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Synergistic *Lemna* Duckweed and Microbial Transformation of Imidacloprid and Thiacloprid Neonicotinoids

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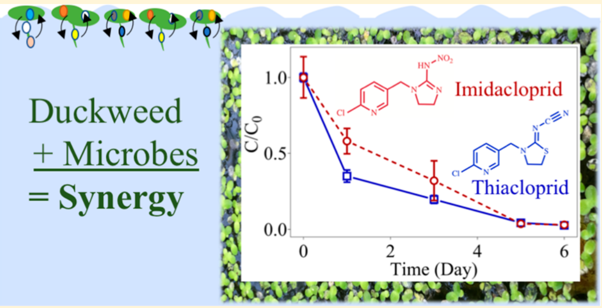
Supporting Information



ABSTRACT: Neonicotinoids are the most widely used insecticides in the world and are commonly measured in aquatic environments, including freshwater wetlands. We report for the first time the synergistic transformation of neonicotinoids by a *Lemna* duckweed and microbial system collected from an agricultural pond in Iowa, USA. Imidacloprid and thiacloprid were removed at statistically indistinguishable rates (0.63 ± 0.07 and 0.62 ± 0.05 day^{-1}, respectively) from hydroponic medium only when in the presence of both duckweed and its associated microbial community. As evidence for this duckweed−microbial synergy, experiments with surface-sterilized duckweed, duckweed-associated microbes, pond water microbes alone, and two other plant species (*Typha* sp. and *Ceratophyllum demersum*) did not yield significant neonicotinoid removal beyond initial biomass sorption. Degradation of imidacloprid and thiacloprid by the duckweed−microbial system generated multiple, known neonicotinoid metabolites (desnitro-imidacloprid, imidacloprid urea, thiacloprid amide, and 6-chloronicotinic acid). Measured metabolites with increased insect or vertebrate toxicity were either absent (imidacloprid olefin) or present only in small amounts (desnitro-imidacloprid; <1% of the parent). The neonicotinoid parent and metabolite mass balance did not fully account for total neonicotinoid removal, suggesting mineralization and/or other unidentified transformation products with unknown toxicity. This novel duckweed- and microbe-facilitated neonicotinoid degradation may represent an important contribution to the environmental fate of neonicotinoids.

INTRODUCTION

Neonicotinoids are the most widely used insecticides in the world.[1] They are applied to a diverse variety of common agricultural crops,[2−4] residential gardens/turf,[1,5] and trees[1,6] and employed for flea and tick control on pets.[1,7] Neonicotinoids have been implicated in numerous ecosystem effects, including the decline of pollinators and insectivorous birds.[8−10] Due in part to their wide usage, large application volume, and solubility in water,[11] neonicotinoids have been reported in surface waters with an average concentration[12] of 0.13 μg/L and much higher maximum concentrations[13] (e.g., 320 μg/L). Neonicotinoids are also present in drinking water, stormwater, groundwater, and wastewater.[4,12,14−17]

Neonicotinoids exert selective toxicity through an electronegative nitro or cyano pharmacophore that preferentially binds to insect nicotinic acetylcholine receptors.[18] This selective binding makes neonicotinoids more target-specific than many preceding pesticides.[16] Nevertheless, modification of the neonicotinoid insecticidal pharmacophore through microbial activity[2,19−24] (largely documented in soil microbes) or an abiotic process such as photolysis[25−27] can alter binding specificity and subsequently impact nontarget organisms. For example, loss of the nitro group from imidacloprid can generate desnitro-imidacloprid,[15,16] which contains a moiety with a positive charge distribution that influences receptor binding and makes this metabolite 317 times more toxic than imidacloprid[16,17] to vertebrates (based on IC$_{50}$). Desnitro-imidacloprid can be further microbially transformed to less-insecticidal compounds imidacloprid urea and 6-chloronicotinic acid (6-CNA);[19] mineralization routes for 6-CNA have also been reported.[19,28,29]

Despite the frequent occurrence of neonicotinoids in surface waters worldwide and the demonstrated capability of soil microbes to transform neonicotinoids, there is a paucity of research on neonicotinoid transformation by aquatic plants and their associated microbial communities.[2,12,19−24] A recent study[30] reported that, of several environmental factors, lower concentrations and a lower detection frequency of neonicotinoids (including imidacloprid) in shallow marsh water were

