Answer to Associate Editor Decision: Reconsider after major revisions (23 May 2018) by Sébastien Fontaine

Comments to the Author: The comments of the two referees made a number of interesting comments with the aim of improving your work. Their comments are united in some aspects such as the lack of clarity on the novelty of this article compared to your previous publication and the lack of robustness of your statistical approach.

You carefully answered to these comments. In one place you suggest that we could add some new data to reinforce your main message. Since the two referees question the robustness of your approach I invite you to add all the data that could reinforce your work when you will submit your revised version.

Dear Sébastien,

We present addition to the text on the increase of stable isotope values, comparing zone of the great cormorant colony in 2014 and 2015 (where former control zone became expansion zone). As we wrote in the answer to reviewers, sample size cannot be increased due to objective reasons, so there is limited possibility for statistics. However, we see no other factor, just colony growth and spread, involved in the observed changes. Additionally we checked cores areas of species in the isotopic space – if they remain the same under increase of number of breeding cormorants and after they appear in new area.

Added text: P7L9-18

"With the expansion of the great cormorant colony in 2015, the isotopic signatures of δ^{15} N in dominant small mammal hair grew in comparison to 2014, though not all differences are significant (Table 2). In *A. flavicollis*, δ^{15} N values increased in all zones (the 7.5 % increase in the ecotone zone is significant at p < 0.05). The increase in the colony is ~1 %, while the expansion zone compared to former control zone is 5.7 %. All are correlated with colony growth and expansion. In *M. glareolus*, δ^{15} N increased by 2.3 % in the ecotone zone and ~1 % in the colony.

 δ^{13} C signatures in the hair of *A. flavicollis* in 2015 decreased in all zones. The decrease in the expansion zone compared to 2014 control zone was 4.5 % (p < 0.1), in the colony zone 3.1 % (p < 0.001) and in the ecotone zone 0.5 %. In the hair of *M. glareolus*, the decrease of δ^{13} C signatures was even stronger - 8.5 % in the expansion zone, 3.3 % (p < 0.1) in the control zone and 2.6 % in the ecotone (Table 2). We suppose that no other factorother than colony growth could account for these changes."

To evaluate significance of these changes, we additionally tested data on stable isotopes, calculating ellipses of species (core areas in the isotopic space) using SIBER package run in R (according Jackson et al., 2011). Non-intersecting ellipses would show, that position in the isotopic space is significantly shifted (thus, confirming significance of the influence of colony impact in 2015 compared to 2014). Package is using Bayesian method, but even so for the graphic representation of ellipses and core areas it requires sample size n=5. This was not fulfilled for *Myodes glareolus* in the control/expansion zone, where 1-2

individuals were trapped (see added Table S1). In this case central position (mean±SE, ‰) of stable isotope ratios in the hair of *Myodes glareolus* could be seen in the Table 2.

Added text: P7L18-22

"Isotopic niches of *A. flavicollis* in 2014 and 2015 (shown as central ellipses) were separated in the colony and between the control and expansion zones, while they partially overlapped in the ecotone (Fig. S2a). The isotopic niches of *M. glareolus* in 2014 and 2015 were separated in the ecotone and had a small overlap in the colony (Fig. S2b). Insufficient sample size did not allow analysis in the control and expansion zones for this species (see Table 1)."

We add four supplements. Fig. S1 is showing normality of the distribution of stable isotopes (two small mammal species, δ^{15} N and δ^{13} C, zones lumped). Figure shows, that namely expansion zone had outliers from normal distribution of δ^{13} C, while δ^{15} N distribution is not normal.

In the Fig. S2 – changes of core areas in the isotopic space of both small mammal species are shown according zones of the great cormorant colony, comparing 2014 and 2015.

Fig. S3 shows central position of stable isotope ratios in litter, cormorant feathers and the hair of *Apodemus flavicollis* and *Myodes glareolus* in different zones of the Juodkrantė colony of great cormorants in 2015.

Introduced Table1 now show number of trapped individuals of both species according zones of the colony in 2014 and 2015. Due to various reasons, not all trapped small mammals were analysed for isotope concentrations in 2014. In 2015, we took hair from nearly all individuals, excluding only those already damaged by insects. To show, that sample size is limited by objective reason, we decided to keep Table 1 in the text. For the Rev#2 such table did not raise objections or comments. New Table 1 also answer comment of Rev#1 for P8L3 "which datasets shoved the influences of great cormorants on small mammals?" Former Table 1 now is presented as supplement, thus we answer comment of Rev#1 to give former Table 1 as Supplement. And so, 2.2 subchapter was re-composed.

To put more emphasis on the immediacy of the observed changes, we re-phrased title of the paper, now it is "Immediate increase in isotopic enrichment in small mammals following the expansion of a great cormorant colony."

Language was re-checked, adjustments done by native speaker. We also added Acknowledgements.

We hope these changes not only answer all remaining questions, but also improve quality of the manuscript.

