than prairie soils, in which significant effects were only observed for 0–5 cm soils (Fig. 4a). Ammonium–N concentration did not differ between earthworm treatments and controls, except for 0–5 cm prairie soils (Fig. 4b). Patterns of total inorganic N mirrored those of nitrate–N concentrations, largely because nitrate dominated the available inorganic forms of N (Fig. 4c). Effects on soil DOC concentrations were consistent and pervasive across all depths of forest and prairie soils. DOC concentrations increased from 71 to 108 % in the earthworm treatment relative to initial conditions (all $P < 0.001$; Fig. 4d).

Field study

Leaf litter

Presence of *A. agrestis* and *A. tokioensis* was associated with accelerated loss of forest leaf litter through time. From July through October, foliage litter mass

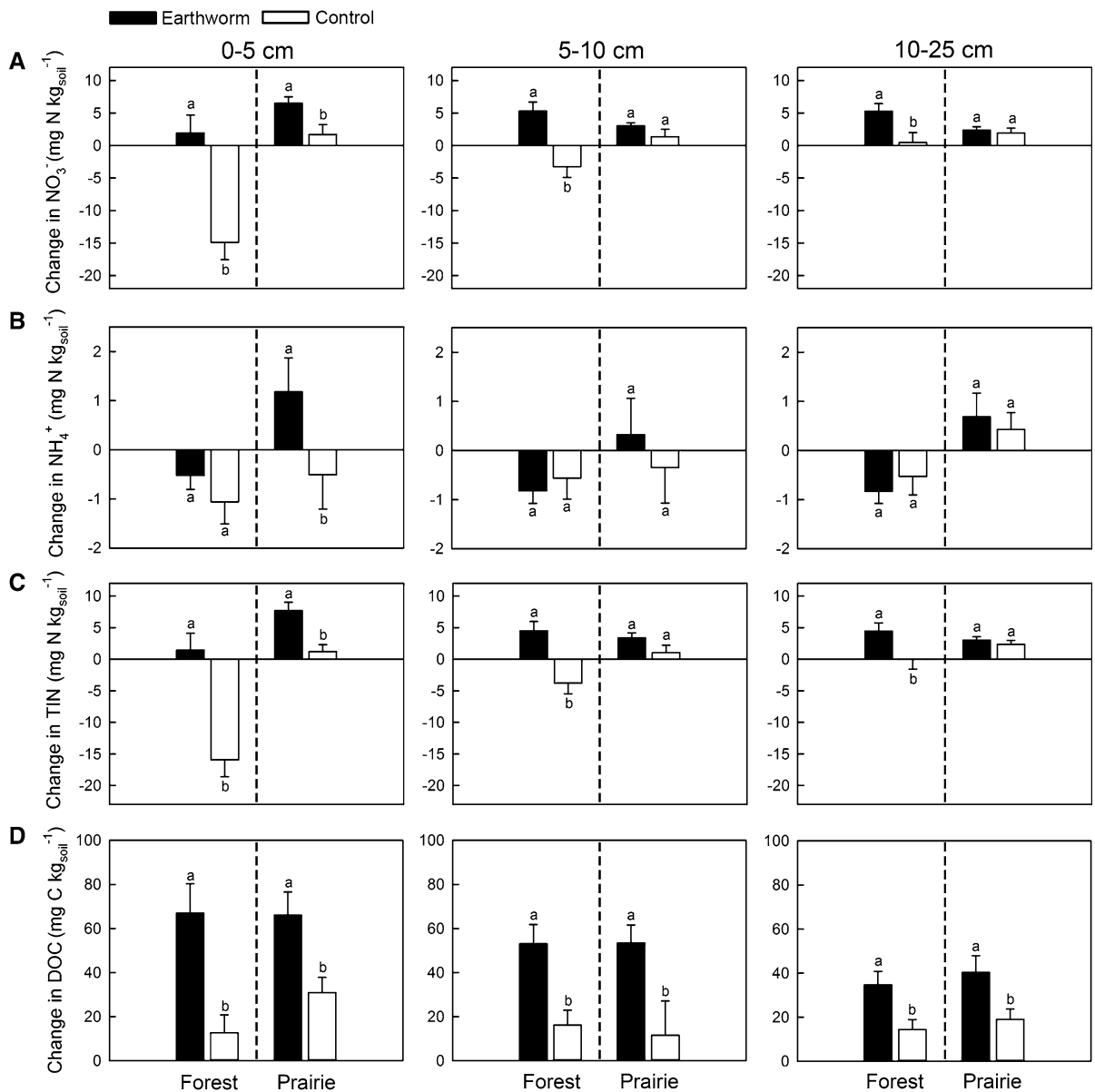


Fig. 4 Effects of *A. agrestis* and *A. tokioensis* on: **a** nitrate (NO_3^-) ($\text{mg N kg}_{\text{soil}}^{-1}$); **b** ammonium (NH_4^+) ($\text{mg N kg}_{\text{soil}}^{-1}$); **c** total inorganic N (TIN) ($\text{mg N kg}_{\text{soil}}^{-1}$); **d** total dissolved organic carbon (DOC) ($\text{mg C kg}_{\text{soil}}^{-1}$) from the mesocosm experiment. Bars represent mean changes as compared to initial conditions

