

Contributions of detrital subsidies to aboveground spiders  
during secondary succession, revealed by radiocarbon and  
stable isotope signatures

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## Abstract

Subsidies from belowground originating from detrital sources add nutrients and energy to arboreal communities. Measurement of this subsidy is required in the understanding of how food web dynamics respond to changes in surrounding environments. Shrub spiders are one of the key predators involved in food web coupling. We evaluate the effects of potential changes in prey availabilities during secondary succession and how different spider feeding guilds used the belowground prey subsidy. We measured the relative importance of the subsidy for the spider feeding guilds, using  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$  and  $\Delta^{14}\text{C}$  isotope measurements. Diet age was calculated from  $\Delta^{14}\text{C}$  values, because old diet ages of spiders indicate the spiders are using prey from detrital food sources. Dominant aerial prey (Diptera) had a distinctively old diet age compared with arboreal prey, which indicates aerial prey was subsidized from detrital food webs. Sit-and-wait spiders tended to have an older diet age than active hunting spiders, which indicates that sit-and-wait spiders depended more on subsidies. Diet age varied only slightly for spiders in stands of different ages, indicating that rates at which spiders use grazing and detrital prey are probably determined more by foraging strategies and not by stand age. A dominance of sit-and-wait predators will lead to higher detrital subsidy inputs to shrub habitats. This study highlights the effect of shrub spider community structure (feeding guild composition) on the volume of the subsidy received from belowground.

**Key words:** Aboveground–belowground linkage · Arboreal spider · Diet age · Feeding guild · Mixing model

## Introduction

Most of the organic matter and energy input into food webs originally comes from plant photosynthesis. Herbivores directly consume some photosynthetic products, leaving the balance of plant production to enter the soil as litter (Swift et al. 1979). Two sub-webs have been defined as the grazing and detrital sub-webs, based on the different pathways through which organic matter is supplied to the food web. These two sub-webs are spatially separated, because photosynthesis occurs aboveground and dead organic matter accumulates at the surface and belowground. However, the interlinking of the aboveground and belowground food webs is becoming widely acknowledged (Wardle et al. 2004; Bardgett and Wardle 2010). Prey-predator interactions, which are driven by the mixed diet of predators, are one of the linkages between the aboveground and belowground food webs. Several studies have shown predators often forage for a variety of prey that use different habitats and food sources (e.g. Vander Zanden and Vadeboncoeur 2002; Collier et al. 2002).

Spiders are one of the most dominant generalist predators in terrestrial ecosystems (Moulder and Reichle 1972; Settle et al. 1996). Miyashita et al. (2003) experimentally demonstrated spiders inhabiting shrub vegetation are subsidized by flying insects that have developed belowground, demonstrating that detrital subsidies have substantial contributions to aboveground food sources. Prey subsidy to shrub spiders has also been reported in an arable field (Von Berg et al. 2010), in a woodland (Pringle and Fox-Dobbs 2008) and in a riparian zone (Akamatsu et al. 2004), implying shrub spiders can generally couple food webs in different habitats.

Forest succession is clearly a powerful driver of consumer communities and

biotic interactions, because plants provide habitats for other organisms, while providing a baseline of energy and a living structural environment. Disturbance brings an abrupt loss of biomass (Bormann and Likens 1979), mixes litter and woody debris with soils, causes leaching of soil organic matter (SOM) and nutrients (Jurgensen et al. 1997), and allows a subsequent reaccumulation process as the rate of production exceeds the community respiration rate (Kira and Shidei 1967). Also, the successional disturbance regime occurs repeatedly, which maintains the ecosystem's state of equilibrium (Bormann and Likens 1979). Therefore, comparing the contribution of prey subsidy between different ecosystems during succession helps to show how ecosystem changes affect donor control of shrub habitat in various environments.