With best wishes

In the name of all authors

Linas Balčiauskas

Expansion of great cormorant colony immediately increased Immediate increase in isotopic enrichment in small mammals following the expansion of a great cormorant colony

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Abstract. Colonies of great cormorants (*Phalacrocorax carbo*) impact terrestrial ecosystems transportingthrough the transport of nutrients from aquatic to terrestrial ecosystems. Deposited guano overload the ecosystem with N and P, change soil $pH_{\overline{2}}$ and damage vegetation. However, The ways how in which small mammals are impacted, however, are notlittle known in details. We aimed to evaluate the effects of an expanding great cormorant colony, testing if the expansion immediately increases

- 5 <u>increased the</u> input of biogens <u>in</u>to the forest ecosystem, and <u>further</u>, if <u>the growing</u> influence of the colony <u>iswas</u> reflected in the basal resources (plants and invertebrates) and the hair of small mammals. <u>Stable carbon δ^{15} N</u> and <u>nitrogen isotope δ^{13} C</u> signatures were analysed in granivorous yellow-necked mice (*Apodemus flavicollis*), omnivorous bank voles (*Myodes glareolus*) and basal resources of animal and plant origin from the territory of a colony of great cormorants, situated near the Baltic Sea, in Wwest Lithuania. We found that biogens transferred by great cormorants to the terrestrial ecosystems affected
- 10 the potential foods of the small mammals and led to highly elevated and variable δ^{15} N values. Increase of the colony in 2015 caused isotopic enrichment of the small mammals in the zone of expansion compared to 2014 levels. An increase of the size of the colony in 2015 resulted in isotopic enrichment of the small mammals in the zone of expansion in comparison to levels in 2014. The increase of δ^{15} N in *A. flavicollis* was 7.5% (p<0.05) in the ecotone and 5.7% in the expansion zone. The decrease in δ^{13} C signatures in *A. flavicollis* was 4.5% (p < 0.1) in the expansion zone and 3.1% (p < 0.001) in the colony. In *M.*
- 15 glareolus, the decrease in δ^{13} C signatures was 8.5 % in the expansion zone, 3.3 % (p < 0.1) in the control zone and 2.6 % in the ecotone. Isotopic niches (central ellipses) of *A. flavicollis* in the colony and between the control and expansion zones were separated in 2014 and 2015, while they partially overlapped in the ecotone. The isotopic niches of *M. glareolus* in 2014 and 2015 were separated in the ecotone and had a small overlap in the colony. For most of the resources tested, the isotopic signatures in the established colony area were significantly higher, than in the ecotone (between colony and surrounding forest)
- 20 and expansion zone with the first year on<u>rest of</u> cormorant <u>nests present. Surprisingly, in_inhabited area. In the colony,</u> the colony $\delta^{15}N$ values in plants (16.9 ± 1.1 ‰) were higher than in invertebrates (13.6 ± 0.4 ‰). In the ecotone, the $\delta^{15}N$ values were 12.0 ± 1.4 ‰ in plants and 14.7 ± 0.04 ‰ in invertebrates, while in the expansion zone <u>-7they were7.2 ± 3.0 ‰</u> and 9.9 ± 3.8 $\frac{3}{5}$ respectively. $\delta^{15}N$ -rich resources led to increased $\delta^{15}N$ values in the hair of *A. flavicollis* and *M. glareolus*. Thus, biogens from the great cormorant colony immediately affected small mammals through their food sources.
- 25 Keywords: δ^{13} C and δ^{15} N, Apodemus flavicollis, Myodes glareolus, Phalacrocorax carbo, colony increase, Lithuania

Copyright statement

1 Introduction

Impact of greatGreat cormorants (*Phalacrocorax carbo*) have one of the greatest impacts on the terrestrial ecosystems is one of the biggest amongof all birds breeding in colonies and transporting nutrients from aquatic to terrestrial ecosystems (Klimaszyk et al., 2015). Cormorant excreta change soil pH, N and P levels and damage vegetation (Kameda et al., 2006;

5 Klimaszyk and Rzymski, 2016), decreasing <u>the</u> diversity of plants (Boutin et al., 2011), and affecting seed germination (Žółkoś and Meissner, 2008). Cormorant faeces may cover up to 80% of vegetation, with as many as 70 % of plant species disappearing in the established colonies, the rest being dominated by nitrophilous plants, such as elder (*Sambucus nigra*), common nettle (*Urtica dioica*), woodland groundsel (*Senecio sylvaticus*) and greater celandine (*Chelidonium majus*) (Goc et al., 2005; Klimaszyk et al., 2015). The abundance and diversity of herbivorous insects also decreases, while other arthropod

10 groups may be abundant (Kolb et al., 2012).

With regard to great cormorant colonies and small mammals, investigations are scarce. In previous studies, we have discovered that <u>the great cormorant colony in Juodkrante</u>, Lithuania, changes the ecology of the small mammals inhabiting the territory, decreasing their diversity and abundance (Balčiauskienė et al., 2014), <u>as well as</u> changing population structure and fitness (Balčiauskas et al., 2015) – allthese changes indicatinge poor habitat, and heightening values of δ^{13} C and δ^{15} N in their hair

- 15 (Balčiauskas et al., 2016). Our continuing interest in the great cormorant colony in Juodkrantė was maintained by Unexpectedly, the fact that it's area and number of breeding cormorant pairs in the colony increased in 2015 due to the absence of deterrent measures. Cormorants built nests in the formerly uninhabited territoryies, giving a unique opportunity to evaluate the immediate effect of the colony formation. As a bigger number of nests should be related to increased in the colonybiological pollution, we hypothesized that stable isotope values will also increase in small mammal hair.
- 20 Measures of limiting breeding success in Juodkrantė great cormorant colony were started in 2004 (Knyva, unpublished) and they withhold colony from expansion. In 2015 measures were not applied, resulting colony growth. First nests appeared in the area, which was free of cormorants in 2014, thus, was used as control zone in Balčiauskas et al., (2016). In 2015 we repeatedly examined δ^{13} C and δ^{15} N distribution in a great cormorant colony, this time including in plants and invertebrates as expected diet sources of small mammals. We analysed the isotope composition in small mammal hair during <u>athe</u> period of cormorant
- 25 colony growth, comparing <u>the</u> isotopic signatures in <u>samples of</u> small mammal hair <u>betweenobtained in</u> 2014 and 2015. We do not analyse species, gender and age based differences of stable isotope values in the hair of small mammals. The aim was to evaluate the effects of the transfer of biogens from the aquatic to terrestrial ecosystem by <u>anthe</u> expanding great cormorant colony. We tested (i) if the expansion of the great cormorant colony immediately increases <u>the</u> input of biogens to the forest ecosystem, and (ii) if <u>the</u> influence of the great cormorant colony is reflected in the basal resources (plants and invertebrates)
- 30 and the hair of small mammals. <u>Novelty The novelty</u> of our investigation was in evaluating <u>speed the immediacy</u> of the impact of <u>the</u> great cormorant colony on small mammals. The results <u>give new insight to understanding for the first time showed</u> how fast biogenic pollution is transferred and what the consequences are to small mammal ecology. The immediacy of the impact