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the most highly correlated with the presence of select dominant plant species, such as a duckweed (*Lemna turionifera*). Other dominant plants, such as cattails (*Typha latifolia*), correlated with higher frequencies of neonicotinoid detection and higher concentrations in shallow marsh water; however, mechanisms were not elucidated with any plant species. Duckweed, and in some cases its associated microbial communities, can transform contaminants in water, e.g., antidepressants,[31] anti-inflammatories,[31] arsenic,[32] and phenols.[33,34] Due to the prevalence of duckweed in aquatic environments worldwide,[35] duckweed-facilitated transformation of neonicotinoids could represent a critical environmental fate mechanism. Therefore, the objectives of this work were (1) to determine if *Lemna* duckweed and/or its associated microbial community can remove imidacloprid and/or thiacloprid from water and (2) to quantify the formation of select environmentally relevant metabolites, including those with greater insect or vertebrate toxicity.

MATERIALS AND METHODS

Plant Sources and Neonicotinoid Removal Experiments. Two aquatic macrophytes, duckweed [*Lemna* spp., tentatively identified as *Lemna turionifera* (Figure S2)] and coontail (*Ceratophyllum demersum*), as well as pond water were collected from a pond in Johnson County, Iowa, in an agricultural region. Plant experiments were conducted in Magenta boxes in a climate-controlled growth chamber using a method similar to previously established methods,[36−39] with 3.1−3.3 g (fresh weight) of plant biomass and 10 mL of filter-sterilized 0.5× Schenk and Hildebrandt medium,[40] pH 6.0 (unless otherwise noted), per box. Experiments were conducted sacrificially in triplicate, unless otherwise noted. Sorption experiments forneonicotinoids to unsterilized duckweed tissue are described in the Supporting Information. Plant tissue was extracted for LC−MS/MS analysis using our previously established methods,[41−44] with minor adaptations noted in the Supporting Information.

The transformation of imidacloprid and thiacloprid and the formation of known metabolites were tested. Imidacloprid is one of the most widely used neonicotinoids,[1] and thiacloprid is structurally similar but contains a cyano group rather than a nitro group as the insecticidal pharmacophore. Treatments consisted of (1) unsterilized duckweed in imidacloprid or thiacloprid-spiked medium, (2) microbes separated from unsterilized duckweed (method details in the Supporting Information) in imidacloprid-spiked medium, and (3) duckweed, sterilized following established procedures[45,46] with minor modifications, in medium with both imidacloprid and thiacloprid. The initial imidacloprid concentrations (10−18.6 μg/L) and thiacloprid concentrations (2.4−6.0 μg/L) in this study were chosen to be within the range of reported worldwide surface water neonicotinoid concentrations.[12]

In addition, unsterilized duckweed was tested in parallel with another unsterilized aquatic plant ("coontail", *C. demersum*) and unfiltered pond water from the same duckweed source pond. Treatments consisted of (1) coontail and pond water, (2) duckweed and pond water, and (3) pond water only. Pond water for all treatments had macrophytes removed and was spiked with imidacloprid. Full details about the experimental design, growth conditions, sampling, sorption testing, and extraction for all experiments are provided in the Supporting Information.

Analytical Methods and Data Analysis. Samples were analyzed via high-performance liquid chromatography (Agilent 1260) coupled to a triple-quadrupole mass spectrometer (LC−MS/MS; Agilent 6460 Triple Quadrupole MS with MassHunter, version B.07.00) operating in multiple-reaction monitoring (MRM) positive mode and electrospray ionization (ESI) as established in our prior work[14,47] (Table S1). Isotopically labeled imidacloprid (d_4) was used as an internal standard (for medium samples) or surrogate (for plant tissue extraction samples). A d_4-imidacloprid-normalized external calibration curve was used to account for surrogate recovery and matrix effects during ionization, unless noted otherwise in the Supporting Information. Full analytical and quality assurance details are provided in the Supporting Information.

GraphPad Prism 8 (GraphPad, La Jolla, CA) was used for all statistics. Matched-pairs *t* tests assessed differences between treatments and controls ($\alpha = 0.05$). Departure from the linear null slope at the 95% confidence interval determined if a significant change in compound concentration occurred over time.