Previous studies indicated the effects of succession cascade to spiders through enhanced prey subsidy. Manipulative studies have shown the abundance of spiders is enhanced by the increased availability of prey from belowground, which occurred in response to the addition of detritus in both forest ecosystems (Chen and Wise 1999) and agroecosystems (Halaj and Wise 2002; Rypstra and Marshall 2005). These manipulative studies indicate that the community of spiders is under bottom-up control in various environments. Also, dipteran prey from soil, a dominant prey subsidy from belowground, occur at much higher densities in ecosystems with a well-developed litter layer compared with densities in grasslands or agroecosystems (Frouz 1999). Shimazaki and Miyashita (2005) compared the availability of a prey subsidy from belowground for web-building spiders in shrubs, and showed the biomass proportion of aerial prey from the detrital food web is higher in forests than in grasslands. These studies imply the availability of belowground subsidy for spiders increases during forest succession,

which may be caused by the reduced litter inputs after disturbance and recovery during succession (Lugo 2008).

Also, diets of spiders in different feeding guilds are thought to be different because of the differences in each guild's foraging methods. The foraging methods used by spiders, like their feeding guilds, are phylogenetically determined (Coddington and Levi 1991), with the different guilds and species using different food sources (Akamatsu et al. 2004; Birkhofer and Wolters 2012). Although previous studies have compared the feeding guild compositions of spider communities along successional gradients (Bultman and Uetz 1982), few studies have shown how spider feeding guilds respond to the changes in altered prey availabilities.

Stable isotope techniques have provided a continuous measure of the mass flows through ecological communities, and they have been used to quantify the relative contributions of each food source for various consumers.  $\delta^{13}\text{C}$  values have been used to detect food sources for consumers because trophic enrichment is 0.5‰ and much lower than  $\delta^{15}\text{N}$  values. In contrast, the stable isotope signature of  $\delta^{15}\text{N}$  has been used to assign organisms to trophic levels and feeding guilds, because trophic enrichment is higher for  $\delta^{15}\text{N}$  than for  $\delta^{13}\text{C}$ , which was 2.3‰ for terrestrial animals (McCutchan et al. 2003). In addition to the trophic enrichment for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , another source of trophic enrichment is the process of decomposition. Decomposition with increased residence time tends to enrich the  $^{13}\text{C}$  and  $^{15}\text{N}$  ratios of SOM (Michener and Lajtha 2007), and because detritivores use decomposed SOM as a food source, their  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values reflect the high  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of those sources (Hishi et al. 2007). Therefore, enrichment through decomposition will result in higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  content in prey

from detrital food webs than in prey from grazing food webs. However, it has been difficult to discriminate between the effect of trophic enrichment and enrichment through decomposition.

Another isotope signature that can be used to trace food web structure is radiocarbon ( $^{14}\text{C}$ ), which allows direct estimation of the mean age of carbon. The radiocarbon approach takes advantage of the elevated levels of  $^{14}\text{C}$  in atmospheric  $\text{CO}_2$  that resulted from nuclear weapons tests in the early 1960s. Since the nuclear test ban treaty of 1963, nuclear bomb-generated atmospheric  $^{14}\text{C}$  in  $\text{CO}_2$  has exponentially decreased (Levin and Hesshaimer 2000). Since we know the  $^{14}\text{C}$  content of organic matter synthesized by primary producers is the same as that of atmospheric  $\text{CO}_2$  at the time of production (Burchuladze et al. 1989; Druffel and Griffin 1995), we can estimate the diet age of consumers, which is defined as the mean time elapsed since C in the consumer's diet was fixed from atmospheric  $\text{CO}_2$  by primary producers. After the seminal study by Tayasu et al., (2002), Hyodo et al. (2006) showed consumers in a grazing food web had a relatively new diet age of 0–4 years, while termites, which consume detrital foods, had an older diet age of a maximum of 18 years. Diet age, which is estimated from  $\Delta^{14}\text{C}$  (bomb-carbon), distinguishes the grazing and the detrital sub-webs, and should give information about the coupling process between the two sub-webs (Tayasu and Hyodo 2010).

In this study, using the diet age of the spiders as a measure to detect prey subsidy from detrital food webs, we reveal the dynamics of forest food webs during forest succession. Also, using stable isotopes, we estimate the contribution rates of each prey group by taxonomic order and body length for shrub spiders, and we test the

following hypotheses. First, the prey subsidy from the detrital food web will have distinctively old diet ages compared with prey from the grazing food webs. Second, if spiders segregate their food sources by feeding guild in every stage of forest succession, the diet ages of spiders will be older in highly subsidized guilds. Third, we hypothesize that if spiders mainly determine their prey based on prey availability, then the prey that are subsidized from belowground will contribute much more to spiders in late successional forest and older diet ages will appear in spiders from old growth forests.