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of the new nests has a practical implication, specifically illustrating potential negative consequences if bird scarring is deployed and the colony moves to a new territory.

2 Material and methods

2.1 Study site

- 5 In 2015, small mammals were trapped and samples of their possible foods were collected in the territory of the biggest colony of great cormorants (*Phalacrocorax carbo*) in Lithuania (Fig. 1), situated near Juodkrantė settlement (WGS 55° 31' 14.22" N, 21° 6' 37.74" E). The colony is in Kuršių Nerija National Park, which has been a UNESCO World Heritage site since 2000. <u>The colony colony existed in 19the 19th century</u>, but due to persecution disappeared in 1887 and returned. Returning only after 100 years. Breeding of , breeding cormorants waswere again registered in 1989, and in 1999 there were over and thereafter the
- 10 colony rapidly expanded reaching 1000 breeding pairs. In 2004 number of breeding by 1999 and 2800 pairs reached 2800. Overin 2004. In the same year, measures to limit breeding success at the Juodkrante great cormorant colony were implemented (Knyva, unpublished) with the aim of limiting colony expansion. Regardless, over 3500 nests have been recorded in the colony each year since 2010, with the exception of 2014 when, due to stringent control measures (firing petards in the nesting period), the number of successful pairs was under 2000. In 2015, control measures were not applied, resulting in colony growth. In that
- 15 year, nests appeared in an area that had been free of cormorants in 2014 and had been used and the number of breeding pairs reached 3800.as control zone in Balčiauskas et al., (2016).

Three zones were defined for this study, <u>namely the colony</u>, ecotone and expansion zone. <u>The colony</u> included <u>the</u> area with the highest concentration of nests and the area of former active influence with dead trees. <u>Zone of The</u> ecotone (<u>position</u> <u>of the trap lines shown in Fig 1a</u>) was situated between <u>the</u> colony and forest to the south (Fig 1a). It's not used by cormorants

- 20 (shown in darker green in Fig 1b). Its position did not change in 2011–2014. In 2015 very few, but a small number of nests appeared_did appear in the ecotone. However, in 2015. Also in 2015, the area of the colony expanded by about 3 ha northward (Fig 1b).), this being the expansion zone. Nests and droppings appeared in the expansion-zone, however,but trees hadshowed no visible influence of the birds. In 2013–2014, this expansion zone was used as trapping control (Balčiauskas et al., 2015; 2016), thus, we comparedwere able to compare results to find colony influence after the firsta single year of cormorant breeding
- 25 in thea formerly freeunaffected territory-

2.2 Small mammal sampling

Small mammals were trapped in September 2015, using lines of 25 snap traps placed every 5 m. <u>ExpansionThe expansion</u> and ecotone zones had two such lines each, and six lines were located in the great cormorant colony (Fig. 1a). Baited with bread and sunflower oil, the traps were <u>openleft</u> for three days and checked every morning. Trapping effort was equal to 750 trap

days and 125 individuals were trapped. <u>Individuals were measured</u>, <u>plus</u> <u>sex</u> and <u>age</u> were recorded during dissection as <u>described elsewhere (Balčiauskas et al., 2015)</u>. <u>StudyThe study</u> was conducted in accordance with the principles of Lithuanian legislation for animal welfare and wildlife.

The dominant species were yellow-necked mice (Apodemus flavicollis) and bank voles (Myodes glareolus), while harvest mice

- 5 (Micromys minutus), root voles (Microtus oeconomus) and short-tailed voles (M. agrestis) were also trapped in very low numbers. Two shrew species, common (Sorex araneus) and pygmy shrews (S. minutus), were trapped occasionally. No small mammals were trapped in The trapped rodent species differ in food preferences, ranging from herbivory in Microtus to granivory in Apodemus and Micromys, and omnivory in Myodes (Butet and Delettre, 2011; Čepelka et al., 2014). In general though, small mammals are mostly omnivorous (Nakagawa et al., 2007), though Sorex typically consume invertebrates
- 10 (Makarov and Ivanter, 2016).

The numbers of rodents trapped in the different zones of the territory (control/expansion, ecotone and the colony itself) in 2014 and 2015 are presented in Table 1, while the age and sex composition of the two dominant species in 2015 are shown in Table S1. No small mammals were trapped in the areas of the great cormorant colony that contained the highest concentration of nests and had experienced long-term influence. In the expanding part of the colony-the, only species, *A. flavicollis* was trapped

15 in numbers <u>in 2015</u>.

Trapped rodent species differ in food preferences, ranging from herbivory in *Microtus* to granivory in *Apodemus, Micromys* and omnivory in *Myodes* (Butet and Delettre, 2011; Čepelka et al., 2014). In general, small mammals are mostly omnivorous (Nakagawa et al., 2007), though *Sorex* typical consume invertebrates (Makarov and Ivanter, 2016).

Individuals were measured, sex and age were recorded during dissection as described elsewhere (Balčiauskas et al., 2015). 20 The age and sex composition of the dominant species is presented in Table 1.