RESULTS AND DISCUSSION

Neonicotinoid Removal and Metabolite Formation via Unsterilized Duckweed. Unsterilized duckweed removed imidacloprid and thiacloprid (Figure 1a) from the liquid medium at significant rates ($p < 0.0001$ for both), whereas no significant losses occurred in the medium of the abiotic negative controls [$p = 0.12$ and $p = 0.29$, respectively (Figure S6)]. Thus, ongoing neonicotinoid removal was driven by biotic processes, with sorption of 2.6−2.7 μg/L to duckweed tissue occurring in the first day (Figure S7). First-order removal rates in the medium for imidacloprid [for $C_0 = 18.6$ μg/L; $k \pm$ standard error = 0.63 ± 0.07 day^{-1} (Figure S4)] and thiacloprid [for $C_0 = 2.4$ μg/L; $k = 0.62 \pm 0.05$ day^{-1} (Figure S4)] were statistically indistinguishable [$p = 0.21$ (Figure S3)]. The similar rates suggest that the chemical differences between the two compounds, including nitro versus cyano pharmacophores, do not significantly impact the removal rate.

Following imidacloprid exposure, the unsterilized duckweed treatment generated small quantities of known imidacloprid metabolites in the medium (no metabolite exceeding 6% of the initial imidacloprid mass at any time point) and in the plant tissue (no metabolite exceeding 0.1% of the initial imidacloprid mass at any time point): desnitro-imidacloprid, imidacloprid urea, and 6-CNA (Figure 1b). These metabolites may be part of the duckweed-facilitated imidacloprid degradation pathway. Previously documented pathways for imidacloprid degradation include bacterial transformation from imidacloprid to desnitro-imidacloprid to imidacloprid urea to 6-CNA to mineralized end products (Figure 1c), which could be occurring here.[19,23,29,48−52] Imidacloprid urea and 6-CNA, both of which are less insecticidal than the parent imidacloprid,[53,54] were present in the treatment but not the abiotic control. The lack of substantial quantities of metabolites that are more vertebrate-toxic than the parent neonicotinoid (i.e., desnitro-imidacloprid) in the unsterilized duckweed treatment, coupled with no measured imidacloprid olefin (more insecticidal than imidacloprid),[54,55] has potentially positive implications for duckweed phytoremediation.

The previously identified, less toxic metabolites thiacloprid amide and 6-CNA were present in the unsterilized thiacloprid duckweed treatment. Thiacloprid amide in the unsterilized

