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Research Article

Signature of Anthropogenic Impacts in Detritus Decomposition and the Freshwater Snail *Melanopsis buccinoidea*

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Abstract: The present study aims to investigate the anthropogenic impacts of crop farming, fertilizing practices, and pesticide use in riparian zones on detritus decomposition and on feeding of *Melanopsis buccinoidea* (Olivier, 1801) on detritus by employing the stable isotope method. For this purpose, (1) Non-treated, (2) fertilizer-treated and (3) pesticide-treated apple *Malus pumila* (Mill) and corn (*Zea mays* L.) leaves were used in duplicate as detritus material and left to decompose for about two and a half months. During the experiment, samples were collected from the detritus every ten days to conduct stable carbon and nitrogen analyses., *M. buccinoidea* individuals were then placed in the media containing different detritus types treatments for 25 days and the stable carbon and isotope values in the muscle tissues of *M. buccinoidea* were measured periodically. The $\delta^{13}C$ values measured in the apple and corn leaves over a period of two and a half months were found to fluctuate and increased in each of the three treatments depending on the time of exposure and only in the fertilizer-treated corn leaves. The $\delta^{15}N$ values of detrita in both plants were observed to have increased in the natural/non-treated and pesticide-treated conditions but decreased in the fertilizer-administered media during two and a half months. Conductivity and pH values in both plants were found to be high and low, respectively. In this medium, $\delta^{15}N$ ratios were lower. The $\delta^{13}C$ values of the *M. buccinoidea* kept in the corn detritus were found to be lower than those of the individuals kept in the apple detritus. It was remarkable to observe during the 25-day experiment that the $\delta^{13}C$ values of the *M. buccinoidea* kept in the corn detritus tended to decrease.

Keywords: Macroinvertebrate, Stable Isotopes, Anthropogenic Impact, Aquatic Ecology

Antropojenik Etkilerin Detritus Ayrışım Süreci ve Bir Akarsu Organizması Olan *Melanopsis buccinoidea* Örneğinde İmzası

Özet: Antropojenik etki olarak tarım faaliyetlerinden akarsu kenarlarında yetiştirilen kültür bitkileri, gübre ve pestisit uygulamalarının detritus ayrışma sürecindeki etkilerini ve akarsuda yaşayan ve bu detritus ortamında beslenen *Melanopsis buccinoidea* (Olivier, 1801) örneğinde akarsu organizmaları üzerindeki etkilerini kararlı izotop yöntemi ile göstermek amaçlanmıştır. Detritus kaynağı olarak kültür bitkilerinden elma (*Malus pumila* Mill) ve mısır (*Zea mays*L.) yaprakları (1) hiçbir uygulama yapılmamış, (2) gübre ve (3) pestisit ile muamele edilmiş olmak üzere ikişer tekrarlı altı grup olarak yaklaşık 2.5 aylık bir ayrışma sürecine tutulmuştur. Deney sürecinde her on günde bir detritus örneği alınarak $\delta^{13}C$ ve $\delta^{15}N$ analizi yapılmıştır. Daha sonra *M. buccionidea* bireyleri bu altı farlı ortamdan alınan detritus içeren ortamlarda 25 gün boyunca tutulmuş ve belirli aralıklarla *M. buccinoidea* bireylerinin kas dokularındaki kararlı karbon ve izotop değerleri ölçülmüştür. İki buçuk ay boyunca elma ve mısır yapraklarında ölçülen $\delta^{13}C$ değerlerinin her üç uygulamada da zamana bağlı bir dalgalanma ile birlikte artış gösterdiği mısır bitki yapraklarında ise sadece gübre uygulamasında artma saptanmıştır. İkibuçuk ay boyunca, her iki bitkide de detritusların $\delta^{15}N$ değerinde doğal ve pestisit koşullarında bir zenginleşme, gübre koşullarında bir düşme görülmüştür. Her iki bitki için gübre ortamındaki iletkenliğin yüksek, pH değerinin ise düşük olduğu belirlenmiştir. Bu ortamda ölçülen ortalama $\delta^{15}N$ oranlarının da düşük olması dikkat çekicidir. Mısır detritus ortamında tutulan *M. buccinoidea* bireylerinin $\delta^{13}C$ değerinin elma detritus ortamında tutulan bireylere göre daha düşük olduğu bulunmuştur. Yirmibeş günlük deneme süresince mısır detritus ortamında tutulan *M. buccinoidea* bireylerinin $\delta^{13}C$ değerlerinde bir azalma eğilimi olması ilginçtir.

Anahtar Kelimeler: Makroomurgasız, Kararlı İzotoplar, Antropojenik Etki, Akuatik Ekoloji

Introduction

Agricultural activities in riparian areas result in a considerable amount of nutrient flow (Vannote, Minshall, Cummins, Sedell, & Cushing, 1980). Type of the plant grown, type and amount of the fertilizer and pesticides used are listed among the highlighted anthropogenic components in this nutrient flow that are likely to affect the aquatic organisms. Increased decomposition rates of the organic waste materials introduced into the stream system are associated with increased invertebrate diversity (Jonsson & Malmqvist, 2000). Besides, Masese et al. (2014) reported that alien species grown on the banks of headwater streams change a stream's physico-chemical properties and functional composition of invertebrates and that stream communities are shaped based on the species of the cultivated plant. The type of introduced organic material in a stream may also cause changes in the trophic design of the functional nutrient groups in the stream over time.

