Urbanization effects on wild bee carbon and nitrogen stable isotope ratios in the Paris region

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ABSTRACT

Urban environments have been shown to have profound, yet still poorly understood effects on key ecosystem processes such as carbon (C) and nitrogen (N) cycling, as well as for key aspects of organism ecology, such as community assembly and network interactions. The study of natural abundances of C and N stable isotopes, $^{13}$C and $^{15}$N, can help infer mechanistic hypotheses on ecosystem processes at play in urban environments. No existing study has investigated whether a visible urban effect on soil and plant C and N biogeochemistry is being transferred to the animal compartment of urban ecosystems through trophic interactions. Here, we report $^{13}$C and $^{15}$N natural abundance values measured on three pollinating species of wild bees (Lasioglossum laticeps, Lasioglossum morio, Lasioglossum nitidulum) sampled in 12 locations along an urbanization gradient in the Île-de-France region (Paris area, France). Our objective was to test whether already recorded isotopic patterns in urban soils and plants in the Paris area would also be visible in pollinators, and to reflect on whether this would be indicative of an urban isotopic signal being transferred to pollinators. Results indicate a significant effect of urbanization on the $\delta^{13}$C and $\delta^{15}$N for all three bee species, with enrichment for both $^{13}$C and $^{15}$N linked to increased urbanization. This could be linked to an urban imprint on the diverse plants on which pollinating insects forage on in the Paris area, or to other factors linked to the physiology and foraging strategies of insects. A better understanding of the drivers influencing pollinator $\delta^{13}$C and $\delta^{15}$N linked to increased urbanization could help better understand urban biogeochemistry and trophic chains, as well as elucidate the origin of specimens and the migratory capacities of bee species, an important yet still elusive question considering the increase of habitat fragmentation.

1. Introduction

Urban environments have been shown to have profound, yet still poorly understood effects on key ecosystem processes such as carbon (C) and nitrogen (N) cycling (Kaye et al., 2006; Lorenz and Lal, 2009; Pouyat et al., 2009). In urban areas, C and N cycling can be influenced by numerous interacting factors including high atmospheric CO$_2$ concentration, high levels of atmospheric N deposition, increased surface temperatures, pollutants, surface sealing, hydrologic changes, increased presence of non-native organisms, and different intentional management practices (McDonnell and Pickett, 1990; Kaye et al., 2006; Grimm et al., 2008; Hahs and Evans, 2015; Alberti et al., 2017). Similarly, urban habitats are known to impose several constraints to organisms (McDonnell and Hahs, 2015), but the mechanistic understanding of organisms’ responses to these constraints is still lacking even for some key aspect of their ecology. For bee communities, for instance, the loss of habitat through urbanization has been shown to reduce bee abundance and richness (Fortel et al., 2014; Geslin et al., 2016a), but counter examples have also been reported (Baldock et al., 2015, 2019; Hall et al., 2017). Despite significant progress in urban ecological research over the last decades, many unknowns remain as to how urban land-use influences key ecosystem processes. This is the case for C and N cycling, as well as for key aspects of organism ecology, such as community assembly and network interactions (Carreiro and Tripler,
The study of natural abundances of C and N stable isotopes, $^{13}$C and $^{15}$N, can help infer mechanistic hypotheses on ecosystem processes. Stable isotopes can act as "ecological recorders" (West et al., 2006) and integrate information on the sources of elements, as well as the transformations they undergo while they cycle in ecosystems, during trophic interactions and other biogeochemical processes (e.g., Peterson and Fry, 1987; Högb erg, 1997; Perkins et al., 2014; Craine et al., 2015). As such, they have already proven useful, albeit arguably still underused, tools in urban ecology (Pataki et al., 2005).

Stable isotope analyses have been used both to trace the assimilation of anthropogenic compounds in ecosystems and biological responses to urban conditions. For instance, the assimilation by urban grasses of fossil fuel CO$_2$, strongly depleted in $^{13}$C compared to background levels, has been analyzed in Paris and Los Angeles (Lichtfouse et al., 2003; Wang and Pataki, 2010). Also, nitrogen compounds produced during combustion processes tend to be enriched in $^{15}$N when compared to the rest of the basin. This result is consistent with several reports indicating enriched $^{15}$N values for deposited N in other urban environments (e.g., Ammann et al., 1999; Pearson et al., 2000; Widory, 2007).