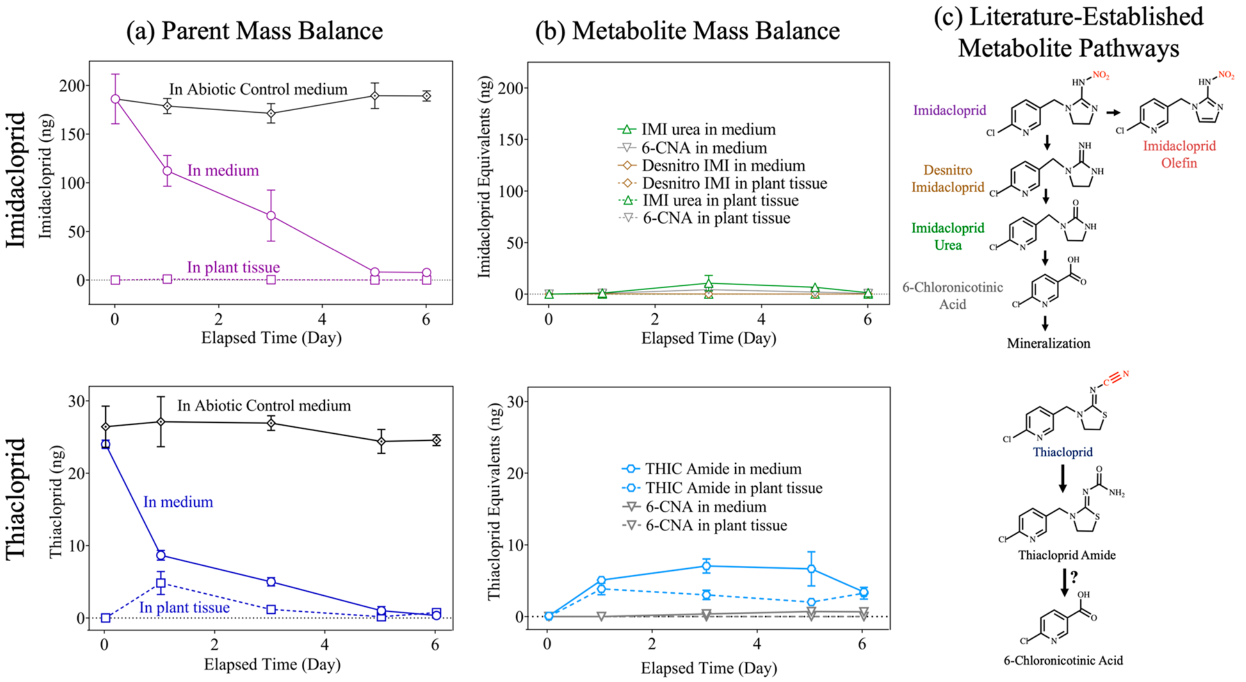

Figure 1. (a) Mass balances of parent imidacloprid and thiacloprid neonicotinoids in the unsterilized duckweed treatments and abiotic controls. Removal of each neonicotinoid represents synergistic duckweed−microbial degradation. Neither imidacloprid nor thiacloprid occurred at consistently high levels in the plant tissue, suggesting overall degradation of the parent compound. Initial conditions: 18.6 μg/L imidacloprid (186 ng) and 2.4 μg/L thiacloprid (24 ng). (b) Measured metabolites in hydroponic medium and plant tissue, reported as molar equivalents of the parent compound. Imidacloprid metabolite results are also presented with an expanded *y*-axis in Figure S5; 5% and 33% of the imidacloprid and thiacloprid mass balances, respectively, were closed on day 6, suggesting the likely presence of other metabolites and/or rapid transformation of these metabolites. (c) Literature-established microbial degradation pathways. The insecticidal pharmacophore is colored red. For imidacloprid, no imidacloprid olefin was found in the treatment or control; the other metabolites were detected. For thiacloprid, both metabolites occurred in the duckweed treatment. Error bars represent the standard error ($n = 3$) with nonvisible error bars obscured by data symbols. Evapotranspiration is accounted for via box weight measurements.

duckweed treatment medium (Figure 1b) represented ≤30% of the initial thiacloprid mass at a given time point. Thiacloprid amide was also present on and/or in the duckweed plant tissue [≤16% of the initial mass (Figure 1b)] but not in the abiotic control medium (Figure S6b), suggesting a biological origin. Thiacloprid amide is less insecticidal than thiacloprid[56] and less acutely vertebrate-toxic than thiacloprid.[57] 6-CNA was found in only the duckweed treatment medium below the LLD (Figure 1b) and only in a single replicate of the abiotic control medium near the LLD (Figure S6b). A small amount of 6-CNA may be abiotically generated from thiacloprid (e.g., by hydroxyl radicals[40,58,59]) in the abiotic control before further degradation.[60] The presence of 6-CNA in only a single replicate at one time point of the abiotic control along with the lack of significant thiacloprid removal in the abiotic control (Figure S6b) indicates that abiotic 6-CNA production is not a major pathway. In contrast, 6-CNA was detected in almost half of all thiacloprid duckweed treatment plant medium replicates, in small quantities. 6-CNA may therefore be a minor or rapidly transformed intermediate metabolite of thiacloprid transformation by the duckweed system. Taken together, the generation of thiacloprid amide and 6-CNA in the duckweed treatment, coupled with the removal of thiacloprid, represents a likely overall decrease in toxicity.

Using the selected commercially available metabolites, 5% and 33% of the initial imidacloprid and thiacloprid masses, respectively, were accounted for at the end of the experiment in the unsterilized duckweed treatments. Therefore, further degradation of the measured metabolites and/or other degradation pathways with unknown toxicological outcomes likely occurred in these systems.