Recently, stable isotopes have been commonly used in the studies on organic material flow and food cycle structures in aquatic ecosystems and operationalized as a tool to identify a trophic structure (Adams & Sterner, 2000; Fry, 2006; Peterson, 1999). The $\delta^{13}C$ values which C3 and C4 plants have assimilated in their tissues are known to be different since they photosynthesize with different enzymatic reactions (Lin & Ehleringer, 1997). The $\delta^{13}C$ ratios in the carbon assimilated by C3 plants in terrestrial ecosystems range from -22‰ to -32‰, while the values concerning C4 plants vary between -8‰ and -18‰ (Schweizer, Fear, & Cadisch, 1999). This difference is observable in the food web. For example, highly variable isotope values have been reported in the muscle tissues of the snail, *Pomacea lineata*, , due to its diet heavily relying on C3 and C4 macrophytes (Fellerhoff, 2002). In each step of the food web, an enrichment of 3-4‰ was realized in the $\delta^{15}N$ ratio (Fry, 2006). It is known that fertilization causes an excess of nitrogen in streams (McClelland, Valiela, & Michener, 1997) and pesticide use alters the isotopic ratio of carbon and nitrogen in freshwaters (Kiriluk, Servos, Whittle, Cabana, & Rasmussen, 1995). Accordingly, this suggests the emergence of various isotopic compositions during the detritus decomposition in water depending on the types of riparian C3 and C4 plants. Addition of fertilizers and pesticides to this process potentially affect the isotopic composition that is likely to appear meanwhile. Moreover, shifts might occur in the trophic position of a heterotrophic organism that will be raised in this different composition.

The present study aims to investigate (1) whether a differentiation in the $\delta^{13}C$ and $\delta^{15}N$ ratios according to C3 and C4 plants in the decomposition of detritus in water will occur and (2) how the isotopic compositions of carbon and nitrogen will be modified upon the introduction of fertilizers and pesticides. Furthermore, the present study also attempts to find out (3) what kind of a change the carbon and nitrogen isotopic composition of a heterotrophic organism will undergo in a detritus media treated with different plants, fertilizers, and pesticides during decomposition. Among the sources of critical anthropogenic effects are the difference of crop types from the natural vegetation and fertilizers and pesticides used for agricultural purposes in riparian areas. The probable effects as allochthonous energy source input into streams can be revealed by identifying the changes in the $\delta^{13}C$ and $\delta^{15}N$ stable isotope compositions. This is believed to make considerable contributions to the management plans for the sustainability of streams.

Material and Method

Material

Apple (*Malus pumila* Mill) and corn (*Zea mays L.*) leaves were used as detritus material and *Melanopsis buccinoidea* (Olivier, 1801) as the heterotrophic organism. Apple as C3 plant and corn as C4 plant are widely grown in riparian agricultural zones. *M. buccinoidea* is an invertebrate species and it is commonly found in agricultural canals, swamps (Farahnak et al., 2006), creeks, ponds and springs (Heller & Abotbol, 1997) and was selected owing to its wide distribution across Çanakkale province.

The leaves of apple and corn grown as cultivated plants were retrieved in an orchard in Kızılkeçili Village of Çanakkale province, to which no pesticide was administered. The leaf samples were collected aseptically and placed in transparent sterilized bags for transportation to the laboratory. *M. buccinoidea* samples were collected in the Tuzla creek in Assos, Çanakkale, and transferred to the laboratory in plastic jars filled with ambient water.

Preparation of the experimental setups with the plant material

21 liters of ambient water (in plastic bottles) was sampled from the water canal on the Terzioğlu Campus of Çanakkale Onsekiz Mart University. After measuring the dissolved oxygen (DO) (mgL^{-1}), pH, conductivity (I) (μScm^{-1}), temperature (S) (ºC), NO3 (mgL^{-1}) and PO4 (mgL^{-1}) the water was filtered through a strainer with 1x1 mm holes, stirred with a spatula, and poured into six 5-liter plastic jars, each to hold 3.5 L of water. Air pumps were connected to each 5 L plastic jar to aerate the water. The collected corn and apple leaves were rinsed with tap water to eliminate the likely presence of organisms and desiccated for 24 h. Then 20 g of leaves were put in the jars filled with the water samples. The apple leaves were put in the three of the six 5-liter plastic jars and the corn leaves in the other three. 20 g of NPK 15:15:15 fertilizer and 0.02 g of GOLDBEN insecticide ((E)-N1 -[(6-chloro-3-pyridyl) methyl]-N_2 -cyano-N_1 -methyl acetamidine, $C_{10}H_{11}ClN_4$) were

diluted and sprayed in each container taken from apple and corn sets. All the experiment setups were kept at the same conditions at ambient temperature.

Experiments with plant material

The experiment lasted from 05 August 2015 to 19 October 2015. Within the course of the experiment one liter of water was weekly added in each jar to compensate for evaporation loss and the water samples taken every ten days were analyzed to measure the aforementioned parameters. Some amount of detritus was collected from each jar with sterilized tweezers every ten days and the detritus samples dried on an aluminum foil in a drying oven at 60°C for 24h were labelled and kept in a desiccator for the stable isotope analysis.