2.3 Baseline sampling

Isotopic signatures were evaluated and isotopic baselines were established from possible dietary items. In 2015, we collected samples of the possible food items at the locations where the small mammals were trapped or <u>at</u> the closest available place. In the most affected zones, plant diversity was extremely restricted, with <u>just</u> a few nitrofilous species present. In total, 45 plant and <u>9nine</u> invertebrate samples were collected. Five litter samples and seven samples of great cormorant feathers and eggshells

25 and <u>9nine</u> invertebrate samples were collected. Five litter samples and seven samples of great cormorant feathers ar were also collected.

Plant samples included leaves of greater celandine (*Chelidonium majus*), sedges (*Carex* sp.), raspberry (*Rubus idaeus*), rush (*Juncus* sp.), blackberry (*Rubus fructicosus*) and billberry (*Vaccinium myrtillus*), leaves and berries of elder (*Sambucus nigra*), alder buckthorn (*Rhamnus frangula*); and European barberry (*Berberis vulgaris*), and oak (*Quercus robur*) acorns.

30 Invertebrate samples included coprophagous dung beetle (*Geotrupes stercorarius*), herbivorous dark bush-cricket (*Pholidoptera griseoaptera*), predatory ground beetle (*Carabus* sp.) and omnivorous land slug (*Deroceras* sp.). Quantifying

of the different foods by volume was not done. Unfortunately, we have no data on <u>the</u> isotopic signatures in basal resoources from the pre-expansion period in 2014.

2.4 Stable isotope analysis

We used <u>the hair of the rodents as metabolically inert samples, these preserving the isotopic record of the animal's diet</u>
(Crawford et al., 2008; Bauduin et al., 2013). Hair samples were <u>cut offtaken</u> with scissors from between the shoulders of allmost of the trapped specimens of *A. flavicollis* and *M. glareolus* (88 and 29 individuals, see Table 1).<u>S1</u>). Individuals spoiled by fly larvae or predators were not sampled for stable isotopes. Each sample was placed separately in bagsa bag and stored dry. Samples were weighed with a microbalance and packed in tin capsules. FewAs few individuals of these species survive longer than <u>lone year</u>; with most over-winter mainlywintering individuals being autumn-born ones (Bobek, 1969), thus, our
samples thus represent cormorant the influence of the cormorants in the year when that the rodents were trapped.

- 10 samples <u>thus</u> represent <u>cormorantthe</u> influence of the <u>cormorants in the</u> year <u>whenthat the</u> rodents were trapped. Environmental samples (including plants, litter, invertebrates and great cormorant feathers and eggs) were stored in a refrigerator at below -20°C prior to preparation and analysis. Samples were dried in an oven at 60°C to a constant weight for 24–48 hours and then homogenized to a fine powder (using mortar and pestle and a Retsch mixer mill MM 400). Pretreatment of hair and other samples was not <u>useddone</u>, as after testing it <u>gaveproduced</u> no change of results. Feathers were cleaned with
- 15 acetone and deionized water prior to measurements. Feather samples were clipped from the vane avoiding the rachis. Stable isotope ratios (δ^{13} C and δ^{15} N) were measured using an elemental analyzer (EA) coupled to an IRMS (Flash EA1112; Thermo Delta V Advantage, Thermo Scientific, USA). Stable isotope data are reported as δ values, according to the formula $\delta X = [R_{sample}/R_{standard} - 1] \times 10^3$, where $R_{sample} = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ of the sample, $R_{standard} = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$ of the standard. 5 % of samples were run in duplicate. The equipment parameters and measurement quality control are detailed elsewhere
- 20 (Balčiauskas et al., 2016).

2.5 Statistical analysis

Normality The normality of distribution of δ^{13} C and δ^{15} N values was tested using Kolmogorov-Smirnov's D. As not all values of δ^{13} C and δ^{15} N were distributed normally, the influences of species and the zone of the colony on the carbon and nitrogen stable isotope values in the mammal hair were tested using nonparametric Kruskal-Wallis ANOVA. Independent groups were

- compared with the same Kruskal-Wallis multiple comparisons procedure (Electronic, 2017). Differences in δ^{13} C and δ^{15} N ratios between 2014 (data from Balčiauskas et al., 2016) and 2015 were tested by multivariate Hotteling's T² test. The minimum significance level was set at p < 0.05. Calculations were performed using Statistica for Windows, ver. 6.0. Environmental samples were analyzed by object group (cormorant, litter, invertebrates, plants) and by the zone (expansion, ecotone, colony). Isotopic baselines were calculated using basal resources as possible foods for rodents grouped according to
- 30 their origin. Reported values are arithmetic means with SE of the $\delta^{15}N$ and $\delta^{13}C$ for all basal resources mentioned above.

The isotopic niches of species, as central ellipses, were calculated using SIBER (Jackson, Inger, Parnell, & Bearhop, 2011) under R ver. 3.5.0 (https://cran.r-project.org/bin/windows/base/rdevel.html) for *A. flavicollis* and *M. glareolus* in the zones, where five or more individuals were investigated for δ^{15} N and δ^{13} C.

3 Results

5 3.1 δ^{13} C and δ^{15} N values in the hair of small mammals inhabiting <u>the</u> great cormorant colony

Distribution The distribution of both δ^{13} C and δ^{15} N values in the hair of *A. flavicollis* was not normal, while in *M. glareolus*-the distribution of δ^{15} N values in *M. glareolus* was also not normal, but the distribution δ^{13} C values corresponded to normal (Fig. S1). Outliers from the normal distribution were values, registered in the expansion zone. (Fig. S1). Kruskal-Wallis ANOVA demonstrated that the distribution of stable isotope values was influenced not only by zone, but also by the species of small

10 demonstrated that the distribution of stable isotope values was influenced not only by zone, but also by <u>the</u> species of small mammal. These factors together significantly influenced the distribution of $\delta^{15}N$ (r² = 0.31) and $\delta^{13}C$ (r² = 0.26, F both p < 0.0001).

