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RESEARCH ARTICLE

Ecological effects of mosquito control with Bti: evidence for shifts in the trophic structure of soil- and ground-based food webs

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Abstract

The microbial control agent *Bacillus thuringiensis* var. *israelensis* (Bti) has been successfully used worldwide to reduce abundances of biting Nematocera (Diptera), often with little direct impact on non-target organisms observed. However, the potential for additional indirect effects on other ecosystem properties, including on trophic linkages within food webs, is poorly known. We investigated the effects of multiple-year mosquito control treatments using the Bti product VectoBac®-G on the stable isotope composition of epigeal and soil-based consumers inhabiting replicate floodplains along the River Dalälven, Sweden. We observed significant changes in the isotopic composition of detritivores feeding at the base of floodplain food webs. Enchytraeid worms were characterised by 3.5% higher δ^{13}C values in treated floodplains, suggesting increased consumption of δ^{13}C-enriched food. The overall range of community-wide δ^{15}N values was 56% greater in the treated floodplains, whilst δ^{15}N values of oribatid mites were elevated by 97%. These results suggest extra fractionation in the transfer of nitrogen through floodplain food chains. We conjecture that the ecological mechanisms driving these food web shifts are (1) the mass mortality of high δ^{13}C *A. sticticus* larvae, which leaves high concentrations of dead mosquito biomass deposited on soils at local scales, after the floodwaters have receded and (2) incorporation of the very high δ^{13}C-enriched corn particles comprising the bulk of the VectoBac®-G product into floodplain food webs. Our results suggest that repeated applications of Bti might have wider, still largely unknown implications for nutrient and energy cycles within floodplain ecosystems.

Keywords *Aedes sticticus Bacillus thuringiensis var. israelensis (Bti)* · Floodplain · Isotopic niche metrics · Soil microarthropods Spiders · Polyunsaturated fatty acids

Introduction

The microbial control agent *Bacillus thuringiensis* var. *israelensis* (Bti) has been successfully used worldwide to reduce emergence of adult mosquitoes (Culicidae) and other nuisance blood-feeding Nematocera (Diptera) from lakes, rivers and floodplains (Boisvert and Boisvert 2000; Brühl et al. 2020; Land et al. 2019; Schäfer and Lundström 2014). Unlike most other aquatic invertebrates, Nematoceran Diptera have an alkaline gut environment, within which Bti produces crystal aggregations containing toxins disrupting the gut wall (Boisvert and Boisvert 2000). This results in mortality of the target organisms in the larval stage, without the often severe environmental impacts of chemical insecticides (Becker et al. 2010; Boisvert and Boisvert 2000). However, impacts of Bti on potentially vulnerable non-target organisms (NTOs), primarily comprising other Nematocera such as the ecologically diverse non-biting midges (Chironomidae), have been observed in some field studies (Allgeier et al. 2019b; Hershey et al. 1998; Vaughan et al. 2008), though not others (Boisvert and Boisvert 2000; Lundstrom et al. 2010). Additionally, the potential for indirect effects on other ecosystem properties, including trophic linkages and the flow of carbon and nutrients through food webs, is increasingly raised as a possible risk associated with the use of Bti (Brühl et al. 2020; Hershey et al. 1998; Poulin et al.

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2010), but has been little assessed in field research (Land et al. 2019).

Indirect effects on food webs might arise when Bti-induced changes in the density and/or behaviour of one or more organism groups result in alterations of food web structure (e.g. number of trophic levels), and/or function, e.g. processing of different resources or biomass production (Land et al. 2019; Truchy et al. 2015). In the case of Bti, both target organisms and the most vulnerable NTOs complete their larval development in aquatic or semi-aquatic habitats before emerging into the terrestrial environment as winged adults, sometimes at very high densities (Armitage et al. 1994; Becker et al. 2010; Carlson et al. 2016). Adult aquatic insects are often notably rich in polyunsaturated fatty acids which are essential for animal growth and development, and comprise an important resource for both vertebrate (birds, bats, amphibians) and invertebrate (spiders, insects) predators (Müller-Navarra et al. 2000; Ramberg et al. 2020). Accordingly, the massive (e.g. 90–100%, Schäfer and Lundström 2014) reductions in the biomass of adult mosquitoes following Bti treatment dramatically reduces a potentially abundant and nutrient-rich food source for terrestrial organisms. Indeed, 66% reductions in the biomass of emerging Nematoceran Dipterans (mosquitoes and chironomids) from French floodplains treated with Bti were associated with reduced breeding success and fitness of house martins (*Delichon urbicum* L.) (Poulin et al. 2010).

Control of mosquitoes using Bti has particularly strong potential to affect ground-based (epigeal) and soil-based food webs. Extreme densities of mosquitoes and other mass-emerging aquatic insects regularly exceed the feeding capacities of their main predators (Yang et al. 2010). As such, a large portion of aquatic insect productivity ends up deposited on riparian or floodplain soils as dead adults (Hoekman et al. 2012). Deposition of dead adult chironomids has been linked with an increase in the number of soil-based detrivores including Collembola and mites (Hoekman et al. 2011), and to increased soil nutrient availability (Gratton et al. 2017). Additional indirect and poorly studied effects on soil food webs might arise from the vector by which the Bti spores are delivered. For example, the widely used formulation VectoBac®-G (Valent BioSciences, USA) consists of pellets derived from the husks of corn (*Zea mays*) cobs treated with a dried culture of Bti, attached with the help of corn oil (Lundstrom et al. 2010). During mosquito control, these pellets are typically distributed by helicopter over water bodies or inundated floodplains, where they are either immediately consumed by biota or, alternatively, enter the pool of organic detritus available to microorganisms and detritivores.

In this study, we focus on the effects of mosquito control using Bti on epigeal and soil food webs of floodplains in the lower River Dalälven catchment, Sweden. Beginning in the final years of the twentieth century, this region suffered increasingly severe outbreaks of the floodplain mosquito *Aedes sticticus* Meigen (Schäfer and Lundström 2014). A Bti-based control program initiated in 2002 has been highly effective at reducing abundances of flying *A. sticticus* by 90–100%, with little evidence of negative outcomes for NTOs including Chironomidae (Lundstrom et al. 2010). Nevertheless, increases in abundance of Bti itself in treated floodplain soils (Schneider et al. 2017), along with substantial increases in density and taxonomic richness of heterotrophic protozoans (Östman et al. 2008), point to potential changes in the structure and function of soil and epigeal food webs.

We used stable isotopes to characterise shifts in the soil- and ground-based food webs of floodplain habitats of the lower Dalälven River catchment, following more than a decade of Bti application (as granular VectoBac®-G) for mosquito control. We studied the isotopic composition of various groups of ground- and soil-based detritivores, representing the probable first scavengers of dead mosquitos in floodplain soils and/or grazers of microbial decomposers of mosquito remains, and of key invertebrate predators (spiders and beetles). As a C4 plant, the corn comprising the bulk of the VectoBac®-G product has a strongly contrasting isotopic composition compared with the C3 plants that comprise the vast majority of terrestrial flora in Sweden (Pyankov et al. 2010). Based on this, we expected that any incorporation of these corn particles into the ground-based food webs might alter the C isotope values of consumers. We hypothesised that:

1) Detritivore δ^{13}C values are expected to be higher in the treated than control floodplains, in line with the deposition of C4-plant-derived particles used in delivery of Bti.
2) Consumer δ^{15}N isotopes will be enriched in the treated floodplains, if the mass accumulation of dead larvae acts as a significant nutrient subsidy in the treated floodplains, lengthening food chains (Hoekman et al. 2012).

We further analysed adult *A. sticticus* for their isotopic composition and polyunsaturated fatty acid (PUFA) content, to gain insight into their value as a food resource for higher consumers (Goedkoop et al. 2007; Müller-Navarra et al. 2000).