Experiments with *M. buccinoidea*

M. buccinoidea individuals transferred to the laboratory were placed in a single aquarium and kept in *in vitro* conditions by adding the canal water to its ambient water for three days. 24 individuals at a time were placed in each of the six 1.5 L plastic jars as accompanied by 1 L of water and each jar was aerated with an air pump. The aforementioned parameters of the water samples in which the specimens kept were measured. No nutrient was fed to the specimens for three days. Three days later, 10 g ± 0.01 g of the detritus taken from the experiment setups which were left to decompose for a month was then introduced into the jars where the *M. buccinoidea* individuals were kept according to their tags.

Operations in the experiment setups with *M. buccinoidea*

The experiment lasted from 05 September 2015 to 19 September 2015. 500 ml of water was added in the jars to compensate for evaporation loss. The water was analyzed three times; at the beginning, middle, and end of the experiment to measure the above mentioned parameters. Individuals were taken out of each jar on 16 September 2015, 20 September 2015 and 28 September 2015 to conduct the stable isotope analysis and the dissected footmuscle tissues were placed on a piece of aluminum foil to dry them at 60°C for 24 h and then stored in a desiccator.

Water analyses

The physicochemical values of the water in the setups containing detrita and *M. buccinoidea* individuals were measured every 10 days and the nitrogen and phosphate analyses were conducted with spectrometry (Spectroquant Pharo 300). Temperature, pH, and dissolved oxygen were measured with WTW Multi 340i/SET model probes.

Preparation of plants and animals for stable isotope analysis

The samples preserved in a desiccator were homogenized with a micro dismembrator and 3 ± 0.005 mg plant and 1 ± 0.005 mg animal samples were weighed using a digital scale (Sartorius). Samples were then tagged onto tin containers in ELISA containers and sent to University of California at Davis Laboratory. For the analysis of the samples whose 13C/12C and 15N/14N ratios were measured with mass spectrophotometry, Pee Dee Belemnite limestone as the standard material was used for carbon and atmospheric nitrogen gas for nitrogen. The ratio of the heavy isotopes to light isotope (R) was calculated for 13C/12C and 15N/14N by employing the following formula and expressed in “per mille”.

$$\delta X = \left[\left(\frac{R_{sample}}{R_{standard}}\right) - 1\right] \times 1000$$

where δX represents $\delta^{13}C$ and $\delta^{15}N$’ (Fry, 2007).

Data evaluation

Because the data were not normally distributed, the Kruskal-Wallis test was employed for the apple and corn plants individually to investigate whether there was a statistically significant difference between the mean $\delta^{13}C$ and $\delta^{15}N$ values measured in the detritus between the interventions during the decomposition. Whether there were time-related differences in isotope compositions was tested with the Kruskal-Wallis test for each treatment and plant species (Sokal & Rohlf, 1995).

Analysis of Variance (ANOVA) was employed to test whether there was a significant difference in the mean values of the physicochemical parameters measured in the setups with apple and corn leaves. The Levene’s test yielded homogeneously distributed data except for the conductivity and phosphate parameters. Because the conductivity data were not homogeneous, a square root transformation was employed before analysis, while the phosphate values were compared by using the Kruskal-Wallis test. The relationship between the physicochemical parameters and isotopic compositions were calculated with Spearman’s Correlation.

Kolmogorov-Smirnov test and Levene's test were used to understand whether the$\delta^{13}C$ and $\delta^{15}N$ values measured in the muscle tissues of the all *M. buccinoidea* individuals were normally distributed and homogenous. Then, the significance of the difference between the mean $\delta^{13}C$ and $\delta^{15}N$ values concerning *M. buccinoidea* individuals in terms of treatments and plant types was tested with the three-factor (time, plant, and treatment) ANOVA and a confidence interval of 95% was set to determine the significance.

Results

Isotopic compositions of the detritus

The mean stable isotope ratio was calculated to be $\delta^{13}C$ = -29‰ ± 0.45, $\delta^{15}N$= 1.25‰ ± 0.70 for the apple leaves and $\delta^{13}C$ = -13.70‰ ± 0.62, $\delta^{15}N$= 11.10‰ ± 6.72 for the corn leaves. No statistically significant between-treatment difference was observed in the $\delta^{13}C$

and $\delta^{15}N$ ratios measured in the decomposition process of the apple leaves ($X^2 = 4.00$; df = 2; $p>0.05$; $X^2 = 4.88$; df = 2; $p>0.05$). A statistically significant between-treatment difference was observed in the $\delta^{13}C$ and $\delta^{15}N$ ratios measured in the decomposition process of the apple leaves ($X^2 = 7.38$; df = 2; $p<0.05$; $X^2 = 31.26$; df = 2; $p<0.001$).