For four tree species growing in parks of New York City, Falka-Raymond et al. (2014) report higher foliar $^{13}$C (e.g., less depleted in $^{13}$C) values than in rural areas, likely reflecting reduced stomatal conductance in response to water stress (water-use efficiency – WUE – strategy). In Los Angeles, Wang and Pataki (2012) found a strong relation between soil moisture and grass $^{13}$C: grasses were more depleted in $^{13}$C as soil moisture increased. A similar result was found for roadside trees in Kyoto by Kagotani et al. (2013), who suggest that isotopic effects linked to WUE could compensate the isotopic imprint of fossil fuel-derived CO$_2$ on the organic matter produced by trees. In Paris, enrichment in $^{13}$C and $^{15}$N has been reported for both street soils and trees, when compared to remote counterparts (Rankovic, 2016).

While data on soils and plants starts accumulating, such results are still scarce for animals. Fujita and Koike (2009) used $^{13}$C and $^{15}$N analyses to follow the transport of nutrients by birds along an urban-rural transect from Yokohama to a mountainous forest-dominated landscape. Smith et al. (2019) gathered data on stable lead (Pb) isotopes in and around Vancouver to study the contamination of bees and honey by urban pollution. Taki et al. (2017) compared stable nitrogen and carbon isotope ratios in Apis ceranae populations (Asian honeybees) across several land uses over an area covering most of Japan. They found urban areas to negatively affect bee $^{13}$C values and positively affect bee $^{15}$N: for $^{13}$C, they hypothesized a possible effect of fossil fuel CO$_2$, while no hypothesis was clearly being put forth for $^{15}$N. However, no existing study has investigated whether a visible urban effect on soil and plant C and N biogeochemistry is being transferred to the animal compartment of urban ecosystems through trophic interactions. Developing this line of investigation would increase our overall understanding of urban C and N biogeochemistry, but also help better understand urban community ecology, through, e.g., the study of food chain length, trophic structure, and even life-history traits (Broso et al., 2009; Perkins et al., 2014).

Wild bees are good candidates to explore such questions. As they are strictly phytophagous organisms at all stages of their life cycle, the $^{13}$C and $^{15}$N values of adult individuals should reflect the $^{13}$C and $^{15}$N of flower-derived (nectar and pollen) food that they received as larvae, and the $^{13}$C and $^{15}$N of flower-derived resources that they forage on as adults. The $^{13}$C and $^{15}$N of individual wild pollinating insects should closely reflect the $^{13}$C and $^{15}$N of their plant diet, notwithstanding other factors (e.g., physiological) that can have more moderate effects (Taki et al., 2017). Furthermore, urban environments are known to strongly influence pollinator communities (Deguines et al., 2016; Geslin et al., 2016a), but it is still hard to study how urban pollinators forage and move inside urban landscapes. This is even more difficult since radio-tracking tools work for larger species, while most insects are very small in size and have limited dispersal abilities. Isotopic data on urban pollinators can thus help explore aspects of their life-history traits that are not easily accessible to investigators.

Here, we report $^{13}$C and $^{15}$N natural abundance values measured on three pollinating species of wild bees (Lasiosglossum laticeps, Lasiosglossum morio, and Lasiosglossum nitidulum) sampled along an urbanization gradient in the Ile-de-France region (Paris area, France). Our objective was to test whether already recorded isotopic patterns in urban soils and plants in the Paris area would also be visible in pollinators, and to reflect on whether this would be indicative of an urban isotopic signal transfer to pollinators. Our hypothesis was that if the soil and plant $^{13}$C and $^{15}$N patterns reported in the Paris area (Rankovic, 2016) were transferred to pollinators, then similar changes should be observed in insect $^{13}$C and $^{15}$N with increasing levels of urbanization, represented by the impervious surface proportion in the landscape (Geslin et al., 2016a). To discuss the likelihood of different hypotheses that could explain these patterns, we also used flora inventories data to test for species composition changes that could affect insect $^{13}$C and $^{15}$N values: we especially looked for the presence of C4 (and CAM) and N-fixating plant species, which could respectively enrich insects in $^{13}$C compared to C3 species and bring their $^{15}$N closer to 0‰ compared to non-N-fixating species (Peterson and Fry, 1987; Högb erg, 1997).