Materials and methods

Study sites and mosquito control

The lower River Dalälven region is characterised by a flat alluvial landscape, encompassing Scandinavia's only true river Delta, and interspersed with extensive wet meadow habitats that are strongly shaped by recurring floods (Holmstedt and Linderheim 2019). High river discharges and subsequent flooding can occur several times over the course of a growing season. Conservation values for the region are high, due to a rich biodiversity of plant and animal life, patches of ancient forest, and the presence of many endangered species and habitat types. Much of the region has received European Union Natura 2000 designation, and is protected in national parks or nature reserves.

Since 2002, the Biological Mosquito Control Project (BMCP) (www.mygg.se) has repeatedly treated several of the floodplain areas to limit the mass emergence of the nuisance mosquito *A. sticticus* using Bti. *Aedes sticticus* is a relatively large-bodied and aggressive mosquito that attacks both humans and livestock, and which has had severe socio-economic effects in the lower River Dalälven region, including on tourism (Schäfer and Lundström 2014). *A. sticticus* lays its eggs in wet-meadow and alder swamp habitats, where their eggs can lay dormant for many years. The eggs typically hatch when a dry period in spring or summer is followed by a flood with water temperature exceeding 8–10 degrees (Schäfer and Lundström 2009). After the floodwater recedes (often after only a few days), the larvae continue their development in remaining temporary standing water habitats. The exact diet of *A. sticticus* larvae in Sweden has not been characterised. However, larval floodplain mosquitoes elsewhere consume mostly particulate detritus and microbes, with algae important only where environmental conditions (light and nutrient levels) favour significant primary production (Merritt et al. 1992).

We sampled epigeal and soil invertebrates from five treated and five control floodplains that are included in the BMCP long-term monitoring program. All are located in and around Färnebofjärden National Park, in close proximity to the main Dalälven river stem (Fig. 1, Tables S1-2). All sampled floodplains are dominated by "wet meadow" habitats, with soils water saturated for much of the growing season and characterised by a cover of herbaceous sedges and grasses (Table 1). Treated floodplains have been subjected to mosquito control starting in 2002, using the granular formulation of VectoBac-G®, applied at a dosage of 13–15 kg per hectare (Schäfer and Lundström 2014). The continuous area of treated habitat encompassing our sampling sites ranged from 1.1 to 11.3 km^2 (Table S2). Prior to 2014 (the year of our study), treatments had been applied during May–August in most years, and most often 2–3 times per year, excepting 2004 and 2013 (no applications) and 2009 and 2012 (four applications) (Schäfer and Lundström 2014, supplemented with information for 2013 from www.mygg.se). Reference floodplains have never been treated, and all are situated more than 1.75 km from the nearest treated area (Table S2).

The ten floodplains were divided into five treated–reference pairs based on their proximity to the main river, soil types and vegetation composition (Tables S1-2, Fig. 1). This paired design was used in our statistical models to account for background variation amongst the floodplains that might have affected our analyses of mosquito control effects. Information on soil type was obtained from the Swedish Geological Survey (SGU, www.sgu.se). Vegetation composition was assessed onsite during field sampling, with the percent cover of different grasses, herbs and woody vegetation recorded in four 5-m^2 quadrats per floodplain. Pairs 1–3 were dominated by grasses and herbs, with pairs 1–2 also characterised by significant growth of *Salix* (willow) (Table S1). Pairs 4–5 are characterised by significant *Sphagnum* cover, which was lacking in pairs 1–3. Overall, neither the elevation nor ground cover of the vegetation categories differed significantly between treated and floodplain categories, except for *Salix* cover which was slightly greater in treated floodplains overall (Table S1).

Field sampling

We collected organisms for stable isotope analysis during July 22–24, 2014, 2 weeks after the most recent mosquito control campaign (undertaken July 9–10 after the second mass emergence of *A. sticticus* during 2014; www.mygg.se). At the time of collection, the flood waters had receded and the floodplains were relatively dry. We focussed on key, predominantly saprophagous, invertebrate groups likely to respond to the deposition of dead mosquitoes and/corn particles in Vectobac G: (1) Annelid worms, including earthworms (Oligochaeta: Lumbricidae) and pot worms (Oligochaeta: Enchytraeidae), and (2) soil microarthropod detritivores, comprising springtails (Collembola) and moss mites (Acari: Oribatida). The two oligochaete families consume decaying plant material and other detritus and associated microflora (fungi, bacteria, actinomycetes, algae), whilst Lumbricidae also feed on living plant material and microfauna (Curry and Schmidt 2007; Didden 1993). The microarthropods Collembola and Oribatida similarly encompass species that predominantly consume decaying vegetation and associated microflora, but also include predacious and carrion-consuming scavenger species (Petersen 2002; Schneider et al. 2004). We further sampled ground-hunting predators, comprising wolf spiders (Lycosidae), fishing spiders (Pisauridae: *Dolomedes fimbriatus*) and rove beetles (Staphylinidae), to assess whether shifts in isotopic composition of detritivores and herbivores can be traced at higher trophic levels. Finally, when available we also collected semi-aquatic Diptera (Tipulidae, Chironomidae), which feed on particulate detritus and algae in moist floodplain habitats (Armitage et al. 1994), and predacious soil mites (Acari: Mesostigmata).

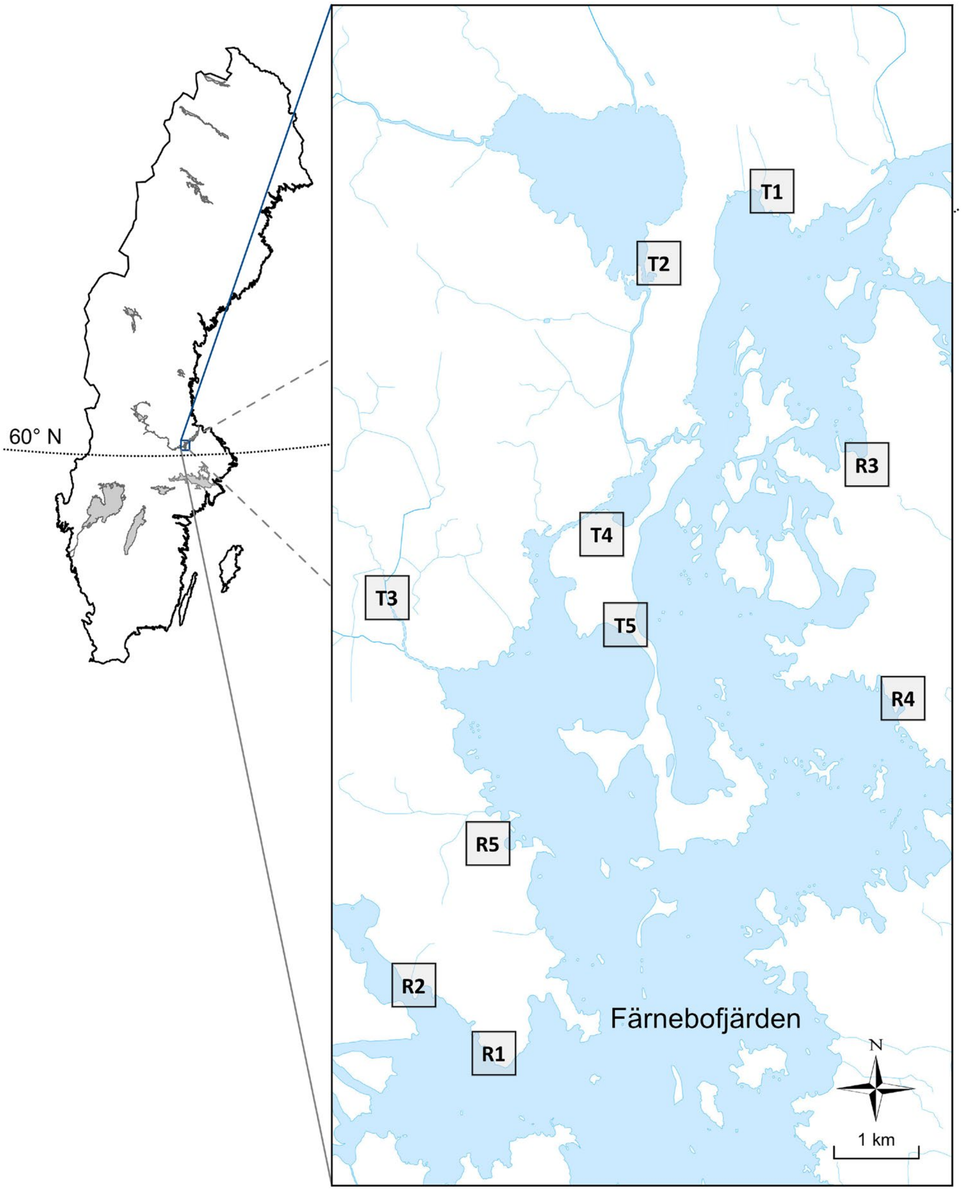


Fig. 1 Map showing the location in Sweden of the Färnebofjärden in the lower River Dalälven showing the paired (by numbers) treated (T) and reference floodplains (R). See Table S1 for detailed information on floodplains and pairs