The analysis of the $\delta^{15}N$ ratios measured in the samples with apple and corn taken from the three treatment groups (non-treated, fertilizer-treated, and pesticide-treated) on different dates revealed that the $\delta^{15}N$ ratios were low in the fertilizer-administered sample of both plants. The $\delta^{15}N$ ratios measured in the decomposition process of the apple leaves were calculated to be 1.53‰ ± 0.22 and 1.35‰ ± 0.15 in the natural and pesticide-treated samples, respectively, whereas 0.89 ‰ ± 0.13 in the samples with fertilizer. The $\delta^{15}N$ ratios measured in the decomposition process of the corn leaves were calculated to be 15.03‰ ± 1.05 and 15.93‰ ± 1.42 in the natural and pesticide-treated samples, respectively, whereas 3.30 ‰ ± 0.16 in the corn samples with fertilizer.

The $\delta^{15}N$ values of the samples collected periodically from the apple leaves in the non-treated setup were found to have fallen in the first three samples, but there was a constant increase in the results of the remaining five samples. A significant difference was observed in view of all the eight dates $X^2=14.51$, df=7, $p<0.05$). No increasing or decreasing regularities were observed between the mean $\delta^{15}N$ values of the samples periodically collected from the apple leaves setup with fertilizer and pesticide on the eight dates and no difference was found out between the measured values ($X^2=13.58$, df=7, $p>0.05$; $X^2=14.16$, df=7, $p<0.05$).

In the samples periodically collected from the non-treated and pesticide-treated corn group on the eight dates, a linear increase in the nitrogen values, except for the decrease in the last sample, was observed ($X^2=14.65$, df=7, $p<0.05$; $X^2=13.75$, df=7, p=0.05).

The decrease realized on the dates of the last sample was considered to have resulted from the possible mold and fungus formation (Newell et al., 2000; Henn and Chapela, 2001). No statistically significant difference was discovered between the $\delta^{15}N$ ratios in the samples from the fertilizer-treated corn leaves ($X^2=13.23$, df=7, $p>0.05$).

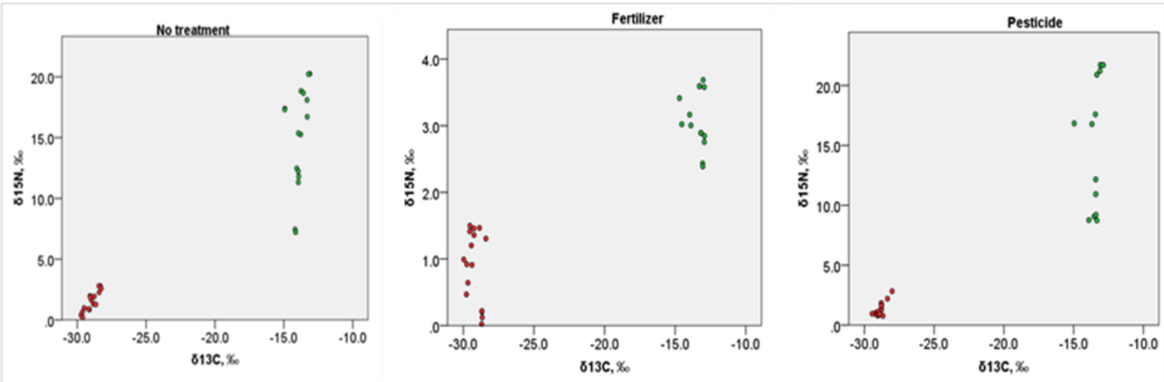

Figure 1. The $\delta^{13}C$ and $\delta^{15}N$ isotopic compositions of apple and corn leaves during the decomposition processes (red circle apple, green circle corn)

Isotopic decomposition of the detritus

The $\delta^{13}C$ values measured in the setups containing apple leaves and subjected to fertilizer administration were found to exhibit time-dependent variations ($X^2=14.743$, df=7, $p<0.05$; $X^2=13$, df=7, $p=0.05$). The analysis of the $\delta^{13}C$ values of the samples collected from the apple leaves treated with pesticide on eight different dates yielded no statistically significant different between the samples except for the ones collected on the last date ($X^2=11.1$, df=7, $p>0.05$).

The analysis of the $\delta^{13}C$ values of the non-treated corn leaves showed that the values gradually increased as from the first sample but decreased in the last ($X^2=14.70$, df=7, $p<0.05$). An increase was observed among the $\delta^{13}C$ values of the corn leaves retrieved from the fertilizer-treated setup on eight different dates ($X^2=14.70$, df=7, $p<0.05$). The samples extracted from the corn leaves with pesticide on these eight dates exhibited no statistically significant differences in the $\delta^{13}C$ values in terms of time ($X^2=11.72$; df=7, $p>0.05$).

Physicochemical parameters

The mean values (±SD) of the physicochemical parameters measured every ten days in the experiment setups of the three treatments with apple and corn leaves are presented in Table 1. The analysis of the differences among the three treatments of apple and corn plants indicated differences between the treatments in terms of pH ($F_{apple}=8.45$; df=2; p= 0.003; $F_{corn}=14.59$; df=2; $p<0.001$) and conductivity ($F_{apple}=1285.6$; df=2; $p<0.000$; $F_{corn}=254.4$; df=2; $p<0.001$). Accordingly, conductivity and pH values in both plants were revealed to be high and low, respectively. It is remarkable that the mean $\delta^{15}N$ ratios measured in this medium were low. In consideration of the physicochemical parameters, a strong positive relationship was found between the pH value measured in the detritus with apple and corn plants and the $\delta^{15}N$ ratios (r=0.601; r=0.595; n=15; $p<0.05$).