2. Materials and methods

2.1. Study sites

Our study was located in Paris and its region (Ile-de-France, France), the most populated region of France with more than 11 million residents (INSEE, 2013). We chose twelve sites located at least 1 km from each other according to their proportion of impervious surfaces within a 500 m radius, in order to cover a gradient of urbanization (Fig. 1). Impervious surfaces coverage, which expresses the proportion of an area covered by buildings, parking, pavements and roads (Marzluff, 2005; Sattler et al., 2010; Liu et al., 2014), has been acknowledged as a key parameter to quantify the urbanization level (Arnold and Gibbons, 1996; Mckinney, 2006, 2008) and is increasingly used in studies linking urbanization and bee communities (Ahrné et al., 2009; Bannaska-Cibicka and Zmihorski, 2012; Geslin et al., 2013, 2016a; Fortel et al., 2014). Among our 12 sites, the proportion of impervious surfaces ranged from 0.06 to 64.31% (mean = 25.25% ± 24.58) with the most urbanized site being located in the center of Paris (Table 1). Three sites were dominated by agricultural landscapes, Grignon (48°50'29.60" / 1°56'24.92"), Ambésis (48°43'44.36" / 1°58'22.54"), and Bezianleu (48°17'07.23" / 2°48'45.24"), while three sites were dominated by semi-natural habitats, St Lambert (48°44'16.28" / 2°01'05.66"), Foljuf (48°16'54.84" / 2°39'58.88"), and Versailles I (48°48'20.74" / 2°05'39.14"). Three sites were dominated by peri-urban landscapes: two sites were located in the town hall garden of small towns, Bonnelles (48°37'07.21" / 2°01'43.59") and Nemours (48°15'54.70" / 2°41'09.80"), and one site in the graveyard of Versailles; Versailles II (48°47'27.00" / 2°08'21.20"). Finally, three sites were located in densely urbanized landscapes in Paris, in public parks: Paris I (48°50'41.93" / 2°20'18.94"), Paris II (48°50'38.15" / 2°21'40.54"), and Paris III (48°49'13.90" / 2°19'52.03").

At each site, we worked on a 500 m radius because it encompasses the estimated mean flight distances for most species of wild bees (Gathmann and Tschamrkte, 2002; Araújo et al., 2004; Zurbuch et al., 2010), and it is widely used in studies linking bee assemblages and landscape composition (Holzschuh et al., 2008; Somsse et al., 2014), especially within urbanization contexts (Ahrné et al., 2009; Bannaska-Cibicka and Zmihorski, 2012; Geslin et al., 2013, 2016a; Fortel et al., 2014).
We captured bee individuals during the spring and summer of 2011 using colored pan traps. These traps are bowls (radius = 7.25 cm, depth = 5 cm) painted with blue, white, or yellow UV-reflecting paints (Westphal et al., 2008). A set of three pan traps (one of each color) was installed in each experimental site. Each pan trap was mounted on a wooden pole, filled with 400 ml of water mixed with a few drops of detergent (surfactant, dishwashing liquid, Colgate-Palmolive Company, New York, NY, USA, same bottle used for all collection sites), and laid in the field for 24 h. Those traps attract bees as they mimic flowers, and bees drown themselves in the liquid.

Once captured, insects were stored in 70% ethanol before being rinsed, dried, mounted, and identified by professional taxonomists (acknowledgement). Ethanol is a good preservative for insects, and it is the isotope ratio of the sample and a standard, calculated as:

$$\delta(%) = \frac{[(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}] \times 1000$$

where $R_{\text{sample}}$ is the isotope ratio ($^{13}C/^{12}C$ and $^{15}N/^{14}N$ for C and N, respectively) of the sample and $R_{\text{standard}}$ the isotope ratio of the standard. C and N isotopic ratios are expressed with respect to the V-PDB (Vienna Pee Dee Belemnita, Craig, 1957) and to air nitrogen (Mariotti, 1983; Mariotti et al., 1984), respectively. The measured values are available in Supplementary Material 2.

### Table 1

Impervious surface (in %) at the twelve study sites, and number of sampled individuals for the three bee species.