Table 1 Means of Layman isotopic niche metrics for the reference and control floodplains, with the probability of divergence between floodplain types estimated based on probability distributions

Niche metric	Reference	Treatment	Probability reference < treated	Probability reference > treated
NR	1.04	2.38	96.55%	
CR	2.44	1.86		78.88%
TA	1.36	2.26	76.98%	
CD	0.82	1.01	78.57%	
MNND	0.75	0.96	67.95%	
SDNND	0.49	0.22		32.05%

Wolf spiders and rove beetles were collected by sweep-netting vegetation near to ground level, whilst *D. fimbriatus* was hand collected by visually searching for individuals. We aimed to collect at least five individuals for each spider and beetle group in each floodplain. Captured spiders and beetles were kept alive in individual jars, cold and in the dark, until further processing in the laboratory.

All soil invertebrates, except Collembola, were collected from two types of habitats in the floodplains: raised tussocks formed of grasses and sedges, and damper depressions forming in between the tussocks. The dryer tussocks were targeted for collection of earthworms and oribatid and predacious mites, whilst pot worms and semiaquatic Diptera were collected from the damp depressions. We sampled two tussocks and two damp depressions per floodplain. Tussocks were harder to locate in the *Sphagnum*-dominated pairs 4 and 5 (Table S1). In these floodplains we nevertheless divided our sampling between raised, drier areas of ground and lower, wetter depressions. To sample soil in the tussocks, we used a spade to first cut off the grassy top, and then dug a c. 800–1000 cm^3 (18 × 18 x c. 2–3 cm depth) soil core. Damp depressions were sampled using an 8 (inner diameter) × 15 cm cylindrical soil corer (754 cm^3). Larger earthworms were sorted immediately from the tussock core in the field and retained in jars with additional soil from the site. All soil core samples were then stored in plastic bags and returned in cool boxes to the laboratory for extraction of soil fauna on the same day. Biota from the tussock samples were extracted over Tullgren funnels for a total of 4 days (Tullgren 1971). Soil cores from the damp depressions were extracted over 3 days using a cold wet extraction technique, with the aid of modified Baermann funnels (Didden et al. 1995). Animals were picked daily during extraction and stored cold in the dark.

Springtails (Collembola) were sampled by collecting a larger soil volume (0.02 m^3) with a spade from two tussock habitats per floodplain. In the field, this soil sample was placed in a bucket of river water, and individuals were picked as they floated to the surface. As we were unable to collect sufficient springtail biomass for isotopic analysis during our first sampling visit, we collected additional springtails during a second sampling trip to the floodplains on 11–12 August 2014. There were no new flood or mosquito control events between these two trips.

We also collected plant, biofilm and soil samples for isotopic analysis, to characterise the basal resources available to earthworms and microdetrivores in the floodplains. Based on our vegetation inventories, *Cirsium palustre* (marsh thistle) and *Carex* spp. were selected to represent common terrestrial litter types, occurring in all floodplains. We collected leaves from multiple specimens of these plants across a 50 m^2 area per floodplain, and stored them cold for return to the laboratory. During our second field visit, we additionally collected samples for analysis of soil isotopes (five soil cores per floodplain) and biofilm from both aquatic and terrestrial plants. Biofilm from terrestrial plants was collected by cutting plants close to ground level, which were then placed in 11L buckets filled with distilled water. To collect aquatic biofilm, submersed fragments of aquatic reeds (*Phragmites australis*) were cut from the water surface down to a depth of 10 cm. Both aquatic and terrestrial plant samples were both placed directly into 11 L buckets filled with 2.5L distilled water. The plants were gently brushed in the distilled water to remove biofilm. The water (1.5–2.5 dL) containing biofilm was then filtered through pre-combusted Whatman glass fiber filters (GF/F, effective pore size = 0.7 μm) to capture biofilm-associated algae and other microbes. The filters were then inspected to remove any larger particles, and placed in 4-ml tubes for transport back to the laboratory in a cool box.

We successfully collected targeted consumer groups from all floodplains, except for lumbriculid earthworms and tipulid dipterans (Table S3). We also obtained four of five targeted resources from all floodplains (Table S3). The exception was biofilm from the adjacent river waters: in two reference and two treated floodplains, there was neither *Phragmites* nor other suitable water plants available for epiphytic biofilm collection.

Unfortunately, we were unable to obtain *A. sticticus* during our two field sampling visits. There was no extensive mosquito emergence during our study visits, which were conducted in between flood events when standing water on the flood plains was minimal. However, the BMCP were able to provide us with 12 adult *A. sticticus* hatched from mature (pre-pupal) larvae. The larvae were collected from temporary standing water habitats at one of our sampling areas (Ista) during an outbreak later in the season. Four of the reared adults were sent for analyses of stable isotopes, and the remainder were allocated for FA analyses. The isotope and PUFA composition of these individuals fully reflect resources consumed during larval development in their natal floodplain habitats, as they were newly emerged and had not fed since collection as mature larvae. Finally, the BMCP provided us with a sample of the control product VectoBac®, which we analysed for stable isotopes.

Stable isotope and fatty acid analyses

Following field work and subsequent laboratory extraction, collected consumer and resource samples were separated into the taxonomic and resource groups described above. After 24–48 h of cold storage, during which invertebrates were not supplied with food, all samples were snap frozen in liquid nitrogen (− 196 °C) and stored in a freezer at − 40 °C. At the time of freezing, all organisms except the staphylinid beetles were alive. After freeze drying, samples were weighed and packaged into 5 × 9 mm tin capsules. Larger animals and plants were homogenised prior to weighing to obtain a representative sub-sample. Target weights were 1.3 mg for invertebrates, 3.5 mg for plants and 6.0 mg for soil. For filters with biofilm, ten pieces of 4 mm discs were clipped out per filter with a hole puncher and stacked in tin capsules. Three samples with blank filters were weighed and sent for analysis, to obtain isotopic values for the filters themselves. Three replicates of each soil and terrestrial plant type were also analysed from each floodplain. Unfortunately, we had insufficient material to repeat this process for the biofilm samples.

Stable isotopes have long been used to provide a picture of an organism's ecological niche, where enrichment in the ^{15}N isotope indicates a higher trophic position, and variation in the ^{13}C isotope indicates whether the basal resource has aquatic or terrestrial origins (e.g. Hoekman et al. 2012; Kupilas et al. 2016). Stable isotopes have been recommended as a tool in evaluation of whole-ecosystem effects of mosquito control using Bti (Boisvert and Boisvert 2000), but remain little utilised (but see Allgeier et al. 2019a). Our samples were analysed for stable isotopes by the Stable Isotope Facility at the University of California, Davis, as described in the supplementary online file S1.

Fatty acids (FA) that are specific for certain algal groups and conservatively incorporated into the tissues of consumers can be used as biomarkers, and certain polyunsaturated fatty acids (PUFA) are considered essential for the growth and development of consumers (Goedkoop et al. 2007; Müller-Navarra et al. 2000). We analysed our adult mosquitos their fatty acid composition following the protocol of Lau et al. (2014), as described in the supplementary online file S1.