Duckweed−Microbial Synergy of the Neonicotinoid Transformation. Degradation of imidacloprid and thiacloprid by the duckweed−microbial system appears to be synergistic. Surface-sterilized duckweed alone (Figure 2) did not significantly remove imidacloprid ($p = 0.13$ for 112 ng at time zero) or thiacloprid [$p = 0.30$ when the $t = 0$ data point is neglected; removal during the first day is likely sorption to duckweed tissue (Figure S7)]. Microbes that had migrated off the duckweed into the liquid medium, which had demonstrated viability and growth [i.e., in parallel plating experiments (Figure S8b)], did not significantly remove imidacloprid in the absence of duckweed [$p = 0.84$ for a nominal C_0 of 10 μg/L (Figure S8a)]. Additionally, unsterilized pond water from the same duckweed source pond, representing the native planktonic microbial community in the pond, did not significantly remove imidacloprid [$p = 0.06$ for a C_0 of 9.3 μg/L representing 559 ng of imidacloprid (Figure 3)]. Thus, degradation of the two neonicotinoids tested required the presence of duckweed and its associated microbial community, suggesting that the duckweed−microbial degradation process is synergistic.

Additionally, the duckweed−microbial synergy appears to be distinct, as not all unsterilized aquatic plants that we tested were able to degrade neonicotinoids. Locally collected *Typha* sp. ("cattail") exposed to imidacloprid-spiked medium did not significantly remove imidacloprid over 7 days [$p = 0.31$, for 158 μg of imidacloprid at time zero (Figure S9)]. Although the

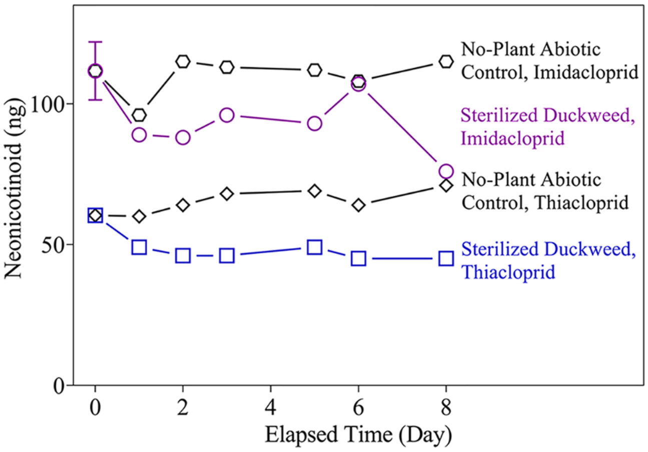

Figure 2. Removal of thiacloprid or imidacloprid from liquid 0.5× Schenk and Hildebrandt hydroponic medium spiked with both neonicotinoids, pH 6.0, by surface-sterilized duckweed. The surface-sterilized duckweed did not significantly remove imidacloprid (p = 0.13) or thiacloprid (p = 0.30 when the t = 0 data point is neglected). Removal during the first day is likely sorption to duckweed tissue, as suggested in a separate duckweed neonicotinoid sorption experiment (Figure S7). Evapotranspiration was accounted for through box weight measurements. At time zero, 112 ng (11.2 μg/L) of imidacloprid and 60 ng (6.0 μg/L) of thiacloprid were present in the medium. Error bars for time zero represent the standard error, with nonvisible error bars obscured by the data symbols (n = 1, except n = 3 for time zero).

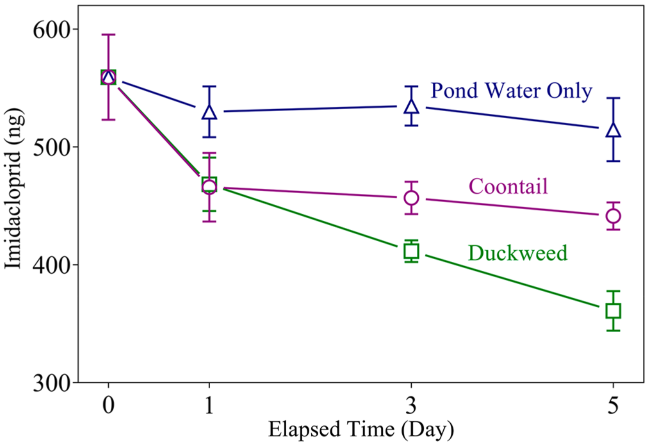

Figure 3. Removal of imidacloprid (C_0 = 9.3 μg/L, 559 ng of IMI) from 60 mL of pond water by unsterilized duckweed, unsterilized coontail (*C. demersum*), and pond water. The unsterilized pond water with a native microbial population did not yield significant imidacloprid removal (p = 0.06), whereas the unsterilized duckweed treatment generated significant, ongoing removal ($p < 0.0001$). Removal in the coontail treatment was not significant (p = 0.09) when the losses during the first day of data, attributed to initial biomass sorption, are neglected. Volume loss due to sampling was factored into the results; evapotranspiration was not measured. Error bars represent the standard error (n = 4).