Moreover, a strong correlation was observed between the $\delta^{15}N$ and conductivity values measured in the corn plants ($r=0.805$; $n=15$; $p<0.05$).

Table 1. Physicochemical parameters measured in apple and corn detritus media.

Parameters	Plant	Treatment		
		Control	Fertilizer	Pesticide
$\delta^{13}C$	*M. pumila*	-28.97 ± 0.50	-29.22 ± 0.50	-28.85 ± 0.32
	Z. mays	-13.88 ± 0,55	-13.49 ± 0.61	-13.00 ± 0.63
$\delta^{15}N$	*M. pumila*	1.53 ± 0.86	0.89 ± 0.54	1.35 ± 0.59
	Z. mays	15.04 ± 4.32	3.28 ± 0.64	15.99 ± 5.51
Nitrate	*M. pumila*	3.49 ± 4.10	2.62 ± 1.84	2.18 ± 0.93
	Z. mays	4.24 ± 1.91	3.20 ± 2.24	3.32 ± 2.09
Phosphate	*M. pumila*	4.74 ± 0.35	5.0 ± 0.0	4.82 ± 0.40
	Z. mays	5.07 ± 0.10	5.00 ± 0.0	5.04 ± 0.10
pH	*M. pumila*	7.62 ± 0.56	6.14 ± 0.97	7.67 ± 0.60
	Z. mays	7.76 ± 0.48	6.02 ± 0.91	7.84 ± 0.50
Dissolved Oxygen	*M. pumila*	5.05 ± 2.38	4.36 ± 3.10	4.22 ± 2.39
	Z. mays	5.14 ± 2.57	5.35 ± 2.62	5.38 ± 2.37
Conductivity	*M. pumila*	1030.29 ± 61.59	7985,7 ± 500,70	1054.71 ± 89.46
	Z. mays	1137.97 ± 503.19	7955,7± 996.70	1269 ± 91.49
Temperature	*M. pumila*	26.0 ± 2.51	25.89 ± 2.59	25.61 ± 2.48
	Z. mays	25.47 ± 2.97	25.67 ± 2.63	25.4 ± 2.67

Isotopic compositions of the *M. buccinoidea* individuals

The $\delta^{13}C$ and $\delta^{15}N$ ratios in the *M. buccinoidea* individuals were found to range from -24.9‰ to -22.2‰ and from 10.6‰ to 12.1‰, respectively. Generally speaking, the $\delta^{13}C$ ratios (-24.4‰) in the muscle tissues of the individuals kept in the corn detritus were lower than those kept in the apple detritus (-23.5‰) (Fig. 3). A difference of 1.22‰ was revealed between the $\delta^{13}C$ ratios of the individuals kept in the non-treated apple and non-treated corn media, while a difference of 0.33‰ and 1.27‰ was discovered in the media with fertilizer and pesticide, respectively. Broadly, although the $\delta^{15}N$ ratios of the individuals kept in the natural apple and corn detritus exhibited similarities, an increase concerning the individuals kept in the apple detritus with fertilizer was observed in comparison with the individuals preserved in the corn detritus with fertilizer. However, a decrease was discovered in the $\delta^{15}N$ composition of the individuals left in the apple detritus with pesticide in contrast to the pesticide-treated corn composition (Fig. 3). The three-way ANOVA indicated that the plant type and process were effective in the variations of the $\delta^{13}C$ ratios of the *M. buccinoidea* individuals (Table 2). Nevertheless, the plant type and treatment collectively were found to affect the $\delta^{15}N$ composition (Table 2). A decrease of 1.22‰, 0.33‰, and 1.26‰ was observed in the $\delta^{13}C$ ratios of the *M. buccinoidea* individuals kept in the non-treated, fertilizer-treated, and pesticide-treated corn detritus, respectively, in comparison with those of the ones kept in the apple detritus.

However, these differences are very low and a difference of 0.05‰ was revealed between the $\delta^{13}C$ ratios of the *M. buccinoidea* individuals kept in the natural apple and natural corn media, while a difference of 0.2‰ and 0.73‰ was discovered in the media with fertilizer and pesticide, respectively.

A decrease by 1.19‰ – 0.92‰ and an increase of 0.33‰ - 0.99‰ in the mean δ13C values measured in the muscle tissues of the M. buccinoidea individuals kept in the apple and corn detritus with different treatments, respectively, were observed to have occurred in terms of plant type and treatment over 25 days (Fig. 4). After the 25-day treatment, the highest decrease (by 1.19‰) was detected to have occurred in the corn-fertilizer media, which was followed by the non-treated apple media (1.32‰). The highest rise (0.99‰) was found in the apple-fertilizer media. Similarly, a decrease of 0.74‰-0.16‰ and an increase of 0.42‰-0.89‰ were calculated in the δ15N values.