Data analysis

Stable isotope data were first evaluated visually, using isotopic biplots and niche space estimation. Isotopic biplots, produced with the R package *siar* (siar: stable isotope analysis in R, Parnell and Jackson (2013)), allow for a visual assessment of the similarity of potential food sources to different groups of consumers. In the production of these isotope biplots, data from the five control areas and from the five Bti-treated areas, respectively, were merged. This resulted in two charts that summarise data for the replicate treated and reference floodplains.

We used the Bayesian framework developed by Jackson et al. (2011) to characterise differences in the dimensions of niche space occupied by consumers (i.e. excluding resources) between treated and reference floodplains. Use of a Bayesian framework facilitates estimation of community wide metrics that account for uncertainty in the isotopic composition of particular community members by incorporating information on their variability. Here, we used individual floodplains within Bti treatment categories as replicates for estimating uncertainty. Consequently, for all niche-related metrics, one value is derived for treated and another for untreated floodplain communities, together with a probabilistic distribution (the posterior) representing the range of possible estimates of the metric.

First, we plotted convex hulls for treated and floodplain communities to visually assess how much and in what direction the communities diverge from one another in the isotopic biplot. We then applied Jackson et al's (2011) Bayesian framework to estimate the niche metrics proposed by Layman et al. (2012):

1) Ranges of $\delta^{13}C$ and $\delta^{15}N$ in the niche space (two metrics: CR and NR, respectively)
2) Total area (TA) of the convex hull, acting as a measure of the total amount of niche space occupied by the community.
3) Mean distance to the centroid of points in two-dimensional space (CD), which acts as a measure of species spread;
4) Mean nearest neighbor distance in two-dimensional space (MNDD), functioning as a measure of density of species packing;
5) Standard deviation of nearest neighbor distance (SDNND), measuring evenness of species packing in two-dimensional space;

Overlap in posterior probability distributions was used to assess the percent probability that each Layman metric differed between reference and treated floodplains (Jackson et al. 2011). A probability value greater than 95% provides strong support for divergence. Uncertainty in each Layman metric estimate is visualised as credible intervals. All Layman metrics, convex hull estimates and probabilities of divergence between floodplains were generated using the SIBER package version 2.1.6 (Jackson et al. 2011) implemented in R version 4.1.1 (R core team, Vienna Austria, http://www.R-project.org/).

We supported our descriptions of isotopic niche space by analysing differences in isotope composition of consumers and resources between treated and reference floodplains (Table S3) using two-sided, paired *t* tests (JMP version 11.0, SAS institute). Two-sided *t* tests were followed by one sided *t* tests to further evaluate the direction (increase or decrease) of change.

Results

Isotopic biplots

The most marked difference in the isotopic biplot between floodplain types was the presence of corn-containing VectoBac®-G in the treated floodplains, which had far higher (less negative) $\delta^{13}C$ values than the in situ resources (Fig. 2). Aquatic biofilm samples were characterised by lower (more

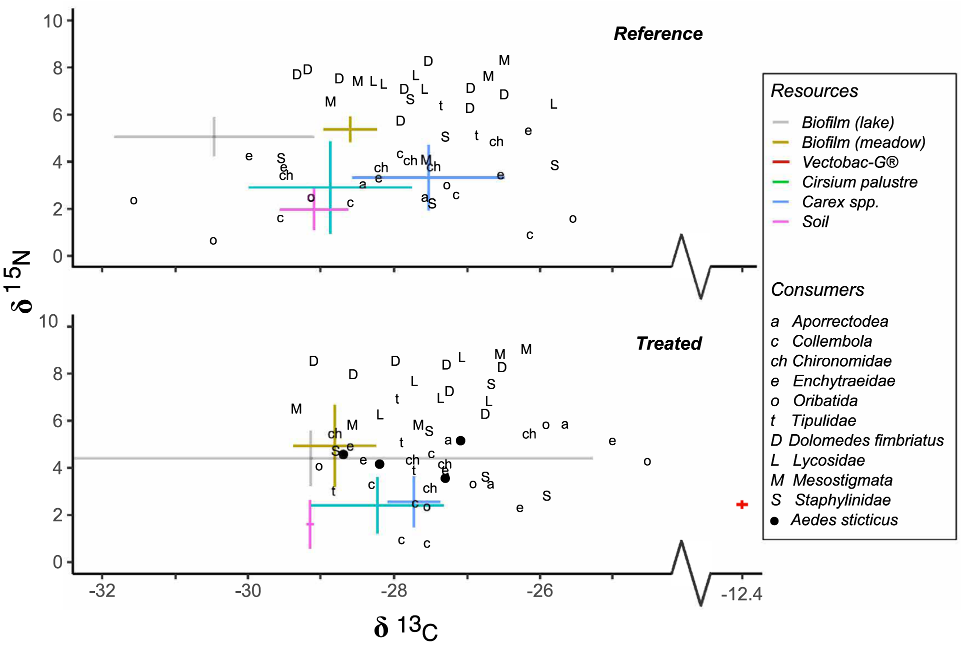


Fig. 2 Isotopic biplots (δ^{15}N and δ^{13}C) for reference (upper panel) and treated floodplains (lower panel) showing basal resources (colors), key taxa of detritivores (small letters) and predators (capital letters), as well as samples of *Aedes sticticus* and VectoBac®-G. Resources plotted as mean values ± 1 SD. For VectoBac®-G, means (± sd) are plotted for three technical subsamples, whilst for adult mosquitoes (*A. sticticus*), data for four individuals are plotted. Both panels are plotted using identical axes, to facilitate comparison

negative) δ^{13}C than the terrestrial plant samples in the reference but not in treated floodplains. Soil δ^{13}C was also lower than all terrestrial plant samples. The large error bar for meadow algae δ^{13}C reflects the wide range, from − 25.41 to − 33.13, of the replicates obtained. The δ^{13}C of the four *A. sticticus* individuals analysed varied from − 27.3 to 28.71, spanning the range of most other detritivores. Finally, detritivore consumers, comprising worms, springtails, oribatid mites and semi-aquatic dipterans, all had lower δ^{15}N values in the biplot space than the predominantly predacious groups, including predacious mites, spiders and beetles (Fig. 2).

Bayesian Layman metrics and niche ellipses

The convex hulls for the treated and reference floodplain communities (Fig. 3) overlapped and were similarly sized (Table 1). However, the reference hull was more spread out along the δ^{13}C axis (Fig. 3a-b), with the treated community hull more spread out along the δ^{15}N (Fig. 3a). Isotopic niche ellipses for individual floodplains are given in Supplementary Information, Fig. S1.

Amongst the Layman metrics, the range of δ^{15}N in the niche space (NR) was 56% higher in treated compared with reference floodplains, with the probability of divergence greater than 95% (Table 1, Fig. 4a). Probabilities of divergence in the δ^{13}C range (CR), total area of the convex hull (TA) and mean distance to the centroid (CD) were all > 75%, with trends for 31% lower CR, 39% higher TA and 18% higher CD in treated relative to reference floodplains (Table 1, Fig. 4b, d). Probabilities of divergence in the mean and standard deviation of nearest neighbor distance (MNND and SDNND) were less than 70% and 35%, respectively (Table 1, Fig. 4e, f).