## **Materials and methods**

### **Study site**

The study sites are located in and near the Ogawa Forest Reserve (OFR) at the southern edge of the Abukuma Mountains, northern Ibaraki Prefecture, in central Japan (approximately 36°56'N, 140°35'E, 580–800 m a.s.l.). The mean annual temperature in Ogawa from 1986 to 1995 was 10.7°C (Mizoguchi et al. 2002). The mean annual precipitation is about 1900 mm. Clear-cutting of natural forest for pulp production is continuing around the OFR, and secondary forests of different stand ages are available. We selected four 50 × 50 m<sup>2</sup> plots within 6 km of the OFR; the fifth site with the oldest trees was the only one on OFR lands. Stand ages were 1, 7, 11, 24 and 105 years at the five sites, based on forest administration department records. Each plot was at least 20 m from the stand edge to avoid lateral inputs from adjacent environments.

### **Sampling and identification**

Sampling was conducted from Jul- to Oct-2008 and from Jun- to Jul-2009. Fresh leaves

collected in Jul-2008 were used to determine the  $\Delta^{14}\text{C}$  values for organic carbon fixed from  $\text{CO}_2$  during 2008 at the study sites. We selected abundant trees species from both the canopy and the understory (less than 2 m tall) and sampled from three ramets (Appendix S1). However, younger age stands (1 and 7 years) were mostly comprised of trees < 5 m tall. Then we sampled fresh leaves from abundant tree species, which were categorized as canopy trees. In older age stands, fresh leaves were collected from ~10 m above the ground. We dried these fresh leaves at 60°C.

We used two methods to sample arthropods and grouped them accordingly. For sweep net sampling (sweeping) we used nylon mesh nets 30 cm in diameter by sweeping from 0.5 m to 2 m aboveground. We categorized prey samples caught by this method as aerial prey. We also beat shrubs to up to 2 m aboveground and collected fallen insects (beating). We categorized prey samples caught by this method as arboreal prey. We stored samples for isotopic analyses in screw vials at room temperature for more than 48 hours, allowing them to excrete gut contents. We washed the samples with distilled water and then freeze-dried them.

We separated the prey samples into six fractions based on taxonomic order and body length to measure isotopic values. Because the biomass of individuals varied widely, mixing entire samples of one taxonomic order would result in a predominance of large individuals for mass ratio calculations. However, it is unreasonable to consider larger prey as important food sources, because spiders have optimal prey sizes that depend on the preferences and abilities of each spider species, such as the size of an orb web (Brown 1981). Therefore, before mixing prey samples, we measured body length of all prey under a stereomicroscope and separated dominant prey orders into three size



classes: 0–5 mm, 5–10 mm, and > 10 mm body length. Diptera dominated aerial prey in every size class, while the dominant order of arboreal prey differed in each size class. Consequently, we decided to measure isotope ratios of prey that were grouped in three size categories for Diptera. We did the same for dominant arboreal prey by size class as follows: Collembola (dominant in the 0–5 mm arboreal prey size class), Hemiptera (5–10 mm), and larvae of Lepidoptera (> 10 mm). We mixed at least three individuals for each prey sample.

Spiders were identified to species using morphological traits. We analyzed individual spiders as long as individual masses provided enough material to accurately measure isotopic values, so intraspecific and interspecific differences could be compared. For two spider species (*Leucauge subblanda* Bösenberg et Strand 1906 and *Yaginumaella striatipes* (Grube 1861)), we measured isotope ratios of multiple individuals to document the variations of isotope values for these two species. We categorized spiders into four feeding guilds, visual pursuit, nocturnal hunting, orb web and crab spiders, based on foraging strategies of the taxonomic families of those spiders. Both visual pursuit and nocturnal spiders forage by actively searching for prey, but visual pursuit spiders find prey by eyesight, while nocturnal spiders find prey at night, apparently by sensing vibrations. Both orb web and crab spiders forage for prey using a sit-and-wait strategy, but orb web spiders catch prey using orb webs, and crab spiders catch prey by using their extended anterior limbs. Appendix S2 contains details about the spider samples.