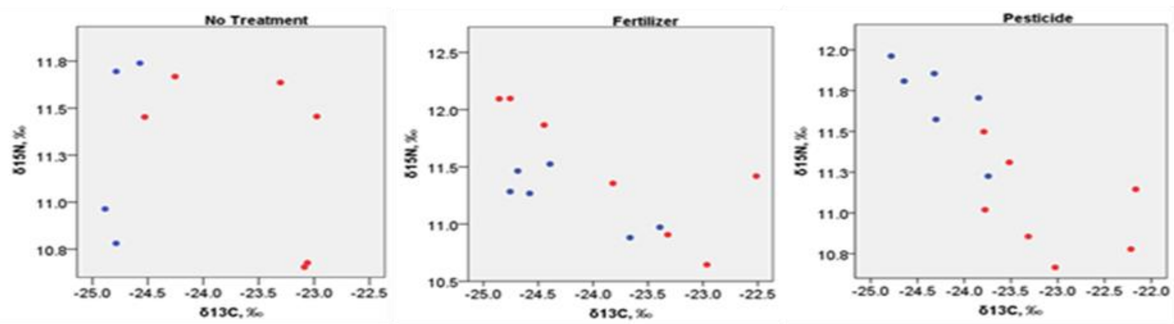

Figure 3. The $\delta^{13}C$ and $\delta^{15}N$ compositions of *M. buccinoidea* specimens kept in apple and corn detritus media (red circle apple, green circle corn)

Table 2. Effects of treatment and plant type stable C and N isotope composition of *M. buccinoidea*

Source	$\delta^{13}C$			$\delta^{15}N$		
	df	F	P	df	F	P
Plant	1	59.91	0.000	1	3.36	0.083
Treatment	2	8.97	0.107	2	0.06	0.942
Date	2	11.39	0.001	2	1.30	0.297
Treatment * Plant	2	11.28	0.001	2	8.25	**0.003**
Treatment * Date	4	2.84	0.053	4	1.17	0.355
Plant * Date	2	1.94	0.000	2	1.31	0.294
Plant * Treatment * Date	3	4.34	0.017	3	10.09	**0.000**

Isotopic compositions of the detrita and *M. buccinoidea* individuals

The $\delta^{13}C$ ratios measured in the muscle tissues of the *M. buccinoidea* individuals kept in the apple detrita indicated an enrichment varying between 4.7‰ and 6.0‰ in comparison with the values measured in the detritus media. This enrichment ranges between 9.6‰ and 10.6‰ in the $\delta^{15}N$ values. A similar enrichment (by 8.1‰) was observable in the fertilizer-treated corn detritus and in the $\delta^{15}N$ values. Nevertheless, a

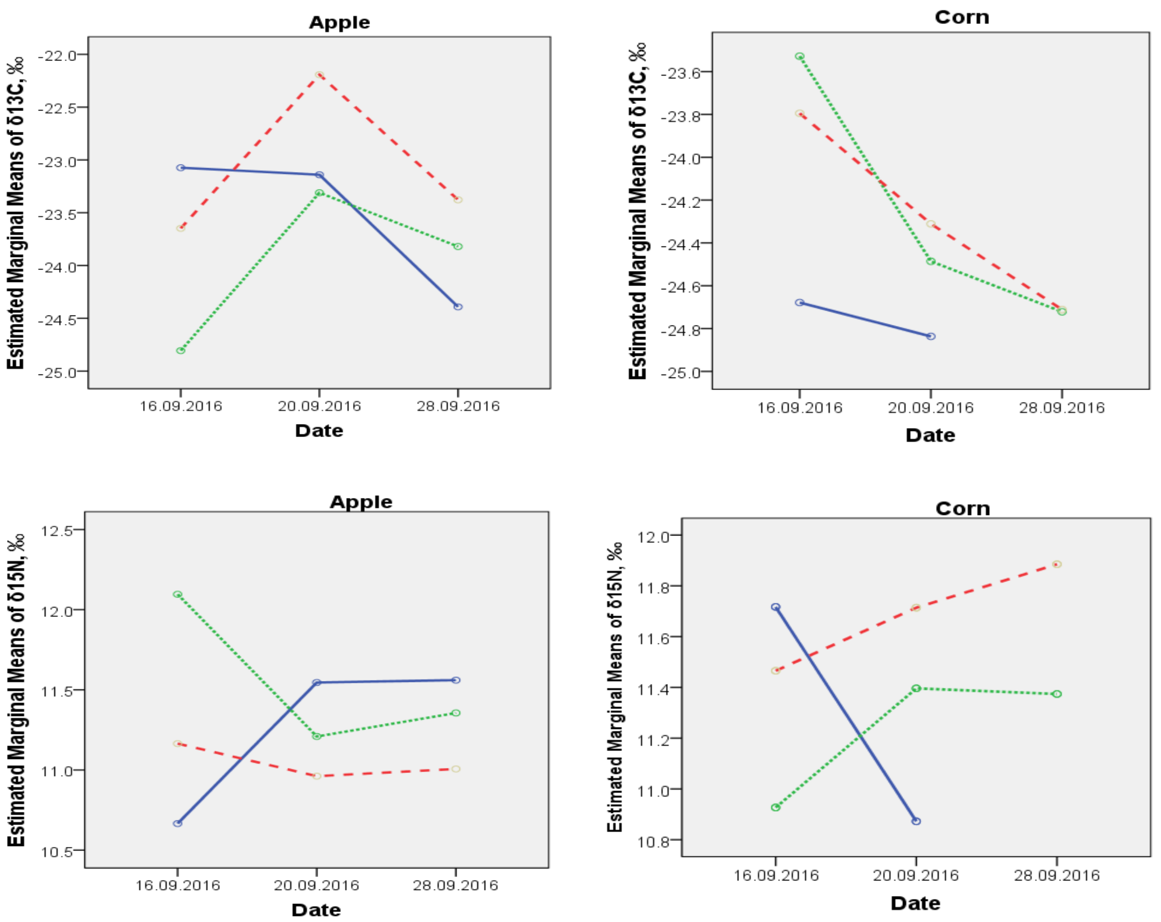

Figure 4. Estimated marginal means of $\delta^{13}C$ and $\delta^{15}N$ ratio of *M. buccinoidea* at apple and corn detritus media (blue: no treatment; green: fertilizer; yellow: pesticide)