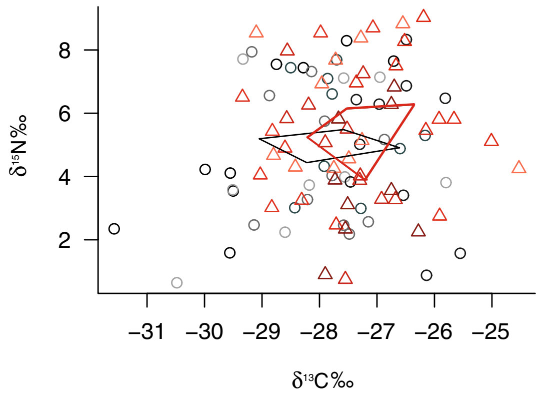


Fig. 3 Isotopic-based convex hulls for reference (black) and treated (red) floodplain communities, pooling individual floodplains within each category. Individual data points from reference floodplains are plotted as circles in grey black, treated floodplains as triangles in red

Consumers and resources

Values of δ^{15}N were 97% higher for detrivorous oribatid mites (Fig. 5a) in treated compared with reference floodplains (Table 2). A similar trend for the pisaurid spider *Dolomedes fimbriatus* (9% higher in treated compared with untreated floodplains, Fig. 5a) was near significance at the 5% level in the two-sided *t* test (Table 2). Values of δ^{13}C were 3.5% higher in reference than treated floodplains for Enchytraeidae (Fig. 5b, Table 2). There were several

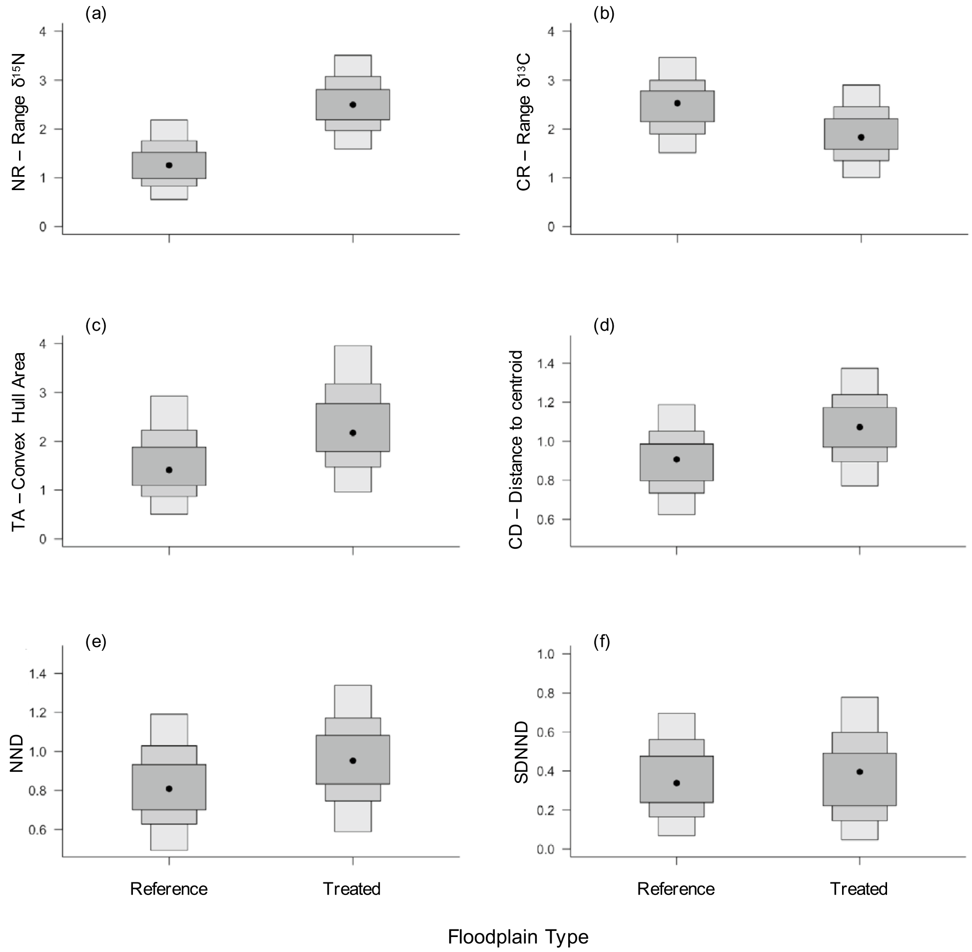


Fig. 4 Bayesian Layman metric modes plotted for each reference and treated floodplains with 95% credible intervals: ranges of **a** $\delta^{15}N$ (NR) and **b** $\delta^{13}C$ (CR) in the niche space, **c** total area (TA) of niche space occupied, based on convex hulls; **d** species spread, based on mean distance to centroid (CD); **e** species packing, based on mean nearest neighbor distance (MNDD); (f) evenness of species pacing, based on standard deviation of nearest neighbor distance (SDNND). Black dots represent the modal estimate, whilst shading in the density plot represents the 50% (dark gray), 75% (light gray) and 95% (white) credibility intervals. See Table 1 for probability of divergence estimates between wetland types

additional trends for lower $\delta^{13}C$ and/or higher $\delta^{15}N$ in other key consumer taxa in treated compared with reference floodplains (Fig. 5, Supplementary material Fig. S1), but these were either not significant in two-sided *t* tests, or could not be evaluated due to a lack of replication (Lumbricidae, Tipulidae) across all floodplains (Table S3).

Resources obtained from all sample sites (i.e. soil, meadow biofilm scrapes, *Carex* sp. and *Cirsium palustre*, Table S1) did not differ in either $\delta^{13}C$ or $\delta^{15}N$ values between treated and reference floodplains (all $t < |1.3|$, $p > 0.3$). The lowest C:N molar mass ratios were observed for *A. sticticus* (5.43 ± 0.51), and lake (9.44 ± 1.51) and meadow (12.07 ± 2.95) biofilms (Fig. 6). VectoBac®-G had the highest C:N ratio (126.7 ± 19.86, Fig. 6). C:N ratios were higher in treated floodplains for *Carex* sp. (27% higher; $t = 4.43$, $p = 0.011$). No other differences in C:N ratios between floodplain types were detected for the remaining resource types (all $t < |1.4|$, $p > 0.23$).

Fatty acid composition of *A. sticticus*

We detected 23 fatty acids (FA) with relative concentrations exceeding 0.05% in adult *A. sticticus* reared from field-collected fourth instar larvae (Table S3). The most abundant FA were the monounsaturated oleic acid (18:1ω9) and hypogeic acid (16:1ω9), and saturated FA palmitic acid (16:0), which accounted for 19–27.4% of total FA. Amongst PUFAs, eicosapentaenoic acid (20:5ω3, EPA) and arachidonic acid (20:4ω6) and their precursors α-linolenic acid (18:3ω3, ALA) and linoleic acid (18:2ω6, LA), respectively, comprised between 2.4 and 4.9%. Finally, adult *A. sticticus* also had detectable levels of docosahexaenoic acid (22:6ω3, DHA), i.e. 0.11% (Table S3).

Discussion

Our isotopic analyses of key invertebrate consumers indicate that more than a decade of mosquito control in the lower Dalälven using a granular formulation of Bti is causing significant changes in the isotopic composition of key organism groups feeding at the base of floodplain food webs. Enchytraeid worms were characterised by significantly higher $\delta^{13}C$ values in treated relative to reference floodplains, and oribatid mites were significantly higher in $\delta^{15}N$ values. Similar trends that were near significance