exact *Typha* species was not identified, this result corresponds to the finding of Main et al.[30] that *T. latifolia* was associated with a higher neonicotinoid detection frequency and a higher concentration in shallow marsh water. Additionally, another macrophyte (*C. demersum*, "coontail") collected from the same pond as the duckweed did not remove imidacloprid beyond initial biomass sorption [p = 0.09 (Figure 3)]. The 17% loss of imidacloprid mass in the coontail treatment medium between days 0 and 1 is similar to the 21% sorption loss in the first day of the test of sorption of imidacloprid to duckweed (Figure S7).

Our results demonstrating pollutant removal with duckweed—microbial combinations and decreased or no pollutant removal with surface-sterilized duckweed are consistent with evidence from prior literature. For example, 4-*tert*-butylphenol was degraded by a duckweed (*Spirodela polyrrhiza*)-associated rhizosphere bacterium stimulated by duckweed root exudates;[33] however, duckweed alone did not degrade the compound. Similarly, a symbiotic relationship between *Lemna aoukikusa* and a rhizosphere bacterium degraded phenol.[34] A combination of predominately *Landoltia punctata* and *Lemna minor* duckweed species enhanced microbial ibuprofen removal.[31] Similar relationships exist that enable transformation of inorganic pollutants; e.g., arsenite oxidation occurred only when *Wolffia australiana* duckweed was grown with its associated bacterial community and did not occur with sterile duckweed.[32] This work is the first documented instance of a duckweed—microbial synergy that degrades neonicotinoids.

Environmental Implications. Duckweed, prevalent in aquatic ecosystems worldwide, may play an important environmental role in degrading insect-toxic neonicotinoids and generating less-insecticidal and less vertebrate-toxic metabolites. The full pathways and toxicological impact of imidacloprid and thiacloprid processing by duckweed and characterization of the duckweed-associated microbial communities remain to be established. This work, however, is consistent with and provides a mechanistic basis for a previously published field correlation[30] between the dominant aquatic plant type and the wetland water neonicotinoid detection frequency and concentration. Further research is needed to determine the impact of these results at field scale. We estimate using our laboratory removal rate with full duckweed coverage that under conditions similar to those of the small farm pond where the duckweed and water were collected, approximately 0.5% (quiescent conditions), 14% (stratified conditions), or 33% (completely mixed conditions) of the imidacloprid mass in the pond could be removed after 5 days (calculation assumptions described in the Supporting Information). Similar results for thiacloprid are expected on the basis of the indistinguishable laboratory removal rates for imidacloprid and thiacloprid we report here (Figure S3). The greater surface area:depth ratio in the pond (151 m^2 of surface area/m of depth) versus the experimental conditions (0.16 m^2 of surface area/m of depth) may yield levels of field removal higher than these estimates. This novel synergistic duckweed—microbial pollutant processing therefore may contribute to regulating the toxicological impact of neonicotinoids in environmental waters, with implications for aquatic life as well as human exposure through drinking water sources.[14,47] This work can also inform engineered natural treatment systems that contain aquatic plants, such as constructed treatment wetlands. The impact of aquatic macrophyte surface coverage, however, must be balanced with other relevant removal processes (e.g., photolysis of neonicotinoids[61,62]) for trace organic contaminants in treatment wetlands.[63] Additionally, future research is needed to further characterize the microbial community of duckweed plants and the neonicotinoid metabolites generated to fully understand and advantageously use the discovered synergistic pollutant transformation potential.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.9b00638.

Additional method details and additional detailed data, results, and analysis in figures, tables, and text (PDF)

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Notes

The authors declare no competing financial interest.

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