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</thead>
<tbody>
<tr>
<td>Impervious surfaces</td>
<td>0.061</td>
<td>0.5</td>
<td>1.33</td>
<td>5.55</td>
<td>7.01</td>
<td>9.33</td>
<td>27.38</td>
<td>33.09</td>
<td>40.57</td>
<td>53.78</td>
<td>60.12</td>
<td>64.31</td>
<td>64.31</td>
<td>64.31</td>
</tr>
<tr>
<td>L. nitidulum</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>L. morio</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>L. laticeps</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>5</td>
<td>5</td>
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2.3. Composition of the local entomophilous flowering community

At each of the 12 experimental sites, we also estimated the composition of plant communities. To do so, we sampled five 2 × 5 m² plots located at increasing distances of the pan traps. The first of the five plots was contiguous to the three pan traps. The other four plots were placed at 50, 100, 150, and 200 m in a randomly chosen direction (north, east, south or west). Plots were divided into 10 cells of 1 m², and the presence/absence of each plant species for each cell was noted. By pooling the five plots at each site, we estimated the abundance of each flowering plant species with an index ranging from 1 to 50. We focused on entomophilous flowering plants species given their importance to bees, which were identified to the species level. We estimated the abundance of Fabaceae species, as members of the Fabaceae family can fix atmospheric N₂ and their δ¹⁵N tends to be closer to 0‰ compared to other species growing in the same surroundings (Högberg, 1997). We also looked for C₄ and CAM species but we only found C₃ species among the entomophilous plant species identified. Its nevertheless worth noting that a corn field (anemophilous, C₄) was present around one of the agriculture-dominated sites (Grignon). We used TAXREF (v12.0), the French Taxonomic Reference for the flora and fauna of metropolitan France and overseas (http://inpn.mnhn.fr/telechargement/referentielEspece/referentielTaxo). The list of species is available in Supplementary Material 1.

2.4. C and N contents and isotope ratios

All sampled individuals were analyzed for δ¹³C and δ¹⁵N. Insects were lyophilized for 48 h and stored in a desiccator until analysis. The whole body of each individual was then ground with pestle and mortar, and about 1 mg of powdered sample was weighed in a tin capsule and analyzed by EA-IRMS (vario Pyro cube, Elementar, Hanau, Germany, coupled with an IsoPrime, Gvi, Stockport, UK) (e.g., Taki et al., 2017).

For isotopic values, results are expressed using the usual delta notation that allows expressing the content in $^{13}$C or $^{15}$N as the relative difference between the isotopic ratio of the sample and a standard, calculated as:

$$\delta(‰) = \frac{([R_{\text{sample}} – R_{\text{standard}}]/R_{\text{standard}}) \times 1000}$$

where $R_{\text{sample}}$ is the isotope ratio ($^{13}$C/$^{12}$C and $^{15}$N/$^{14}$N for C and N, respectively) of the sample and $R_{\text{standard}}$ the isotope ratio of the standard. C and N isotopic ratios are expressed with respect to the V-PDB (Vienna Pee Dee Belemnita, Craig, 1957) and to air nitrogen (Mariotti, 1983; Mariotti et al., 1984), respectively. The measured values are available in Supplementary Material 2.
2.5. Data analysis

Statistical analyses were performed with the R software (R Development Core Team, 2013). The effects of the proportion of impervious surface on the measured δ13C and δ15N was analyzed with a linear model. Models were run either for each species separately or for all three species, in the latter case including species as an additional explanatory factor. The p-values were obtained with a Fisher test. We systematically checked for normality of residuals and data were log transformed in case of non-normal variance (Anova) using a Fisher test. All species we studied and leads to an increase in their δ15N values.

3. Results

We collected 45 individuals of L. morio, 34 of L. nitidulum and 28 of L. laticeps along our urbanization gradient (Table 1).

Regarding the δ13C of bees, an increase was observed for all three bee species with the increase of impervious surfaces proportion around sites (F = 22.46, P < 0.001) (Table 2, Fig. 2). There was no significant effect of the interaction between species and the proportion of impervious surface. A significant increase of δ13C was observed with the proportion of impervious surfaces for individuals of L. morio (F = 9.72, P = 0.013) and L. laticeps (F = 21.98, P < 0.001), but this relation was however not significant for L. nitidulum (F = 1.72, P = 0.19; Table 2, Fig. 2). For the three sites that had respectively the smallest (average of 0.6%) and highest amount of impervious surface (average of 59.3%), the average δ15N values were respectively 1.6‰ and 6.2‰, a difference of 4.6‰.