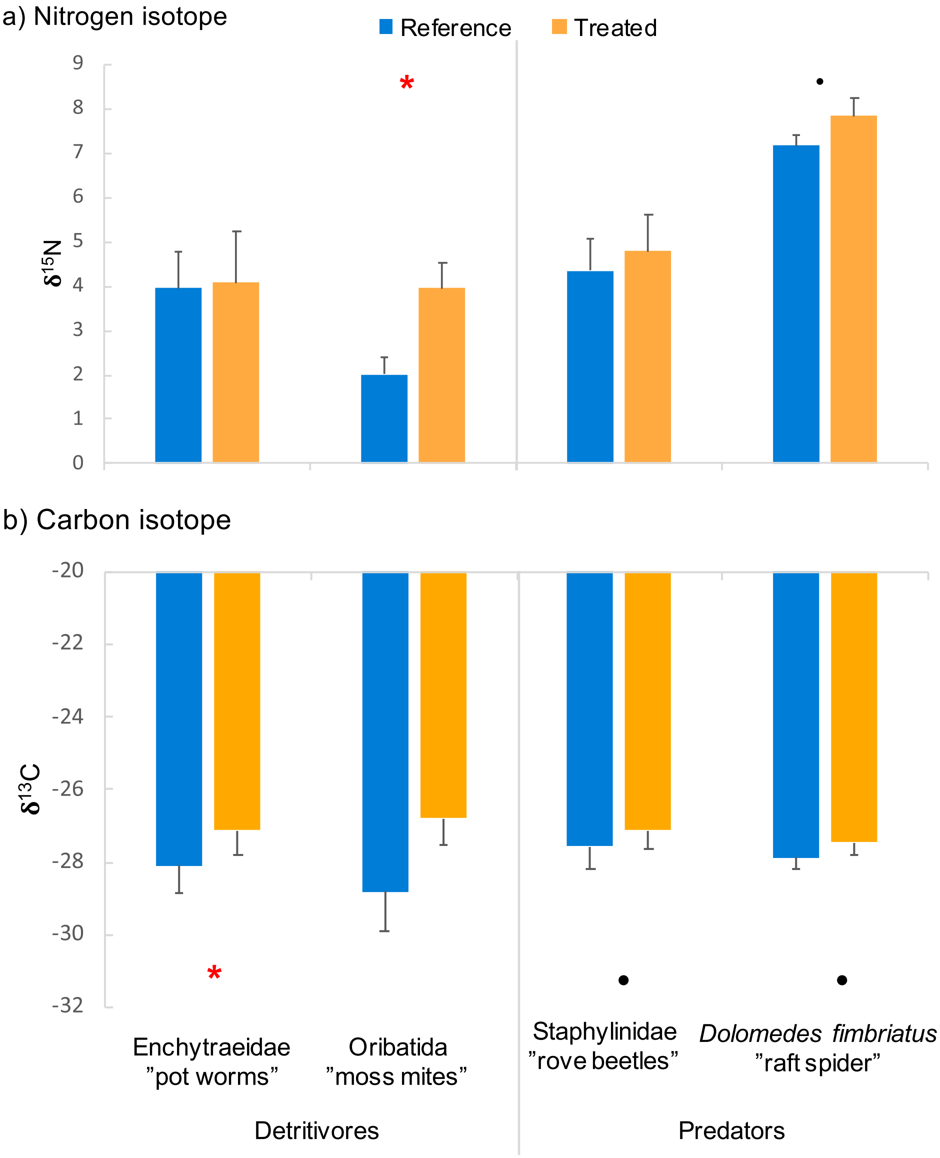


Fig. 5 Differences in mean ± 1 SE values for $\delta^{13}C$ (**a**) and $\delta^{15}N$ (**b**) of key consumer taxa in treated (orange) and reference floodplains (blue). Significance levels from two-sided paired *t* tests of differences (Table 2) indicated: $*p < 0.05$, $\bullet p < 0.1$

Table 2 Results from paired *t* tests for each taxon assessing whether δ13C and δ15N values differ between the treated and reference floodplains (two sided tests) and whether these values are elevated or lowered in treated relative to reference floodplains (one sided tests)

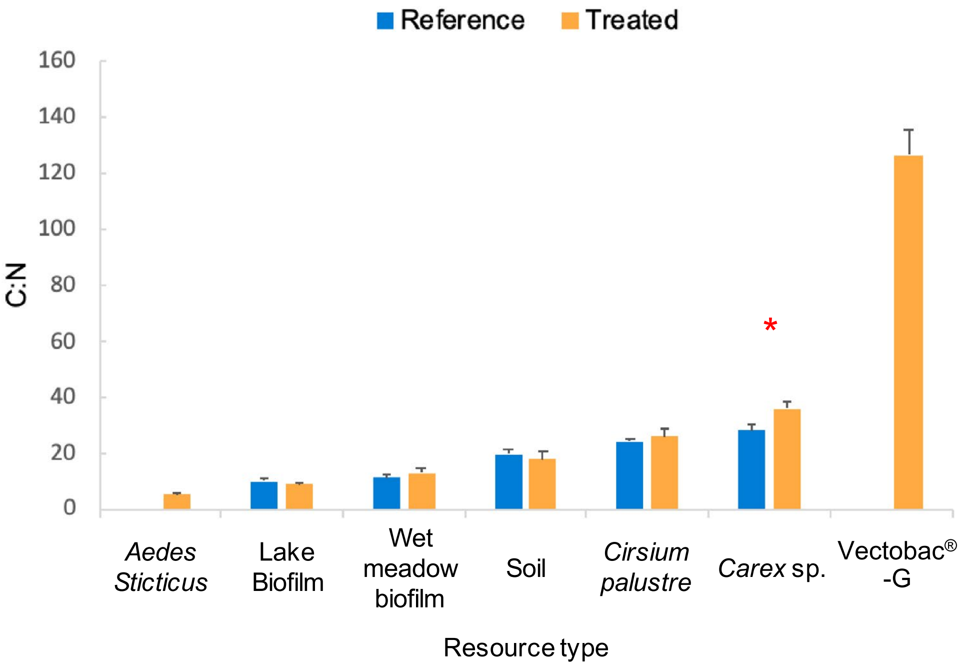
	Taxon	$\delta^{13}C$			$\delta^{15}N$		
		2-side $p>\vert t]$p	1 side $p>t$	1 side $p<t$	2-side $p>\vert t]$	1 side $p>t$	1 side $p<t$
Detritivores	Enchytraeidae	**0.011**	Ns	**0.005**	Ns	ns	ns
	Chironomidae	ns	ns	ns	ns	ns	ns
	Collembola	ns	ns	ns	ns	ns	ns
	Acari: Oribatida	ns	ns	ns	**0.021**	**0.011**	ns
Predators	Lycosidae	ns	ns	ns	ns	ns	ns
	Dolomedes fimbriatus	ns	ns	*0.094*	*0.069*	**0.034**	ns
	Acari: Mesostigmata	ns	ns	ns	ns	ns	ns
	Staphylinidae	ns	ns	*0.063*	ns	ns	ns

Note that no tests were conducted for the earthworms Aporrectodea or Diptera Tipulidae, since in both cases individuals were sampled from only 2 of the reference sites, and 3–4 of the treatment floodplains

All *t* tests conducted with 4 degrees of freedom

Significant tests with $p < 0.05$ highlighted in bold, other results near significance ($p < 0.1$) highlighted in italic

Fig. 6 C:N molar mass ratios of seven resources found in the reference (blue/dark bars) and treated (orange/light bars) floodplains, ordered from lowest to highest. Biofilm, soil and plant samples were all collected directly from the floodplains, and the mean ± SE of these resources is calculated at the floodplain level. *A. sticticus* were sampled prior to pupation from one of the treated floodplains only (Ista mire), whilst the Vectobac®-G sample represents the product used for mosquito control in the floodplains. The mean ± SE for the *A. sticticus* and Vectobac®-G are calculated based on three and five analytical replicates. Significant differences between floodplain types based on paired *t* tests indicated: $*p < 0.05$



were apparent in predacious staphylinid beetles and the raft spider *D. fimbriatus*. Finally, our Bayesian analyses of isotopic niche metrics indicate a 96.5% and 79% probability of divergence between floodplain types in the ranges of community $\delta^{15}N$ and $\delta^{13}C$ values. Variation in key soil characteristics (soil types, depth, isotopic signature) and the composition of ground cover amongst our floodplains were minimal, and did not differ systematically between floodplain types. Accordingly, we conclude these observed food web shifts are a consequence of the repeated, large-scale applications of VectoBac®-G in the treated floodplains, i.e. 3–5 times annually during all but 2 years over the period 2002–2014. Ecological mechanisms potentially explaining these results include (1) incorporation of the high $\delta^{13}C$ value corn particles, used as a vector in the VectoBac®-G product, into floodplain food webs, and (2) the mass mortality of *A. sticticus*, which results in high concentrations of dead larvae deposited on floodplain soils at local scales.