## Isotope analyses

We measured stable isotope ratios (‰) using an EA1108 elemental analyzer (Fisons Instruments, Milan, Italy) connected to a Delta S mass spectrometer (Finnigan MAT, Bremen, Germany) with a ConFlo II interface (Finnigan MAT, Bremen, Germany). We corrected data with international standard substances (Tayasu et al. 2011). Standard deviations of the measurements of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were 0.10‰ and 0.09‰, respectively.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures are reported as relative to carbonate in Vienna Pee Dee Belemnite for atmospheric carbonate and nitrogen, respectively, using the following equation:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} (\text{‰}) = \left( R_{\text{sample}} / R_{\text{standard}} - 1 \right) \times 10^3 \quad (\text{Eq. 1})$$

$$R = {}^{13}\text{C}/{}^{12}\text{C} \text{ or } {}^{15}\text{N}/{}^{14}\text{N} \quad (\text{Eq. 2})$$

We performed graphitization for radiocarbon ( $^{14}\text{C}$ ) measurement following the method of (Kitagawa et al. 1993). We measured  $^{14}\text{C}$  concentrations using an accelerator mass spectrometer (AMS, National Electrostatics Corp., Middleton, WI, USA) in the National Institute for Environmental Studies following the method of Uchida et al. (2004) and reported it as a  $\Delta^{14}\text{C}$  value. The following equation defines  $\Delta^{14}\text{C}$ :

$$\Delta^{14}\text{C} (\text{‰}) = \delta^{14}\text{C} - 2 (\delta^{13}\text{C} + 25) (1 + \delta^{14}\text{C}/1000) \quad (\text{Eq. 3})$$

We corrected  $^{14}\text{C}$  concentrations for the ongoing radioactive decay of the international reference standard (oxalic acid) since AD 1950 and corrected for  $\delta^{13}\text{C}$  signatures of  $-25\text{‰}$  (Stuiver and Polach 1977). Owing to the correction applied for  $^{14}\text{C}$  concentrations, fractionations of  $^{14}\text{C}$  were cancelled by the use of  $\delta^{13}\text{C}$  values, when represented by  $\Delta^{14}\text{C}$ .  $\Delta^{14}\text{C}$  signatures were reported with an error (‰) for each analysis,

which was within 5‰ (2.8‰ on average).

## **Source contribution estimation and diet age estimation**

For whole prey samples, we compared  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between arboreal and aerial prey to test how arboreal and aerial prey sources could be distinguished isotopically. Then, we estimated the contribution of each aerial prey relative to the entire group of prey using a dual isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) MixSIR (ver. 1.0.4) model (Moore and Semmens 2008; Semmens et al. 2009). We defined each source category based on the order of the prey and used four sources to estimate contribution rates. We set trophic enrichment of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in the modeling to  $0.5 \pm 1.37\text{‰}$  and  $2.3 \pm 1.52\text{‰}$  (mean  $\pm$  standard deviation), respectively, using the value for terrestrial animals reviewed by McCutchan et al. (2003). For the analyses of contribution rates of aerial prey, medians of the contribution rates were used.

We estimated the diet age using the following method. First,  $\Delta^{14}\text{C}$  of atmospheric  $\text{CO}_2$  was estimated to be exponentially decreasing (Eq. 4; Hyodo et al. 2006):

$$\Delta^{14}\text{C}(t) = 417 \times \exp\{(1974 - t) / 16\} \quad (\text{Eq. 4})$$

where  $t$  is represented in dominical year.

We made some modifications to Eq. 4 because the  $\Delta^{14}\text{C}$  value of local  $\text{CO}_2$  was determined by the mixing of two sources. One of the sources follows the globally observed trend expressed in Eq. 4, and another source is  $\text{CO}_2$  that originated from fossil fuel, of which the  $\Delta^{14}\text{C}$  value is almost constant ( $-1000\text{‰}$ ). We assumed the rates of

CO<sub>2</sub> from local fossil fuel combustion in atmospheric CO<sub>2</sub> were constant through time and denoted by  $P$  in the following equations:

$$\Delta^{14}\text{C}_{\text{sample}} = (1 - P) \times \Delta^{14}\text{C}(t - D) - 1000 \times P \quad (\text{Eq. 5})$$