Table 2

Results of ANOVA models testing for the effects of the proportion of impervious surfaces on the measured δ13N and δ13C. Models were run either for all species pooled together (including a species effect, and a Fabaceae presence effect), or for each species separately (including a Fabaceae presence effect). Df: degrees of freedom, significance levels: ***: P < 0.001; **: P < 0.01; *: P < 0.05).

<table>
<thead>
<tr>
<th>Factor</th>
<th>Df</th>
<th>Mean Square</th>
<th>F Value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All species</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>δ13N Species</td>
<td>2</td>
<td>44.72</td>
<td>7.80</td>
<td>&lt;0.001  ***</td>
</tr>
<tr>
<td>Fabaceae</td>
<td>1</td>
<td>34.43</td>
<td>6.01</td>
<td>0.016   *</td>
</tr>
<tr>
<td>Impervious surfaces</td>
<td>1</td>
<td>273.77</td>
<td>47.76</td>
<td>&lt;0.001  ***</td>
</tr>
<tr>
<td>δ13C Species</td>
<td>2</td>
<td>7.03</td>
<td>5.62</td>
<td>0.004   **</td>
</tr>
<tr>
<td>Impervious surfaces</td>
<td>1</td>
<td>27.28</td>
<td>21.81</td>
<td>&lt;0.001  ***</td>
</tr>
<tr>
<td>Lasioglossum laticeps δ13N Fabaceae</td>
<td>1</td>
<td>48.02</td>
<td>8.95</td>
<td>0.006   **</td>
</tr>
<tr>
<td>Impervious surfaces</td>
<td>1</td>
<td>137.17</td>
<td>25.58</td>
<td>&lt;0.001  ***</td>
</tr>
<tr>
<td>δ13C Impervious surfaces</td>
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<td>21.95</td>
<td>21.98</td>
<td>&lt;0.001  ***</td>
</tr>
<tr>
<td>Lasioglossum morio δ13N Fabaceae</td>
<td>1</td>
<td>1.30</td>
<td>0.20</td>
<td>0.65</td>
</tr>
<tr>
<td>Impervious surfaces</td>
<td>1</td>
<td>121.23</td>
<td>18.86</td>
<td>&lt;0.001  ***</td>
</tr>
<tr>
<td>δ13C Impervious surfaces</td>
<td>1</td>
<td>9.5</td>
<td>9.72</td>
<td>0.013   *</td>
</tr>
<tr>
<td>Lasioglossum nitidulum δ13N Fabaceae</td>
<td>1</td>
<td>7.75</td>
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<td>0.21</td>
</tr>
<tr>
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<td>25.08</td>
<td>5.24</td>
<td>0.028   *</td>
</tr>
<tr>
<td>δ13C Impervious surfaces</td>
<td>1</td>
<td>1.91</td>
<td>1.72</td>
<td>0.19</td>
</tr>
</tbody>
</table>

4. Discussion

4.1. A significant effect of urbanization on the δ13C and δ15N of wild bees

Our results show a significant effect of urbanization on the stable isotope content of bees. Both the δ13C and the δ15N increased with the proportion of impervious surfaces in a 500 m radius around specimens’ collection point. The variation in stable isotope abundance explained by the proportion of impervious surfaces was even higher than that explained by the difference between species (3.9 and 6.1 times higher for δ13C and δ15N, respectively). Isotopic response to the urbanization gradient slightly varied among species (Figs. 2 and 3), with a stronger effect on L. laticeps and a weaker effect on L. nitidulum. However, this difference was not significant in the analyses (as revealed by the non-significant interaction term either for the δ13C or the δ15N). The urban environment thus seems to induce similar effects on the three Lasioglossum species we studied and leads to an increase in their δ13C and δ15N.

Furthermore, while samples were collected in the same region and within the same season, the difference in values observed at the extremes of the gradient were quantitatively important for both δ13C and δ15N. For δ13C, the difference between the least and most urbanized sites, was of the order of magnitude (1.8‰) of the differences found between rural and urban populations of kit foxes (Newsome et al., 2010) and Tetramorium ants (Penick Clint et al., 2015), and between human populations in Europe and the United States (Bol and Pfieger, 2002). For δ15N, the difference is also high (4.6‰): it is of the same order of magnitude, for instance, as the experimental values reported in the literature for trophic level changes inside plant-invertebrate food chains spanning four trophic levels (maximum Δ of 4.81‰) (Perkins et al., 2014). For the same bee species, living in the same region, urbanization was thus related to marked patterns for δ13C and δ15N values, and we discuss possible causes below.