for the earthworm treatment (black) and control (white), and error bars are standard errors (SE). Bars with different lowercase letters indicate a significant difference ($\alpha = 0.05$) between the earthworm treatment and control from Tukey's multiple comparison test

declined by 84 % in invaded forests (most declines occurred from September to October), compared to 43 % in the adjacent uninvaded forests ($P < 0.01$, Fig. 5a). Litter depth also decreased more rapidly in invaded forests relative to uninvaded forests (79 vs. 43 %, respectively; Fig. 5b; online Appendix C Table C1).

Soil nutrient pools

Changes in soil nutrient pools closely paralleled loss of leaf litter. In invaded forests, soil organic matter, total C and total N content in 0–5 cm soils did not change noticeably over the first three months, but increased sharply from September to October (Fig. 6a,

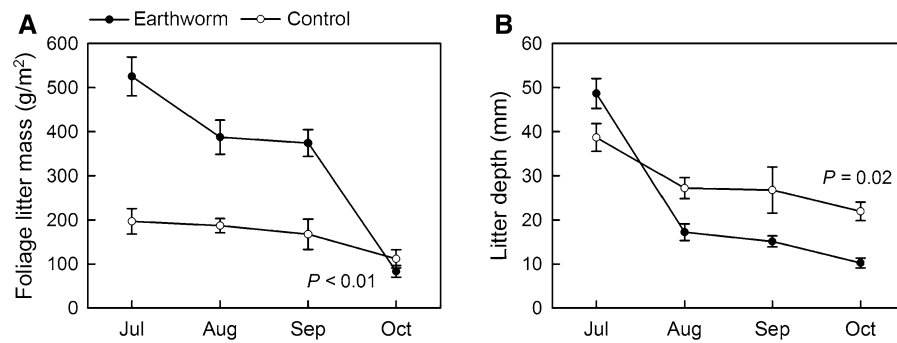


Fig. 5 Temporal changes in **a** foliage litter mass (g/cm^2); **b** litter depth (mm) in forest invaded by *A. agrestis* and *A. tokioensis* (solid circle), and adjacent non-invaded forest (open circle) from the field study. Sampling occurred monthly from

July to October 2014. Errors represent standard errors ($N = 9$), and P values are the significance level of the interaction term between month and absence/presence of earthworms