In the absence of mosquito control, the vast majority of *A. sticticus* larvae will emerge as adults, and subsequently disperse in search of mates and (in the case of females) blood meals (Fig. S2). Those that escape predation can then be expected to die after swarming (for males) and oviposition (which occurs in moist soils), with their corpses being distributed over a wide geographic area. Dispersal distances > 11 km have been documented for female *A. sticticus* (Mirta et al. 2011). In contrast, under a scenario of repeated Bti treatment events, adult abundances are reduced by 90–100% in the lower River Dalälven (Schäfer and Lundström 2014), thus preventing wider dispersal of mosquito biomass (Fig. S3). Instead, dead larval biomass remains concentrated in the remaining, temporary, standing water habitats after the floodwater recedes (Fig. S2). This dead biomass likely enters soil- and ground-based food webs, either as a result of scavenging from detritivores, or as a result of microbial degradation (Gratton et al. 2017; Hoekman et al. 2012). Our results indicate that dead *A. sticticus* larvae represent a labile and relatively nutrient-rich resource, characterised by a low C:N ratio and moderate concentrations of long-chain PUFAs (Table S4). When made available, such high-quality food resources are preferentially consumed by soil detritivores, altering their isotopic composition (Scheu and Schaefer 1998; Tiunov and Scheu 2004). The dead *A. sticticus* larvae accumulating in floodplains might also be attractive to predators and/or scavengers, including oribatid mites and staphylinid beetles (Schneider et al. 2004; von Berg et al. 2012).

The ground corn particles used in VectoBac®-G also represent an additional C input to the epigeal and soil-based food webs of the treated floodplains. VectoBac®-G has an extremely high C:N ratio, similar to that of some of the most nutrient-poor, naturally occuring, plant litter species (Frainer et al. 2015; Handa et al. 2014). However, the percentage of C in corn cob comprised of refractory lignin is relatively low, varying from c. 5–15% depending on growing conditions (Demirbaş 2005; Mourtzinis et al. 2014; Pointner et al. 2014). This is similar to the lignin content of those plant litter species categorised as “fast-decomposing” by Handa et al. (2014), and is also at the lower end of the range observed previously for floodplain herbaceous vegetation (9.6–29.4%, Britson et al. 2016). Furthermore, the VectoBac®-G granules also incorporate an unknown quantity of corn oil, used to aid attachment of Bti (Lundstrom et al. 2010), which might enhance the resource quality of the particles. Thus, although nutrient-poor compared with dead *A. sticticus* and meadow biofilms, the C characterising the corn substrate used in VectoBac®-G is likely to be relatively labile for decomposing organisms inhabiting a soil matrix that is otherwise rich in highly refractory (Curry and Schmidt 2007).

Carbon characteristics of resources and consumers

Freshwater biofilms generally have lower δ^{13}C values than terrestrial plants, reflecting the higher presence of light ^{12}C isotopes taken up during algal photosynthesis (Marty and Planas 2008). However, our biofilm samples were not as ^{13}C-depleted as expected, especially in the treated floodplains, given that clean algal samples often have δ^{13}C values < -30 (Marty and Planas 2008). It is, thus, likely that our aquatic biofilm samples also included particulate C of terrestrial origin. This "contamination" is a common problem in field-based aquatic isotope studies (Kupilas et al. 2016; Marty and Planas 2008). Interestingly, soil δ^{13}C values were on average 4% more negative than those of terrestrial plants. This likely reflects the long-term influence of flooding on the soils of the wet meadows. Each time the floodwaters recede, carbon derived from aquatic primary producers and thus depleted in ^{13}C is deposited on the floodplain (e.g. as stranded algae or submerged aquatic plants), thus reducing soil δ^{13}C (Hein et al. 2003; Tockner et al. 1999). Finally, our analyses confirm that the corn used as a substrate in the Bti control agent VectoBac®-G consitutes a unique C-source for the River Dalälven floodplains, with δ^{13}C values that are substantially higher than any other analysed resource.

Carbon isotopes of most consumers were similar to or lighter than the δ^{13}C values of aquatic biofilm, and did not closely match with any of the terrestrial resources. This provides further support for the importance of C derived from aquatic primary producers in these floodplain food webs, and points towards the capacity of organisms to select for C of aquatic origin. Aquatic derived C is typically more palatable (i.e. characterised by more labile C compounds, lower amounts of secondary plant compounds and higher nutrient content) than that arising from terrestrial leaf litter. Indeed, Lau et al. (2014) showed that autochthonous resources are the main driver of secondary production even in dystrophic lakes, as algae supply consumers with fatty acids that are essential for consumer growth and reproduction. The higher palatability of aquatic-derived resources to terrestrial consumers is further seen in the low C:N ratios of lake biofilms. As expected, dead *A. sticticus* also had a low C:N ratio, which is likely to make it attractive to decomposers and scavengers alike.

The δ^{13}C of newly emerged *A. sticticus* adults was highly variable amongst individuals, spanning the full range of terrestrial resources. Our PUFA analyses confirm *A. sticticus* consumes a mixed diet, with important long-chain PUFAs synthesised by algae (e.g. EPA 20:5ω3) making up around 5% of total FA, and the bacterial indicator FA vaccenic acid (18:1ω7) making up 2%. This is in line with values reported by Sushchik et al. (2013) for mosquito adults in floodplains in the Novosibirsk region in Russia, and those for detritivorous benthic invertebrates from boreal lakes, such as the isopod *Asellus aquaticus* (Lau et al. 2012). Finally, docosahexaenoic acid (DHA) is common in aquatic invertebrate predators (e.g. Lau et al. (2012)), and suggests that *A. sticticus* also preys on smaller invertebrates. Overall, these results suggest that much of the C ingested by the mosquitoes in their temporary floodplain habitats is terrestrial in origin, obtained through filtration of bacteria colonising particulate C (Merritt et al. 1992). However, the PUFA and isotope results indicate this diet is patchily supplemented with freshwater algal productivity, likely reflecting an uneven deposition of algal cells over the floodplains during flooding events (Hein et al. 2003; Tockner et al. 1999).

Consumer isotopes: comparing treated and reference floodplains

Transitions between trophic levels typically involve a δ^{15}N increase of about 3‰ (from 2.2 to 3.4 ‰) (Vander Zanden and Rasmussen 2001). Accordingly, our isotopic analyses suggest our sampled invertebrates spanned at least three trophic levels. The overall increase in the range of δ^{15}N values characterising consumers in the treated floodplains suggests that repeated applications of VectoBac®-G have increased the amount of fractionation involved in nutrient transfer through floodplain food webs, and hence increased food chain lengths. The marked increase in δ^{15}N values of oribatid mites suggests that some of this extra fractionation occurs at the base of these food webs, possibly mediated by protozoans and other micro/meiofauna that feed on bacteria and decaying organic matter (Griffiths 1994). The use of Bti has led to an increase in single-celled protozoans in soils of Dalälven floodplains (Östman et al. 2008). This result was attributed to reduced feeding by mosquito larvae on these organisms (Östman et al. 2008), but might also reflect the ready availability of dead mosquito larvae for protozoans to colonise. We, thus, hypothesise that the observed additional N-fractionation for the oribatid mites in treated floodplains reflects an increased transfer of N from decaying mosquito larvae to higher trophic levels, mediated at least in part through protozoans.

Enchytraeid worms were significantly higher in δ^{13}C in treated relative to reference floodplains. We sampled Enchytraeidae from the moist depressions in between grassy tussocks characterising our floodplains. These are the habitats where standing water is likely to persist longest after the floodwaters have receded, and hence are where both dead mosquito larvae and the corn particles comprising the bulk of the VectoBac®-G product are most likely to accumulate. Enchytraeidae are one of the main detritivore groups feeding on particulate C and its associated microbes in moist soils (Didden 1993), and are capable of searching out patches of more labile C within a soil matrix otherwise characterised by highly refractory C (Curry and Schmidt 2007). Accordingly, the increase in enchytraeid δ^{13}C values in the treated floodplains might be especially attributable to consumption of the corn particles, given these are substantially richer in ^{13}C values than any other floodplain resource. The trend for an overall reduced δ^{13}C range in consumers of treated floodplains suggests a shift from consumption of a broader diversity of C sources towards the readily available C delivered in the form of the VectoBac®-G corn particles, and/or the abundant C introduced locally by the mass morality of larval mosquitoes. However, more research is required to confirm the evidence of divergence in not only the δ^{13}C range but also total isotopic niche area (77% probability) and species spread (79% probability) between floodplain types observed in our study.