$\Delta^{14}\text{C}_{\text{sample}}$ ,  $t$  and  $D$  indicate any  $\Delta^{14}\text{C}$  value of samples obtained from a single site that originate from locally photosynthesized organic matters, sampling year and diet ages, respectively. From Eq. 4 and Eq. 5, we can calculate  $D$  from Eq. 4 and 5, for given  $P$ :

$$D = T + 16 \times \left\{ \ln \left( \left( \Delta^{14}\text{C}_{\text{sample}} + 1000 \times P \right) / (1 - P) \right) - \ln 417 \right\} - 1974 \quad (\text{Eq. 6})$$

We obtained foliar samples, which are thought to show  $\Delta^{14}\text{C}$  values of local atmospheric <sup>14</sup>CO<sub>2</sub> concentrations during the sampling period  $t$  (equals 2008.7, in this study). Therefore, when  $\Delta^{14}\text{C}_{\text{sample}}$  equals foliar  $\Delta^{14}\text{C}$  values,  $D$  equals 0. By substituting the values, we solved Eq. 6 for  $P$  at each study site and calculated diet ages of all other samples.

## Statistical analyses

We applied two-way analysis of variance (ANOVA) to test whether or not each study site (1-, 7-, 11-, 24-, 105-year-old forest sites) and each category of samples (shown in parentheses below) are the determinants of foliar  $\Delta^{14}\text{C}$  (canopy and understory tree leaves), diet ages of prey (arboreal and aerial prey), and diet ages of two species of spiders (*L. subblanda* and *Y. striatipes*), which were repeatedly measured for their  $\Delta^{14}\text{C}$  content. We applied one-way ANOVA to test  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of aerial versus arboreal prey as separate source values. We created a generalized linear mixed model (GLMM) to analyze the relationships between diet ages of individual spiders and

feeding guild of the spiders and the change in food sources during secondary forest succession. The full model included the following explanatory variables: stand age as a continuous variable and the feeding guild of spiders as a categorical variable. We also incorporated spider species as a random and categorical variable into the full model. For the GLMM analysis, the Akaike information criterion (AIC) least model was selected as the best fit model to explain diet ages of spiders. All the statistical analyses were conducted by R version 2.13.0 (R development core team 2011). Package “lme4” was used for GLMM analysis.

## **Results**

### **Stable isotope ratios of prey and spiders**

Overall means and standard deviations of carbon and nitrogen stable isotope ratios for aerial prey were  $-25.6 \pm 0.8$  and  $4.5 \pm 1.8\text{‰}$ , whereas the values for arboreal prey were  $-29.2 \pm 1.9$  and  $-1.8 \pm 1.9\text{‰}$ . Carbon and nitrogen isotope ratios of aerial prey were significantly different from arboreal prey (one-way ANOVA,  $F_{1,56} = 43.4$ ,  $P < 0.001$  for  $\delta^{13}\text{C}$  and  $F_{1,56} = 117$ ,  $P < 0.001$  for  $\delta^{15}\text{N}$ ), and the food sources were clearly separated by the differences in isotope signatures between aerial prey and arboreal prey (Fig. 1). Carbon and nitrogen isotope ratios, which were offset for trophic enrichment, were located within polygons of the stable isotopes biplots of the four prey categories in each study site, indicating the source mixing models can be applied.

The MixSIR source mixing model generated variable contribution rates of aerial prey, which had a median from 0.11 to 0.68 (from minimum to maximum). The high variation of the contribution rate was accounted for by the wide range of carbon

and nitrogen isotope ratios of spiders, even within a single site.

### **Plant $\Delta^{14}\text{C}$ as a basis for diet age calculation**

The  $\Delta^{14}\text{C}$  values of canopy leaves and understory leaves were  $43.0 \pm 6.4$  and  $46.2 \pm 5.8\text{‰}$  (mean  $\pm$  standard deviation), respectively (Fig. 2a). The  $\Delta^{14}\text{C}$  of leaves were significantly different between study sites but not between canopy trees and understory trees (two-way ANOVA,  $F_{4,18} = 3.3$ ,  $P = 0.034$  for study sites and  $F_{1,18} = 1.3$ ,  $P = 0.26$  for canopy or understory trees). Thus, we used mean values of each study site as the  $\Delta^{14}\text{C}$  value of current production, shown as foliar  $\Delta^{14}\text{C}$  in Eq. 6.