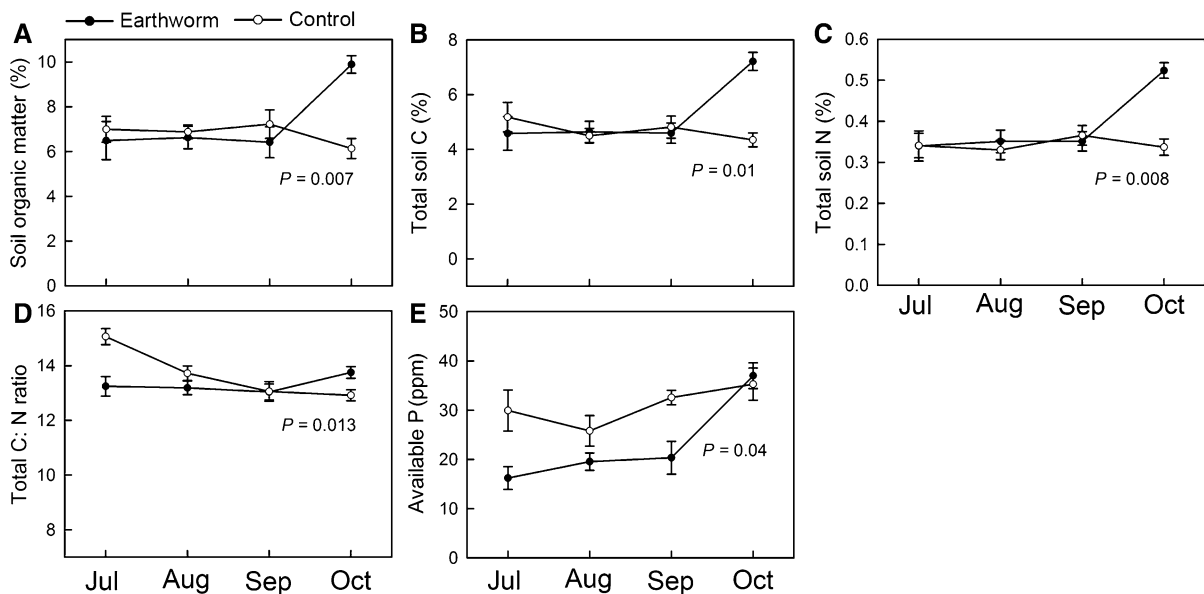


Fig. 6 Temporal changes in 0–5 cm soil: **a** organic matter (%); **b** total C (%); **c** total N (%); **d** total C:N ratio; **e** available P (ppm) in forest invaded by *A. agrestis* and *A. tokioensis* (solid circle), and adjacent non-invaded forest (open circle) from the

field study. Sampling occurred monthly from July to October 2014. Errors represent standard errors ($N = 9$), and P values are the significance level of the interaction term between month and absence/presence of earthworms

b, c); in contrast, in uninvaded forests, these soil attributes remained constant or declined slightly. Soil C:N ratio did not change significantly in 0–5 cm soils in invaded forests, but declined from 15.1 to 12.9 in uninvaded forests (Fig. 6d). We also found that available P in the upper 5-cm of soils increased by 128 % in invaded forests from July to October (vs. 18 % in uninvaded forests) (Fig. 6e). Similar to the mesocosm experiment, effects on these nutrient pools

were insignificant for soils deeper than 5-cm (online Appendix C Table C2 and Fig. C1).

Consistent with the mesocosm experiment, presence of *A. agrestis* and *A. tokioensis* substantially altered inorganic N and DOC concentrations in field conditions (Fig. 7). Nitrate–N concentrations increased in 0–5 and 5–10 cm soils over time in invaded forests but remained unchanged or declined in uninvaded areas (Fig. 7a). Effects on ammonium–N were also significant in the