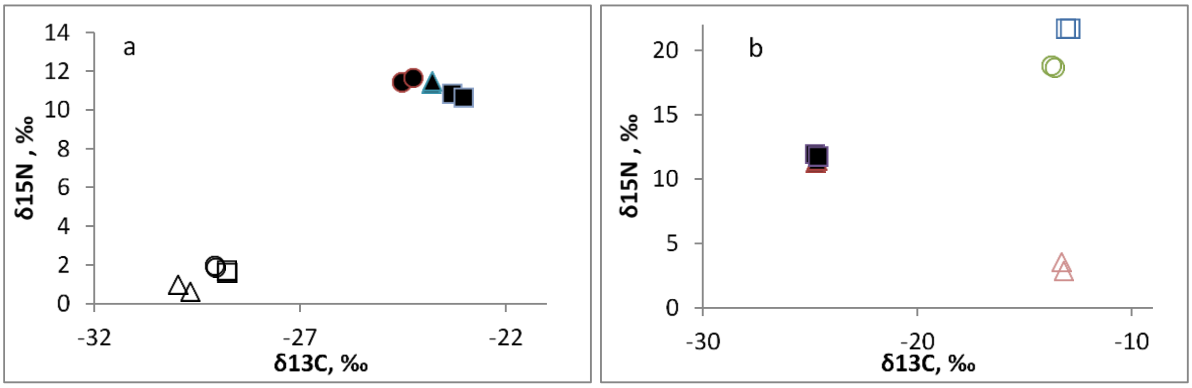

Figure 5. The isotopic compositions of apple (a) and corn(b) detritus (empty) and *M. buccinoidea* specimens (full). (○ Natural, Δ Fertilizer, □ Pesticide)

decrease was realized to have occurred in the carbon and nitrogen isotope compositions measured in the *M. buccinoidea* individuals kept in the corn detrita in comparison with the detritus media.

Discussion

The $\delta^{13}N$ and $\delta^{15}N$ ratios measured in apple and corn leaves are congruent with the data available in the related literature but were observed to be lower than the ones calculated by Hilderbrand et al., 1998; Lin and Ehleringer, 1997). In consideration of time and treatments in this study, the $\delta^{15}N$ ratio of the corn plant was found to exhibit a broad variation (2.39‰ to 21.71‰). Similar to the variation of 19.32‰ in the $\delta^{15}N$ ratios, Cloern, Canuel, & Harris (2002) reported a variation of 16.3‰ calculated in the halophilous wetland C4 plants in their natural habitats. The anthropogenic nitrogen inputs lead to a wide variation in the $\delta^{15}N$ composition particularly in the case of corn plant.

A time-dependent difference was observed in the isotopic compositions in all the treatments during the decomposition of both plant types. While an enrichment was observed in the $\delta^{13}C$ ratio during the detritus decomposition of the apple leaves, a depletion was realizable in the non-treated and pesticide-treated detrita during the decomposition of the corn leaves and an enrichment in the fertilizer-treated detrita. Likewise, the literature reports an increase in the $\delta^{13}C$ value during the decomposition of the *Pinus resinosa* leaves (Melillo et al., 1998), a decrease of around 2‰ in the $\delta^{13}C$ value during a one-year incubation of the seagrasses (Fourqurean & Schrlau, 2003), and also no significant change in the isotopic signature during the decomposition of *Desmodium ovalifolium* and *Brachiaria humidicola* (Schweizer et al., 1999), which suggest that the change in isotopic signature occurring in the decomposition can be associated with plant type.

Mc Clelland et al. (1997) note that the NO_3^- input in the system resulting from human and animal waste has led to an enrichment (10 to 20 ‰) in the $\delta^{15}N$ ratio in groundwater and the synthetic fertilizer introduction has caused a decrease (-3‰ to +3‰) in the $\delta^{15}N$ ratio. Similarly,Nishida & Sato, (2015)indicated a low $\delta^{15}N$ value of synthetic fertilizers. The lower $\delta^{15}N$ ratios of 0.64‰ and 11.74‰ in the apple and corn plants, respectively, in the fertilizer-treated setups of the present study are similar to the entries available in the literature.

The relationship between the pesticide amounts in freshwaters and $\delta^{15}N$ values has been discussed (Guo et al., 2008), yet no statistically significant relationship has been reported. Likewise in this research study, no significant difference was found between the $\delta^{15}N$ ratios measured in apple and corn leaves treated with pesticide and the values obtained from the control samples. In this study, the absence of a significant difference in the $\delta^{15}N$ ratios measured in relation to the pesticide treatment in comparison with the control group can be associated with the low dose used towards bioaccumulation and insufficient accumulation in the leaves. Also the half-life of the pesticide may play a role on the insufficient accumulation of pesticide in the leaves.