4.2. Urban environmental factors that could explain $\delta^{13}C$ and $\delta^{15}N$ patterns

The increase of bee $\delta^{13}C$ following the increase of impervious surfaces could be reflecting the effects of urban conditions on plants. As recalled in the introduction, the assimilation of fossil fuel CO$_2$, strongly depleted in $^{13}C$ compared to background levels, has been reported for grasses in Paris and Los Angeles (Lichtfouse et al., 2003; Wang and Pataki, 2010). However, our results indicate an opposite trend, of $^{13}C$ enrichment rather than depletion with increased urbanization. This could reflect a plant response to increased water scarcity with the increasing amount of impervious surfaces, which leads to less water infiltration in soils and an increased urban heat island effect (increased temperature). This was observed for grass species in the Los Angeles area (Wang and Pataki, 2010), and tree species in Kyoto and New York City (Kagotani et al., 2013; Falxa-Raymond et al., 2014). Kagotani et al. (2013) explicitly proposed that isotopic effects linked to WUE could compensate the isotopic imprint of fossil fuel-derived CO$_2$ on the organic matter produced by trees. In Paris, Rankovic (2016) found that urban conditions result in an enrichment of soil and tree $\delta^{13}C$ and $\delta^{15}N$. 

Fig. 2. Bee $\delta^{13}C$ for all three studied species following the percentage of impervious surface around capture sites.

Fig. 3. Bee $\delta^{15}N$ for all three studied species following the percentage of impervious surface around capture sites.
hypothesized that it was linked to the response of trees to urban drought (documented by David et al., 2018) and the subsequent transfer of $^{13}$C-enriched tree root organic matter to soil. The urbanization effects on $^{13}$C observed for wild bees are of a similar order of magnitude as the hypothesized drought effect reported for plants. If this urban climate-related effect on plant $^{15}$N was widespread across urban landscapes, the observed pattern on bee $^{13}$C could be explained by a transfer of this signal from urban plants to their consumers. Urban areas also seem to favor plants with C4 photosynthesis (Duffy and Chown, 2016). This specific photosynthesis pathway has an impact on the $^{13}$C of plants, which reflects on their nectar consumers (Peterson and Fry, 1987; Proctor et al., 1996). In our case, no C4 plants were found in the plant communities of collection sites, thus excluding this hypothesis to explain the observed pattern in bee $^{13}$C.

The urbanization effect observed on $^{15}$N could also be explained by urban biogeochemical factors. Wang and Pataki (2010) report that annual grasses in the mostly urbanized areas of the Los Angeles basin were strongly enriched in $^{15}$N when compared to the rest of the basin. This result is consistent with several reports indicating enriched $^{15}$N values for deposited N in other urban environments (e.g., Ammann et al., 1999; Pearson et al., 2000; Widory, 2007). In Paris, Widory (2007) found that atmospheric particulate N (ammonium and nitrate) had a $^{15}$N as high as 10‰ on a yearly average. Direct measurements from vehicle exhaust yielded a $^{15}$N for particulate N of 3.9–5.6‰ (Widory, 2007). In Paris, Rankovic (2016) reported an enrichment (age-related enrichment for urban ecosystems, and also compared to peri-urban sites under the same species) of street soil and tree $^{15}$N (total soil N and foliar N) values and suggested that it was linked to the entrance of strongly $^{15}$N-enriched urban reactive N in soil-tree systems and its subsequent transformations in soils (notably, high rates of nitrification and denitrification). The difference between tree foliar $^{15}$N measured in urban areas and in streets was of about 5‰, with urban values being close to 7‰, which is similar to the orders of magnitude observed here for pollinators. As for $^{13}$C, if urbanization biogeochemical effects leading to increased plant $^{15}$N are widespread, it could be reflected in wild pollinator $^{15}$N, as observed here.

4.3. Factors linked to bee physiology and foraging that could explain $^{13}$C and $^{15}$N patterns

Besides plant-insect transmission, other factors could also explain the patterns we observed, more related to animal physiology and foraging behavior.