Quantities of VectoBac®-G compared with possible abundances of dead mosquito larvae

We are unable to definitively distinguish whether the shifts in the isotopic characteristics of soil- and ground-dwelling food webs between treated and untreated floodplains of the lower Dalälven are primarily attributable to the labile C introduced with VectoBac®-G, or the prevention of *A. sticticus* emergence and the subsequent accumulation of dead larvae on floodplain soils. An approach for separating these mechanisms is the use of isotopic mixing models for quantifying the contribution of different resources in consumer biomass (Layman et al. 2012; Phillips et al. 2014). However, isotopic mixing models are prone to strong biases when the isotopic range of potential resources falls well outside the niche space of consumers (Phillips et al. 2014), as is clearly the case with the corn particles comprising Vectobac-G. Nevertheless, the question of the impact of the corn particles is non-trivial, since alternative Bti products are available that do not use corn as the substrate. Here, we explore the potential for both mechanisms to change the isotopic composition of resources in floodplain soils using data on the C and N content of the corn granules and mosquitoes, respectively.

Our analyses reveal that VectoBac®-G consists on average of approximately 50% C (500 g/kg) and 0.5% (5 g/kg) N. At the typical application rate of 15 kg/ha (Schäfer and Lundström 2014), the spreading of VectoBac®-G in the lower Dalälven floodplains adds 7050 gC/ha, and 75 gN/ha per treatment event, all in a relatively labile, albeit nutrient poor, form. These figures accumulate with repeated applications in 1 year. After ten applications, 75 kg of C and 0.75 kg of N will have been added per hectare (though the final fate of these particles will depend on the influence of subsequent winds, rainfall and flooding). Estimates of mosquito biomass in treated and reference floodplains are more challenging, since we lack data on mosquito production per hectare. In one of the most extreme mass emergence years (2000), some 192,000 individual female mosquitoes were caught from June–August in carbon dioxide traps covering 99 ha of floodplains area. According to our analyses, an adult *A. sticticus* contains on average 0.416 mg C and 0.04 mg N. Upscaling these figures and assuming a sex ratio of 1:1 (Lounibos and Escher 2008), we estimate that the mosquito biomass captured in the traps in 2000 represented approximately 0.64 gC/ha and 0.14 gN/ha. This is, of course, an underestimate of emerging mosquitos, given that CO_2 traps only capture a fraction of all flying adults and are particularly inefficient at attracting males (McPhatter and Gerry 2017). If we assume that similar-size populations of mosquitoes instead had been killed as larvae, this C and N would remain concentrated in temporary pools in treated floodplains. There are clearly multiple sources of uncertainty in these calculations, including the lack of data on abundances of males, and uncertainties in the sampling efficiency of the carbon dioxide traps. Nevertheless, these figures serve to demonstrate the potential impact of dead mosquitoes on soil- and ground-based food webs compared with that of the distribution of the corn particles in VectoBac®-G. Ultimately, it is likely that the two effects are partially linked, as consumers that feed on dead mosquito larvae will also consume carbon from any VectoBac®-G remaining in the mosquito's digestive tract.

Conclusion

The findings of our spatially replicated field study point towards changes in the trophic structure of the soil- and ground-based food webs in the floodplains of the lower Dalälven, associated with the long-term, repeated applications (2002–2014) of the mosquito control agent VectoBac®-G. In a previous aquatic mesocosm experiment, Bti application as a suspension resulted in an increase in isotopic niche areas of predatory dragonflies (Allgeier et al. 2019a). In contrast, the effects of Bti on our terrestrial, floodplain communities were clearest at the base of the food web. This is exemplified by the increased δ^{13}C and δ^{15}N values of detrivorous enchytraeid worms and oribatid moss mites, respectively. Additionally, the increased δ^{15}N range characterising treated floodplain consumer communities indicates an increase in the number of trophic fractionation steps involved in the processing of nitrogen. We conjecture that the increase in the overall δ^{15}N range in the treated wetlands arises from an extra trophic step involved in decomposition of the dead mosquito larvae, which is likely to be mediated not only by the organism groups studied here, but also by other key organisms involved in the processing of detritus (e.g. protozoans, nematodes and microorganisms). In contrast, the increase in the δ^{13}C of enchytraeid worms might be more likely to reflect integration of the more palatable C originating from the corn particles composing the VectoBac®-G product, into soil- and ground-based food webs. Further research is required to assess these hypotheses.

The potential ecological significance of our observed shifts in isotopic values of some key consumer groups can be assessed by comparing with previous studies of terrestrial and other invertebrates living in close association with aquatic ecosystems. Riparian spider δ^{15}N values were 9% higher in an agricultural compared with forested catchment in New Zealand (Collier et al. 2002), and river restoration was associated with 7–11% increases in δ^{15}N values of riparian spiders and beetles (Kupilas et al. 2020). More marked 70–400% shifts in δ^{15}N values were observed in detritivorous millipedes, omnivorous beetles and predatory spiders living adjacent to rivers with marine-salmon-derived nutrient enrichment, relative to references (Hocking and Reimchen 2002; Rammell et al. 2021). Finally, 50–150% increases in δ^{15}N values were observed in benthic macroinvertebrates along gradients of increasing anthropogenic nutrient enrichment in Sweden (Bergfur et al. 2009; Frainer and McKie 2015), similar to observations elsewhere (Clapcott et al. 2010; Smucker et al. 2018). Differences in δ^{13}C values in the same studies ranged less widely, from 0 to 9% (anthropogenic nutrient enrichment, Bergfur et al. 2009; Frainer and McKie 2015), 0–4% (marine derived nutrients, Hocking and Reimchen 2002; Rammell et al. 2021) and 0–1% (river restoration, Kupilas et al. 2020). Collier et al (2002) observed a more marked 28–31% difference in δ^{13}C values between an agricultural compared with forested catchment in New Zealand, which is attributable to strong differences in tree cover that were not a factor in our study. Finally, Tiunov and Scheu (2004) found that enrichment of soil with refractory C (glucose) increased earthworm δ^{13}C values by 7%.

Accordingly, the 97% increase in δ^{15}N values observed in oribatid detritivore mites and the 3.5% increase in δ^{13}C observed for enchytraeid worms, fall within the mid-range of shifts observed previously in studies of key environmental drivers on riparian and floodplain invertebrate consumers. Furthermore, isotopic shifts in the range observed here have previously been linked to altered nutrient and energy flows, including across habit boundaries (Hladyz et al. 2011; Hocking and Reimchen 2002; Kupilas et al. 2016, 2020). On the other hand, statistical support for divergence in the isotopic characteristics of most other consumer groups (i.e. apart from Enchytraeidae and Oribatida) was weak, and the isotopic niche space occupied by our untreated and treated floodplain communities substantially overlapped. Thus, we suggest our results are best characterised as providing evidence that the food webs of our treated floodplains might be in transition, with effects of mosquito control manifesting unevenly amongst organism groups, trophic levels and floodplains. Further research with a greater level of replication and after more years of VectoBac®-G treatment is, therefore, required to establish how extensive and established the shifts observed here become, and to evaluate the potential wider implications for nutrient and energy cycles within floodplain ecosystems and linkages with adjacent habitats.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00027-023-00944-0.

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Author contributions BGM, WG and AT conceived and designed the study. AF and TN conducted field sampling and laboratory analysis. The data were analysed by BGM and AF. The first draft was prepared by BGM and WG. All authors contributed to draft revision.

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Data availability The datasets used and/or analysed during the current study are available as supplementary material.

Code availability Not applicable.

Declarations

Conflict of interest The authors declared that they have no conflict of interest.

Consent to participate Not applicable.

Consent for publication Not applicable.

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