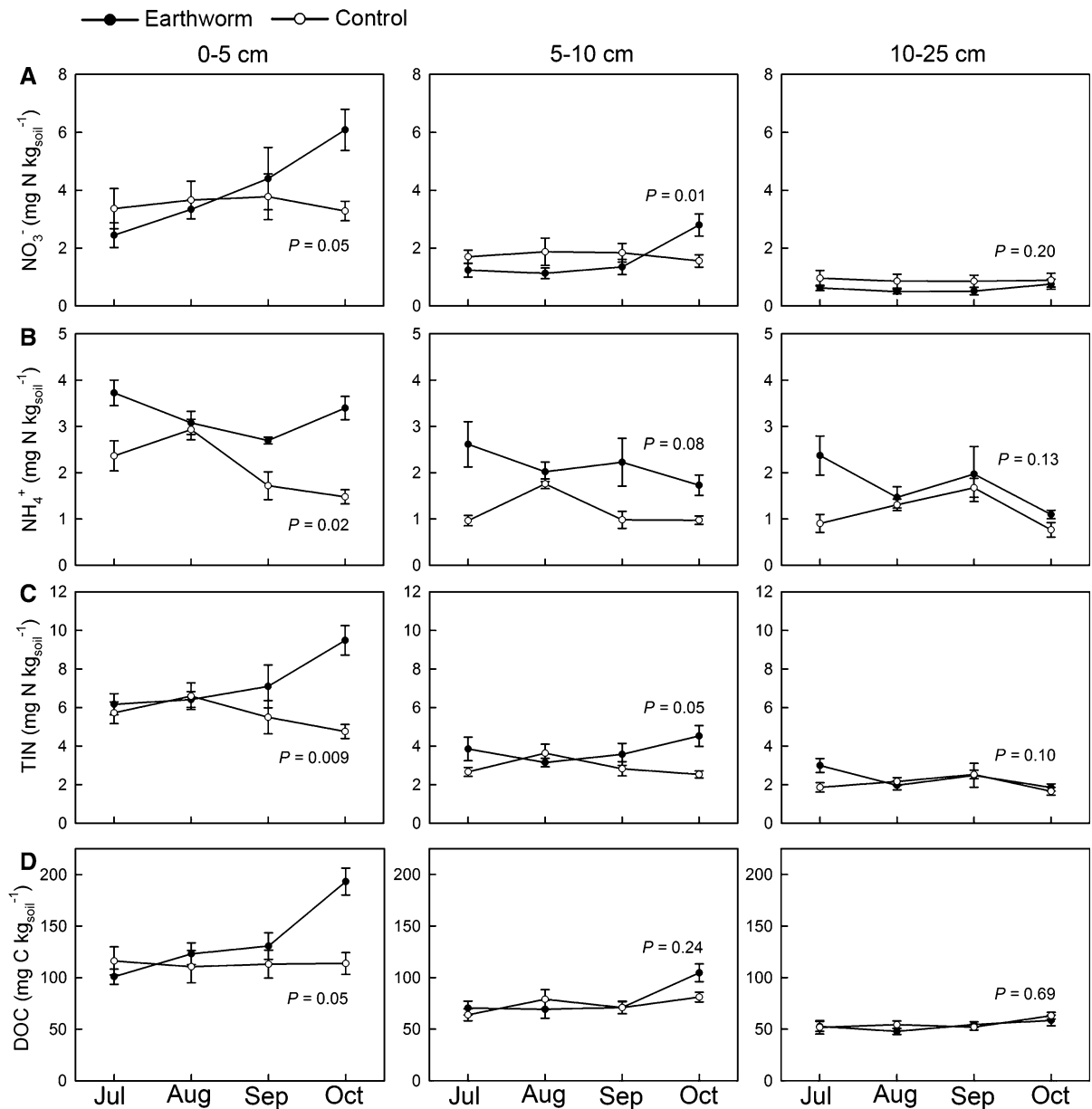


Fig. 7 Temporal changes in: **a** nitrate (NO_3^-) ($\text{mg N kg}_{\text{soil}}^{-1}$); **b** ammonium (NH_4^+) ($\text{mg N kg}_{\text{soil}}^{-1}$); **c** total inorganic N (TIN) ($\text{mg N kg}_{\text{soil}}^{-1}$); **d** total dissolved organic carbon (DOC) ($\text{mg C kg}_{\text{soil}}^{-1}$) in forest invaded by *A. agrestis* and *A. tokioensis* (solid circle), and adjacent non-invaded forest (open circle) from the

field study. Sampling occurred monthly from July to October 2014. Errors represent standard errors ($N = 9$), and P values are the significance level of the interaction term between month and absence/presence of earthworms

upper 5-cm of soils, where ammonium–N initially declined but spiked in the last month (Fig. 7b). Changes in total inorganic N again were akin to those of nitrate–N concentrations, with increases of 54 and 18 % from July through October in 0–5 cm and 5–10 cm invaded soils, respectively (vs. 17 and 5 % declines in respective

uninvaded soils; Fig. 7c). DOC concentrations in 0–5 cm soils increased by 91 % in invaded forests, but was unchanged in uninvaded areas ($P = 0.05$; Fig. 7d). Effects on DOC concentrations at deeper soils (i.e., 10–25 cm) were weaker than those found in the mesocosm experiment.

Discussion

Invasion of *A. agrestis* and *A. tokioensis* substantially reduced leaf litter and enriched soil nutrient pools in temperate deciduous forest and prairie soils. The rapid transformation of organic matter into more mobile forms may change nutrient availability for plants and could potentially make ecosystems more susceptible to nutrient losses. Effects of Asian jumping worms were also significant in the prairies (at least in the experimental microcosms, as the worms had not yet been observed in situ in the prairies), which are of great conservation value (Samson and Knopf 1994). Thus, effects of Asian jumping worms might rival those of better-studied European invasive earthworms—the major drivers of ecological changes in northern temperate and boreal forests (Bohlen et al. 2004c). Given the potential northward expansion of *Amyntas* spp. (Bohlen et al. 2004a), this study provides timely insights into their ecological impacts.