In 2015, <u>the</u> influence of the zone (both species pooled) was significant for the distribution of δ^{15} N (Kruskal-Wallis ANOVA, H_{2.119} = 18.62, p = 0.0001) and δ^{13} C (H_{2.119} = 6.30, p = 0.043). Between-species differences of the stable isotope values in

15 the hair of the rodents in the colony area were highly significant for δ^{13} C (H_{1,119} = 21.69, p < 0.0001) and for δ^{15} N (H_{1,119} = 6.67, p = 0.01). δ^{15} N values were highest in the hair of *M. glareolus* trapped in the ecotone and colony zones, while <u>highest</u> in *A. flavicollis* in the expansion zone. δ^{13} C signatures in the hair of *A. flavicollis* were higher than in *M. glareolus* in all territories, including the expansion zone (Table 2).

With the development expansion of the great cormorant colony in 2015, the isotopic signatures, mostly of $\delta^{15}N_7$ in dominant

- small mammal hair grew compared in comparison to 2014, though not all differences are significant (Table 2). In *A. flavicollis*, δ^{15} N values increased in all zones (the 7.5 % increase in the ecotone zone is significant at p < 0.05). The increase in the colony is ~1 %, while the expansion zone compared to former control zone is 5.7 %. All are correlated with colony growth and expansion. In *M. glareolus*, δ^{15} N increased by 2.3 % in the ecotone zone and ~1 % in the colony. δ^{13} C signatures in the hair of *A. flavicollis* in 2015 decreased in all zones. The decrease in the expansion zone compared to
- 25 2014 control zone was 4.5 % (p < 0.1), in the colony zone 3.1 % (p < 0.001) and in the ecotone zone 0.5 %. In the hair of *M*. *glareolus*, the decrease of δ¹³C signatures was even stronger 8.5 % in the expansion zone, 3.3 % (p < 0.1) in the control zone and 2.6 % in the ecotone (Table 2). We suppose that no other factorother than colony growth could account for these changes. Isotopic niches of *A. flavicollis* in 2014 and 2015 (shown as central ellipses) were separated in the colony and between the control and expansion zones, while they partially overlapped in the ecotone (Fig. S2a). The isotopic niches of *M. glareolus* in
 20 2014 and 2015 are separated by the second decound be and the second decound decound be and the second decound decound decound be and the second decound de
- 30 2014 and 2015 were separated in the ecotone and had a small overlap in the colony (Fig. S2b). Insufficient sample size did not allow analysis in the control and expansion zones for this species (see Table 1).

3.2 Basal resources

Average<u>Comparing average</u> baseline data of plants and invertebrates <u>ofbetween</u> the expansion zone, ecotone and colony (Table 3) showed the highest), considerable differences were noted in the δ^{15} N of plants (Kruskal-Wallis ANOVA, H_{2,45} = 13.89, p = 0.001) but not <u>in</u> invertebrates (H_{2,9} = 2.76, p = 0.25). In plants, δ^{15} N values were highest in the colony (difference from

5 expansion zone, p < 0.002; difference from ecotone, p = 0.062). δ^{13} C values showed no significant differences between zones in either plants or in the invertebrates (Table 3).

Out of ten plant species, the most ¹⁵N-enriched were: *Chelidonium majus*, $\delta^{15}N = 19.6 \pm 2.0$ ‰ (from 8.4 ‰ in the expansion zone to 25.7 ‰ in the colony), *Sambucus nigra*, $\delta^{15}N = 16.9 \pm 2.6$ ‰ (from 9.2 ‰ in the ecotone to 22.5 ‰ in the colony), *Juncus* sp., $\delta^{15}N = 13.8 \pm 4.7$ ‰ and *Rhamnus frangula*, $\delta^{15}N = 13.2 \pm 1.8$ ‰ (from 8.1 ‰ in the expansion zone to 16.3 ‰ in

10 the colony). $\delta^{15}N$ values in *Carex* sp. (average $\delta^{15}N = 12.3 \pm 0.9 \%$) did not differ significantly across zones. The most $\delta^{13}C$ -enriched plants were *Carex* sp. with $\delta^{13}C = -26.7 \pm 0.7 \%$ and *Sambucus nigra*, $\delta^{13}C = -27.5 \pm 0.6 \%$, values in the expansion zone and colony did not differ. *Chelidonium majus* was among the leass $\delta^{13}C$ -enriched plants, $\delta^{13}C = -29.5 \pm 0.4 \%$.

Of the investigated invertebrates, the most δ^{15} N enriched were *Carabus* sp. and slugs, δ^{15} N = 14.9 ‰ and 14.0 ‰ respectively.

15 Dung beetles showed the highest variation of δ^{15} N, being two times lower in the expansion zone than in the colony (6.2 % versus 13.3 %). The stable carbon isotope ratio in slugs was about 1 % higher than in arthropods.

 δ^{15} N values in the litter were highest in the colony (δ^{15} N = 16.3 ‰), followed by <u>the</u> ecotone and expansion zones (δ^{15} N = 8.2 ‰ and 7.3 <u>‰,</u> we respectively). δ^{15} N values in the cormorant eggshells and feathers were lower than in rodent hair, and <u>did</u> not <u>depending from depend on</u> zone (Fig. <u>\$2\$3</u>).