$^{13}$C-enriched sugar sources may exist in urban areas due to a direct anthropogenic effect: human food waste (Newsome et al., 2010; Penick Clint et al., 2015). Like many other insects, honeybees are known to forage on food wastes rich in sugars from corn or cane origins. Since human wastes may be more abundant in urban areas, this effect could impact the $^{13}$C of animals consuming these food sources. Such an effect of food wastes in urban areas on $^{13}$C was observed on ants in North America but could not be shown on honeybees (Penick Clint et al., 2015, Penick et al., 2016). In the latter case, a bias could be induced by the beekeepers provisioning their hives with corn syrup both in urban and rural areas (Penick et al., 2016). Such provisioning does not occur with the three non-managed bee species studied here. We are not aware of any observation of these small wild bees foraging on food waste. However, although unlikely, we cannot entirely exclude this hypothesis to explain the patterns of $^{13}$C highlighted in this study. As most carbohydrates consumed by bees do not contain N, potential foraging on food waste for sugar should have little effect on bee $^{15}$N.

Hydric stress may also modify animal metabolism, inducing a further increase in the $^{15}$N (Ambrose and DeNiro, 1986). Protein catalysis under food stress has also been related to increase in $^{15}$N (Hobson, 1999). Hence, hydric stress could also have a direct change in the metabolism of bees, and food stress could also induce such a pattern in their $^{15}$N.

In addition, plant community composition, hence bees’ diet composition, varies between urban and non-urban areas. In our case, both plant species richness and amount of nitrogen fixing Fabaceae were negatively correlated with the proportion of impervious surface (data not shown). However, the Fabaceae abundance was included in our models. Furthermore, in other models, not shown, we tested the effect of plant diversity overall, and did not find and effect that was distinguishable from the effect of Fabaceae alone. This low impact of plant species composition, and especially the abundance of Fabaceae, on the $^{15}$N of bees, could also be influenced by plant choices of the bees studied here: most Fabaceae are pollinated by long-tongue bees, able to reach the nectar at the bottom of their long flowers. Even if they are polylectic, these _Lasioglossum_ bees are both small and with a short tongue, hence they might not be able to forage on many Fabaceae plants (Pellissier et al., 2012). The presence of a corn field around one rural site might possibly have influenced the bee $^{13}$C (leading to an enrichment of bees from this rural site, compared to sites without corn fields), since bee can occasionally forage on anemophilous plants, seeking for pollen (Saunders, 2018). However, only one capture site was concerned, and a potential effect of corn would have weakened the observed effect of urbanization, rather than causing the reported pattern. The results nonetheless highlight that plant composition, hence food resources, changed throughout the gradient, with potential effects on isotope ratios, albeit surpassed by other factors linked to urbanization.

5. Conclusion

A strong effect of the increase of impervious surfaces was observed for the $^{13}$C and $^{15}$N of three species of wild bees in the Paris area. This could be linked to an urban imprint on the diverse plants on which pollinating insects forage on in the Paris area, or other factors linked to the physiology and foraging strategies of insects (e.g., sugars in human food). In the first case, this would imply that an urban isotopic signal is transmitted from primary producers to their animal consumers and can thus further imprint urban trophic networks. In the second case, this would have implications for our understanding of the physiological stresses imposed by urban environments on pollinating insects, and potential changes in foraging strategies as a potential response to urban constraints and opportunities. To further discriminate among the processes that could explain the observed patterns, further studies could, alongside isotopic data on pollinators, also sample plants and other potential food sources at the sampling sites. This would help improve the understanding of urban biogeochemistry and trophic chains. A better understanding of the factors influencing pollinator $^{13}$C and $^{15}$N could also help elucidate the origin of specimens and the migratory capacities of bee species, an important yet still elusive question considering the increase of habitat fragmentation.

Author contributions

_Benoît Geslin_ & _Aleksandar Rankovic_ conceived the study, were involved in laboratory and statistical analyses, and wrote the paper. _Adrien Perrard_ performed statistical analyses and graphical work. _Benoît Geslin_ & _Isabelle Dajoz_ realised the field work. _Anne Barbillon_ & _Veronique Vaury_ realised the laboratory analyses. _Luc Abbade_ help in conceiving the study and writing the paper. All authors corrected and proofread the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actao.2020.103545.

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