Earthworm effects on leaf litter and soil nutrient pools

Effects of *A. agrestis* and *A. tokioensis* on leaf litter were consistent with our hypotheses and other studies that earthworm invasion accelerates leaf litter loss in northern temperate forests (Hale et al. 2008; Fahey et al. 2013). However, the magnitude of effects might exceed those of other earthworm species. In the present study, for example, *A. agrestis* and *A. tokioensis* caused a 63 % greater reduction in forest foliage litter mass in earthworm treatment than control in the 4-month experiment, and a 41 % more decline in litter mass in invaded than uninvaded forests under the field conditions. Greiner et al. (2012) reported 23 and 40 % more losses in forest leaf litter mass due to *Amyntas hilgendorfi* and *Lumbricus rubellus*, respectively, compared to uninvaded areas. In central New York, USA, Suarez et al. (2006) found 31 % more litter mass loss in hardwood forests invaded by *Lumbricus rubellus* and *Octolasion tyrtaeum* than control plots after 540 days of experiment. Great effects of *A. agrestis* and *A. tokioensis* on leaf litter corroborate findings of Snyder et al. (2011), and may be due to their high population densities (~ 10 times of other non-native earthworms) (Ikeda et al. 2015), dietary flexibility and superior feeding on the gram-positive soil bacteria essential to earthworms for litter

digestion (Zhang et al. 2010). We also found significant effects on prairie litter, albeit of smaller magnitude than in forests, perhaps because of the lower C:N ratio of the deciduous foliage litter (Snyder et al. 2009).

Our results of nutrient enrichment in the upper 5-cm of soils agree with previous findings on Asian jumping worms (Snyder et al. 2009; Greiner et al. 2012), but contradict research on other earthworm species (Alban and Berry 1994; Suarez et al. 2004; Bohlen et al. 2004b; Eisenhauer et al. 2007; Crumsey et al. 2013). Such differences are possibly due to the relative dominance of two mechanisms that control earthworm effects on nutrient dynamics: (1) earthworm mixing and incorporation of litter nutrients into soils; (2) earthworm-induced nutrient losses through gaseous fluxes (e.g., CO₂, N₂O) (Speratti and Whalen 2008) and leaching (e.g., dissolved organic matter) (Bohlen et al. 2004b). It is likely that given high densities of *Amyntas*, the incorporation of litter nutrients into soils exceeds earthworm-induced nutrient losses, leading to a transient enrichment of nutrient pools in topsoils. Differences in effects can also be attributable to earthworms of distinct functional groups: *A. agrestis* and *A. tokioensis* are epigeic and exert greater effects on O horizon and topsoils, whereas for anecic species (which feed on surface litter but form deep vertical burrows, e.g., *L. terrestris*), effects on surface soils can be trivial (Edwards 2004). Finally, incubation time might be another factor. Our study focused on effects during one growing season, and thus revealed short-term effects. Established earthworm populations with a long history of invasion might lead to consequences that differ fundamentally from short-term effects (Bohlen et al. 2004a; Zhang et al. 2013).

Our hypotheses that invasion of *A. agrestis* and *A. tokioensis* increases soil inorganic N and DOC concentrations were supported (Figs. 4, 7). Increases in inorganic N are likely due to enhanced mineralization in earthworm guts, and addition of organic materials into soils that provide substrates for mineralization (Scheu and Parkinson 1994; Costello and Lamberti 2009; Lubbers et al. 2011). Other studies also found elevated inorganic N in topsoils due to invasive earthworms under experimental (Hale et al. 2008; Greiner et al. 2012) and field conditions (Szlavecz et al. 2006). The fact that inorganic N increased at greater depths than previously reported suggests that

Asian jumping worms might have stronger effects on N transformations and pools than other earthworm species.

Soil DOC concentrations may be altered by *A. agrestis* and *A. tokioensis* through both biotic and/or abiotic mechanisms. Biotic factors include (1) enhanced mineralization by earthworm activities that promote DOC production (Kalbitz et al. 2000); (2) earthworm-induced increases in soil organic matter that acts as prime sources of DOC (Currie and Aber 1997; Tipping et al. 1999; Sackett et al. 2013); and (3) secretion of labile C in mucus and casts of earthworms. Burrowing activities of earthworms could also create macropores and facilitate DOC movement across soil profiles. In addition, increased macropores could reduce contact time between DOC and mineral soils as water percolates, reducing DOC adsorption and increasing DOC levels at deeper soils (Kalbitz et al. 2000). We suspect that biotic factors are primarily responsible for production of DOC in soils, and abiotic changes caused by earthworms are probably the main reason for DOC increases at deeper soils.