20 **3.3 Comparison of isotopic signatures in the hair of small mammals and in possible diet resources**

Comparison of isotopic signatures in the hair of *A. flavicollis* and *M. glareolus* with baseline δ^{13} C and δ^{15} N values in possible food sources from the expansion, ecotone and colony zones showed that <u>noted</u> differences were related to bird influence.

In the expansion and ecotone zones, invertebrate isotopic signatures were higher than in plants in terms of both δ^{13} C and δ^{15} N. 25 In the great cormorant colony, most plants were highly enriched in ¹⁵N due to over-enrichment and tended to have δ^{15} N values

well above the invertebrate δ^{15} N range (Fig. 2).

Compared to average plant and invertebrate baseline values, <u>the</u> higher average of $\delta^{15}N$ and $\delta^{13}C$ values in the hair of *A*. *flavicollis* and *M*. *glareolus* waswere related to the zones where <u>the</u> rodents were trapped (Table 4). For *A*. *flavicollis* trapped in the expansion and ecotone zones, the average $\delta^{15}N$ was over 5 % higher than the plant baseline, <u>thoughbut</u> the plant baseline

30 in the colony was higher than the δ^{15} N value in the hair. Compared to the invertebrate baseline, enrichment of 15 N in the rodent hair enrichment of 15 N-was 2.5–3 ‰. Concerning *M. glareolus*, 15 N enrichment was highest in the ecotone when compared to

the plant baseline, but highest in the colony zone when compared to <u>the</u> invertebrate baseline. As for ${}^{13}C$, enrichment was highest in the ecotone zone for both *A. flavicollis* and *M. glareolus* (Table 4).

4 Discussion

4.1 Immediacy of the great cormorant colony impact

- 5 Our main finding showed that great cormorants influenced small mammals in the very first year of the appearance of breeding colony, and possible food objects (plants and invertebrates) also were subjected to increased $\delta^{15}N$ and decreased $\delta^{13}C$ and $\delta^{15}N$ concentrations. Moreover, in 2015 the year of colony increase and expansion we found, that the increased influence of the great cormorant colony <u>already</u> limited even small mammal distribution. Small mammals were not trapped in the area with the highest concentration of nests. HoweverBy contrast, representatives of three small mammal species werehad been trapped in
- 10 the same place in earlier years, when <u>the number of nests was lower due to scaring measures</u>. In the expanding part of the colony, <u>a</u> single individual of *M. glareolus* was trapped in 2015, <u>and the while</u> only <u>a single species</u>, *A. flavicollis*, was trapped in numbers. In 2014, before colony expansion, four small mammal species were trapped in the same area.

4.2 Biogenic pollution is disclosed by stable isotope concentration in the hair of small mammals

Investigations of into the influence of great cormorant colonies have recently received more attention (see Ishida, 1996; Goc et al., 2005; Kameda et al., 2006; Nakamura et al., 2010; Klimaszyk et al., 2015; Klimaszyk and Rzymski, 2016). However, the impact of such colonies on plant and animal species is insufficiently investigated (see Bostrom et al., 2012; Kolb et al., 2012). Small mammal ecology in the colonies was investigated for the first time investigated in Lithuania (Balčiauskienė et al., 2014; Balčiauskas et al., 2015). Some) and some aspects of isotopic enrichment of small mammals in great cormorant colonies were reviewed in Balčiauskas et al., (2016). We found, that biogenic pollution of resulting from the birds reach the dominant species of small mammals. Stable stable isotope ratios in their hair depend on the powerdegree of cormorant influence, being strongest in the area with cormorant nests (see data in Table 2), and dependingalso on the colony size. However, we had no data of regarding stable isotopes in basal resources and we were not aware, how fast may be such the influence could be.

4.3 Stable isotopes show small mammal diet differences in various zones of cormorant colony

Stable isotope analysis (SIA) can be used to investigate the trophic structure of food webs and various aspects of animal diet (Boecklen et al., 2011; Koike et al., 2016). SIA using mammal hair is a method suitable for diet analysis and for comparing intrapopulation groups as well as different species. When analyzing diet, hair isotopic signatures are compared with the

signatures of possible food sources (Bauduin et al., 2013). In identifying diet sources, carbon and nitrogen isotope ratios are most widely used. The tissues of animals do differ in isotopic composition as a result of differences in their diet. Nitrogen values help in identifying the trophic position, since these values increase by 3–5 ‰ between levels of the food chain (Kelly, 2000; Smiley et al., 2016).

5 _The stable isotope ratio in the hair reflects the dietary isotope composition and trophic level, depending on the ingested food (Cassaing et al., 2007).

As <u>the</u> hair of the rodents trapped in the Juodkrantė colony, ecotone, and expansion zone differed in δ^{15} N and δ^{13} C values, rodents obviously consumed foods with different isotopic signatures, the more diverse diet being in the expansion zone as reflected by much higher variance of δ^{13} C values. Enrichment of plants and invertebrates was strongest in the territory of <u>the</u>

10 colony (see Fig. 2).

25

Species-related differences in the isotopic signatures of the two dominant rodent species may be explained by diet differences and microhabitat use, both supporting coexistence (Stenseth et al., 2002; Cassaing et al., 2013). Previous experience with live-trapping and marking of *A. flavicollis* in the cormorant colony (Jasiulionis, unpubl.) letallowed us to conclude, that migration movement between zones was very limited: we did not found find any marked animals using several zones during the same

15 year. In the resource-scarce territory of the great cormorant colony, any spatial segregation could lead to changes in the diet of the rodents. Considering the isotopic signatures in the hair of the small mammals as dietary proxies (according to Fernandes et al., 2014) reflecting the proteins of the food sources (Perkins et al., 2014), we found that diets differed in the various zones of the colony and dependingalso depended on the small mammal species. Therefore, differential exploitation of resources minimizesd competition (according Bauduin et al., 2013).