The $\delta^{13}C$ ratio measured in the corn detritus (-13.7‰) was found to be much higher than the ratio measured in the apple detritus (-29.01‰), which is congruent with what is available in the literature(Hauer & Lamberti, 2007). Assuming that the *M. buccinoidea* individuals kept in the above conditions fed on the detritus in the media, the values pertaining to isotopic compositions of the individuals kept in the apple and corn detrita are expected to be closer to these values (Fry, 2006). However, the $\delta^{13}C$ ratios measured in the muscle tissues of the *M. buccinoidea* individuals left in the corn detritus in this study were observed to be lower than those of the individuals kept in the apple detritus. These results may suggest that the detritus as nutrient resource was not influential for the *M. buccinoidea* individuals.

Freshwater macroinvertebrates can be categorized into functional feeding groups according to their morphological and behavioral adaptations(Cummins, 1973). The functional grouping of macroinvertebrates is characterized by their way of feeding but not by their feeding mechanisms and most are labeled omnivorous because they feed on plants and animals(Cummins & Klug, 1979). Additionally, the highly variable isotope ratio in macroinvertebrates results from their use of various nutrient resources originating from C3 and C4 macrophytes (Fellerhoff, 2002). In this research, contrary to what was expected, the $\delta^{13}C$ ratios of the *M. buccinoidea* individuals kept in the C4 detritus were found to be lower than those of the ones stored in the C3 detritus. This can be accounted for by the mostly-carnivorous way of feeding of the *M. buccinoidea* individuals. Coat, Monti, Bouchon, & Lepoint, (2009) report that the $\delta^{15}N$ values in omnivorous, herbivorous-detrivorous, and carnivorous sea shrimp juveniles range between 8.8 and 10.2‰, 5.0 and 8.4‰, and 11.0 and 12.7‰, respectively. The results of this study indicate that the *M. buccinoidea* individuals are in the upper trophic level according to the $\delta^{15}N$ range records (10.6‰ - 12.1‰). However, basic studies are needed to support the feeding patterns of this species in its natural habitat.In addition, Heller & Abotbol, (1997) note that *M. buccinoidea* individuals are generalist omnivorous feeders. They feed on layer of biofilm, also consume the decaying leaves, if existed. However, it can be inferred from the $\delta^{15}N$ ratios measured in this study that the *M. buccinoidea* individuals did not directly feed on the plant detritus.

In the study on the *M. buccinoidea* individuals, the experiment was discontinued on day 25 due to the death of all the individuals except for the ones in the apple+fertilizer detritus. It can be claimed that this reportedly omnivorous species survived thanks to the favoring physicochemical parameters and its capability to find food in the media.

The $\delta^{15}N$ ratio in organisms can be used as an indicator of the trophic level (Adams & Sterner, 2000). It was concluded that plant type and pesticide/fertilizer treatments were effective in shaping the trophic levels of the *M. buccinoidea* individuals kept in the C3 and C4 detritus media. The feeding strategy of the model organism *M. buccinoidea* might have affected this result. Furthermore, the ratio of the fertilizer and pesticide might be effective in the results of the experiment. It is suggested that the experiment be duplicated on macroinvertebrates directly feeding on detritus input in a stream.

Overall, some time-dependent differences were observed in the $\delta^{13}C$ ratios measured in the *M. buccinoidea* individuals kept in various media and a decrease in the $\delta^{13}C$ ratios measured in the muscle tissues of the individuals in the setups exposed to fertilizer and pesticide, especially in the corn detritus. A study on the mollusks in a tropical stream reports a positive relationship between the concentrations of some pesticides and the $\delta^{13}C$ ratio (Coat et al., 2011). In the present study, the *M. buccinoidea* individuals were subjected to the detritus treated with pesticide and no more pesticide was introduced to the media, and the water added was pesticide-free. The decrease in the $\delta^{13}C$ ratio in the muscle tissues of the *M. buccinoidea* individuals might have resulted from the pesticide concentration having been diluted over time. In addition, the half-life of pesticide may also be effective for the present result.

Conclusion

The leaves of the C3 plant apple and C4 plant corn commonly grown in riparian zones enter freshwaters and are used as a heterotrophic energy resource in detrita in an aquatic ecosystem. It can be concluded that isotopic composition change during detritus decomposition and pesticide treatments cause no differentiation in isotopic composition, but that fertilizer treatment lead to differentiation especially in corn with a quicker decomposition process. The qualitative analyses indicated that corn plant decomposed more quickly than the apple plant and the decomposition of corn leaves into fine particles in the pesticide-treated group took place the most quickly. Accordingly, corn leave inputs from riparian zones into freshwaters as energy resources enter the food web in stream systems more quickly. The analysis of the $\delta^{13}N$ and $\delta^{15}N$ stable isotope ratios of the *M. buccinoidea* individuals showed that fertilizer- and pesticide-treated detritus introduced into water had no significant influence on the $\delta^{13}N$ and $\delta^{15}N$ values of the *M. buccinoidea* individuals.

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