Increased inorganic N and DOC may lead to nutrient losses through different pathways. One possibility is leaching. Increased nitrate and DOC in soils may exceed plant uptake, and can be susceptible to potential leaching and transport into aquatic systems, which may degrade water quality. Also, large soil aggregates and macropores could further facilitate preferential flows and leaching process (Greiner et al. 2012). Other studies have also founded increased nutrient leaching in forest and agricultural soils as a consequence of earthworm invasion (Bohlen et al. 2004b; Dominguez et al. 2004).

Comparison between forest and prairie ecosystems

Stronger effects in forests than prairies could be explained by differences in litter (quality, quantity, composition) and soil (physical–chemical structure, nutrient) properties. Deciduous forest litter normally has lower C:N ratio and is a preferable food source as compared to prairie graminoid litter. Differences in litter stoichiometry can further cascade into effects on soil nutrients (Fahey et al. 2013). In addition, forest soils are relatively easier to burrow than prairie soils that are often more compact and contain dense root systems. Forest soils are also more moist than prairie soils (averaged moisture of $35 \pm 4\%$ in forest vs.

$25 \pm 3\%$ in prairie mesocosms over the experimental period), which may facilitate microbial activities in forest soils.

Nonetheless, the significant effects on prairie soils are consistent with the stimulated ligninolytic enzyme activities by *A. agrestis* (Bellitürk et al. 2015), and provide evidence that *A. agrestis* and *A. tokioensis*—native to Asian grasslands—may survive and affect prairies. It further suggests that, while U.S. forests are considered susceptible to invasion of non-native earthworms, prairies that support diverse life forms and ecosystem functioning (Samson and Knopf 1994; Werling et al. 2014) may also be vulnerable to invasion of Asian jumping worms. One caveat is that our study only examined effects on prairies under experimental conditions but not in the field, mostly because no populations were detected in the prairies at the time of this work. Future studies should monitor multiple habitats, including prairies, to quantify rates and patterns of the spread of Asian jumping worms and understand their ecological effects under natural conditions.

Temporal dynamics of earthworm effects

Effects of *A. agrestis* and *A. tokioensis* did not change linearly over time and were most pronounced towards the end of the growing season (Figs. 6, 7). This temporal pattern aligns well with the life history of many epigeic *Amyntas* spp. that emerge from cocoons in spring, grow rapidly to sexual maturity and peak in biomass in the fall (Greiner et al. 2012; Ikeda et al. 2015). Another factor that might reinforce this pattern is the new litter fall occurring from September to October, which adds food sources for earthworms.

Methodological considerations

Effects of *A. agrestis* and *A. tokioensis* from our mesocosm experiment and field study were consistent, but the magnitude of effects sometimes differed. Such differences are not surprising. First, plant uptake is an important nutrient flux in the field, but negligible in mesocosms. Second, mesocosms are semi-closed systems as compared to field conditions, with little or no external inputs such as litter fall. Third, differences in shading could also affect soil hydrologic conditions and earthworm performance. Although we installed shade cloth above mesocosms, soils were not

as fully shaded as below the closed forest canopy or the dense, tall prairie vegetation. Last but not least, in the mesocosms, a fixed number of earthworms were introduced into a confined space. Under field conditions, earthworm movement is not constrained, and soils at a particular location can be affected by variable number of earthworms. Nevertheless, the consistent results from both methods provide confidence in the effects of *A. agrestis* and *A. tokioensis* detected in this study.

Conclusions

Non-native earthworm invasion is a major driver of ecological change. Effects of European invasive earthworms in North America are well understood, but non-native Asian earthworms are increasingly discovered. We found that *A. agrestis* and *A. tokioensis* significantly reduced litter and enriched nutrients in surface soils. Effects were observed in both forest and prairie soils and may rival those reported for other invasive earthworms. The rapid transformation of litter and soil nutrients into labile and soluble forms may make invaded ecosystems more susceptible to nutrient losses. Effects on litter and soils may further cascade to other taxa, such as understory herbs, soil micro- and macro-fauna, and affect important ecological processes like plant establishment and growth.

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