20 4.4 Pathways of small mammal enrichment in stable isotopes

Possibility of There is just a possibility that the observed rodent enrichment in δ^{15} N from-was a result of eating cormorant tissues is not clear. Dead chicks, broken eggs and eggshells are constantly present on the ground underneath the nests in the breeding season, so could serve as food source. Moreover, δ^{15} N values in great cormorant eggshells and feathers were lower than the δ^{15} N values in the hair of rodents from the same zone (See Fig. S2). Furthermore, there are observations of *Microtus* or *Myodes* voles eating auklets' eggs and chicks (Drever et al., 2000). In our case, however, the difference in δ^{15} N values between the cormorants and rodents iswas not biggreat, questioning the possibility of consumption of cormorant tissue in any significant amount. So Thus we support the opinion of Millus and Stapp, 2008, that cormorant influence on small mammals is not direct, but is mediated through disturbance of influence onto their food resources (Millus and Stapp, 2008).

TwoThe two possible pathways of marine nitrogen are (1) through guano-fertilised plants or (2) invertebrates that have fed on guano, guano-fertilised plants or cormorant remains (Harper 2007). According to Szpak et al. (2012), ¹⁵N enrichment of plants may range from 11.3 to 20 ‰ after fertilization by guano of seabirds. Plants enriched in guano ¹⁵N may occur at distances

exceeding 100 m from nesting sites and colonies (Millus and Stapp, 2008). FewA few plant samples (*Carex* and *Sambucus*)

were highly enriched in ¹⁵N atin the expansion zone of Juodkrantė colony in 2015, the first year of the presence of cormorant presence.nests.

Rodents usually eat foods that are most abundant (Bauduin et al., 2013) or have preferences characteristic to the species (Fisher and Türke, 2016; Schneider et al., 2017), here were choices in the Juodkrante great cormorant colony are limited to several

- 5 plant species (mainly nitrophilous) and invertebrates. Nitrophilic plants usually grow abundantly, being the food source for herbivores living in the territory of a colony (Cassaing et al., 2007). Even after birds cease to use <u>a</u> territory, <u>the</u> isotopic signatures of the litter and plants remain high (Kameda et al., 2006). Enrichment of plants by ¹⁵N is a result of uptake of nitrogen from biopolluted soil enriched by marine-derived N from great cormorant excreta. In such situation, δ^{15} N is not a straightforward indicator of the trophic level (Drever at al., 2000). On seabird islands, herbivores often exhibit heightened δ^{15} N
- 10 signatures (Stapp et al., 1999; Drever et al., 2000).

Typically, diet-tissue fractionation is from 2.5 % to 3.4 % for nitrogen (Perkins et al., 2014). Trophic fractionation from 3 to 5 % for nitrogen occurs at every trophic level in seabird colonies (Cassaing et al., 2007). However, in Juodkrantė_± it may exceed 5 % in comparison to plants. Trophic fractionation for carbon of *A. flavicollis* was inat a predictable level, up to 4.6 % in comparison to plants, and up to 3 %, compared to the invertebrate baseline. Enrichment in ¹³C of *M. glareolus* was lower,

15 up to 3.6 ‰ compared to plants, and up to 1.9 ‰, compared to the invertebrate baseline (see Table 4). These values are similar to or even higher than those observed by Sponheimer et al. (2003), with a mean diet–hair fractionation of +3.2 ‰ and a range of +2.7 to +3.5 ‰ in mammalian herbivores.

6 Conclusions

- This study seeks to understand, how the influence of biologic pollution from the great cormorant colony reaches small
 mammals, and how fast this influence is registered after birds appear in the territory. The general conclusion is that in Juodkrantė, Lithuania, the great cormorant colony affected the terrestrial ecosystem starting from the autotrophs and ending with the consumers (two species of rodents). Increase of <u>An increase in</u> the number of breeding pairs in 2015 led to increased δ¹³C⁵N and δ¹⁴⁵Ndecreased δ¹³C values in the hair of *Apodemus flavicollis* in the territory of the colony, ecotone and in expansion zone. In the colony expansion zone, the influence was visible after the firsta single year of nest appearance. In the resource-limited territory under the great cormorant nests, differences in isotopic signatures were related to the species of rodents, pointing out to the differences in their diet. We conclude that despitethe influence of the nutrient transport from water to land ecosystems by great cormorants, their influence_is indirect, resulted by resulting from the biological pollution from of guano on rodent foods. Our results show that scaring cormorants from the colonies may have opposite effect. Negative negative consequence displaced birds may build their nest in other habitats and, as such, a negative, impact of thean emerging
- 30 <u>new colony is spanningcould result spread through the entire ecosystem and reachingimpact small mammals in the first year.</u> The immediacy of the impact from new nests has practical implications, indicating the potential benefit of deterring birds

from the nests during the actual breeding period as a management measure to limit growth in numbers, rather than scarring them before breeding, which may result of colony fragmentation and the moving to new territories.

Data availability

Data used in this paper are available upon request from the corresponding author.

5 Supplement link

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Author contributions

MJ, LB1 and LB2 trapped small mammals and collected baseline data. MJ and RS analysed stable isotopes. LB1 analysed data statistically. LB1 and LB2 wrote <u>the</u> first draft. All authors provided substantial input to the design of the study and discussion of the results.

Conflict of interest

The authors declare that they have no conflict of interest.

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Figure 1. Trapping design in the great cormorant colony in Juodkrantė (a) and colony expansion in 2015 (b). <u>ZonesA – position of</u> the trap lines in 2015: 1 – expansion, 2 – ecotone, and 3 – the colony in 2015 (a); <u>B</u>: yellow line – colony area in 2014, red line – colony expansion in 2015 (b).



Figure 2. Isotopic signatures of potential animal and plant foods compared with isotopic signatures in the hair of age groups of *Apodemus flavicollis* and *Myodes glareolus*, trapped in the expansion (a) and ecotone (b) $zones_7$ and Juodkrantė great cormorant colony (c) in 2015. Ad – adults, sub – subadult animals, juv – juveniles.

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Table 1 Age and sex structure of the two dominant small mammal species trapped in the Juodkrantė great cormorant colony in 2015.

Table 1 Numbers of trapped individuals of the two dominant small mammal species in the Juodkranté great cormorant colony in 2014 (before colony expansion) and in 2015 (the year colony expanded).

| Species Sex | Adult Subadult Juvenil | es | | | | | |
|----------------------|------------------------------|------------------------------|----------------------|---------------------|-----------|--|-----------|
| A | Apodemus | <u>Myodes glareolus</u> Male | | ,18 ,1 | 7 11 | | |
| Year | FemaleControl/expansion* | 12Ecotone | Colony ₁₅ | 15Control/expansion | Ecotone | | Colony |
| 2014Myodes | Male <u>13</u> | 7 <u>28</u> | <u>564</u> | 5 <u>1</u> | <u>7</u> | | <u>25</u> |
| glareolus | | | | | | | |
| 2015 | Female <u>17</u> | <u>633</u> | -43 | <u>62</u> | <u>20</u> | | <u>8</u> |
| control zono in | 2014 become expansion zone i | n 2015 | | | | | |

5 control zone in 2014 became expansion zone in 2015

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Table 2 Central position (mean±SE, ‰) of stable isotope ratios in the hair of *Apodemus flavicollis* and *Myodes glareolus* trapped in the Juodkrantė colony of great cormorants. Data for 2014 recalculated from Balčiauskas et al. (2016). Significance of differences according Hotteling's T² multivariate test, * - p < 0.1, ** - p < 0.05, *** - p < 0.001.

| Species | Zone | Year | δ^{13} C, ‰ ± SE | δ^{15} N, ‰ ± SE |
|----------------------|-----------|------|-------------------------|-------------------------|
| Apodemus flavicollis | Control | 2014 | -24.20±0.08 | 12.26±1.04 |
| | Expansion | 2015 | -25.30±0.33* | 12.96±0.94 |
| | Ecotone | 2014 | -24.37±0.13 | 15.97±0.45 |
| | | 2015 | -24.51±0.10 | 17.16±0.26** |
| | Colony | 2014 | -24.08±0.12*** | 16.52±0.90 |
| | | 2015 | -24.82±0.12 | 16.67±0.50 |
| Myodes glareolus | Control | 2014 | -25.82 | 14.30 |
| | Expansion | 2015 | -28.02 ± 0.85 | 6.70±0.91 |
| | Ecotone | 2014 | -24.85±0.30 | 17.48±0.64 |
| | | 2015 | -25.49±0.16 | 17.87±0.30 |
| | Colony | 2014 | -24.89±0.31* | 17.99±0.91 |
| | | 2015 | -25.72±0.17 | 18.17±0.26 |



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Table 3 Isotopic signatures of basal resources (plants and invertebrates) in the Juodkrantė great cormorant colony, ecotone and expansion zones in 2015

| Basal resources | Zone | n | δ^{13} C, ‰ ± SE | δ^{13} C, ‰ min–max | δ^{15} N, ‰ ± SE | δ^{15} N, ‰ min–max |
|-----------------|-----------|----|-------------------------|----------------------------|-------------------------|----------------------------|
| Plants | Expansion | 9 | -28.6 ±0.9 | -32. 825.2 | 7.2 ± 3.0 | -3.3 - 25.1 |
| | Ecotone | 10 | -29.1 ±0.7 | -31.625.2 | 12.0 ± 1.4 | 8.0 - 22.5 |
| | Colony | 26 | -28.1 ± 0.3 | -31.425.2 | 16.9 ± 1.1 | 8.4 - 27.7 |
| Invertebrates | Expansion | 2 | -28.3 ± 0.8 | -29.127.5 | 9.9 ±3.8 | 6.2 – 13.7 |
| | Ecotone | 2 | -26.9 ± 0.1 | -26.926.8 | 14.7 ± 0.0 | 14.6 – 14.7 |
| | Colony | 5 | -27.6 ± 0.3 | -28.426.5 | 13.6 ± 0.4 | 12.8 - 14.9 |

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Table 4 Nitrogen $(\Delta \delta^{15}N)$ and carbon $(\Delta \delta^{13}C)$ trophic fractionation between *Apodemus flavicollis* and *Myodes glareolus* and their possible food sources in the Juodkrantė great cormorant colony.

| Zone | Baseline for comparison | $\Delta \delta^{15}$ N, ‰ | | $\Delta \delta^{13}$ C, ‰ | | |
|-----------|-------------------------|---------------------------|--------------|---------------------------|--------------|--|
| | | A. flavicollis | M. glareolus | A. flavicollis | M. glareolus | |
| Expansion | Plants | 5.78 | -0.48 | 3.31 | 0.59 | |
| | Invertebrates | 3.05 | -3.21 | 2.95 | 0.23 | |
| Ecotone | Plants | 5.15 | 5.86 | 4.58 | 3.60 | |
| | Invertebrates | 2.49 | 3.20 | 2.34 | 1.37 | |
| Colony | Plants | -0.23 | 1.27 | 3.32 | 2.41 | |
| | Invertebrates | 3.11 | 4.61 | 2.76 | 1.86 | |



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