### **Diet ages of prey and spiders**

Diet ages of aerial prey (Diptera) were always older than those of arboreal prey (Collembola, Hemiptera, larva of Lepidoptera) at any stand age, excluding one case (Fig. 3) where the Diptera of size class 5–10 mm was 1.5‰ lower in  $\Delta^{14}\text{C}$  value than Hemiptera at the 105-year site, which was within the precision of the AMS. The diet ages of aerial prey were significantly older than arboreal prey, but the measurements between the study sites were not significantly different (two-way ANOVA,  $F_{1,24} = 62.1$ ,  $P < 0.001$  for prey category and  $F_{4,24} = 0.7$ ,  $P = 0.60$  for study sites).

Two spider species were repeatedly measured in nearly every study site. The first, *L. subblanda*, an orb web spider, had a diet age of  $6.5 \pm 1.9$  years. The second, *Y. striatipes*, a visual pursuit predator, had a diet age of  $3.6 \pm 2.3$  years (Fig. 2b). *Y. striatipes* was not collected in the 1- and 7-year-old sites. Diet age was significantly different between spider species, but was not significantly different between study sites

(two-way ANOVA,  $F_{1,16} = 12.5$ ,  $P = 0.0028$  for species and  $F_{4,16} = 2.01$ ,  $P = 0.14$  for study sites).

Diet ages of all spiders were within the ranges between minimums and maximums of diet age of prey, excluding one study site (Fig. 3). Simple linear regression revealed spider diet age increased when increasing proportions of aerial prey were used as a food source (adjusted  $r^2 = 0.08$ ,  $P = 0.03$ ). This correlation corresponds to the positive correlation between diet age and the  $\delta^{13}\text{C}$  value (adjusted  $r^2 = 0.28$ ,  $P < 0.001$ ), and the positive correlation between diet age and the  $\delta^{15}\text{N}$  value (adjusted  $r^2 = 0.11$ ,  $P = 0.009$ ) for spiders (Fig. 4).

Feeding guild factored in with the random effect of species was selected as the least AIC model (AIC = 237, df = 6, whereas the full model was AIC = 244, df = 7) in the GLMM analysis. The estimated diet ages, with the standard error, by feeding guild for visual pursuit, nocturnal, orb web and crab spiders were  $1.2 \pm 2.1$ ,  $3.5 \pm 1.5$ ,  $5.5 \pm 1.8$ , and  $5.7 \pm 2.5$  years, respectively. The factor based on stand age, measured as years after clear-cutting, was not selected during the AIC model selection.

## Discussion

### Source mixing models using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

$\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of the aerial prey (Diptera) were higher than those of the arboreal prey by 3.5‰ and 6.0‰ on average, respectively. One of the possible reasons for the higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for aerial prey is that aerial prey were dominated by predators, which would be expected to naturally have higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. However, it is very unlikely that Diptera forage for arboreal prey for which isotope

ratios were measured in this study, because this hypothesis assumes a high  $^{13}\text{C}$  enrichment at each trophic level. Another more convincing possibility is that the increase in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for aerial prey is caused by the fact their food originates from decomposed litter.  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment through decomposition has been generally observed (Michener and Lajtha 2007; Hishi et al. 2007).

#### **Diet age estimation from $\Delta^{14}\text{C}$**

Previous studies reported site-specific  $\Delta^{14}\text{C}$  signatures of current year photosynthetic production among sites and attributed the variations to anthropogenic  $\text{CO}_2$  emissions (Shibata et al. 2005; Pataki et al. 2010). These studies indicate it is necessary to consider the local atmospheric  $^{14}\text{CO}_2$  variations caused by anthropogenic carbon dioxide emissions, which fluctuate at a local scale when comparing  $\Delta^{14}\text{C}$  values between sites. Our results indicate the difference in  $\Delta^{14}\text{C}$  values was about 10‰ between the study sites, which is equivalent to a shift of 3–4 years in diet ages since 2008. In contrast,  $\Delta^{14}\text{C}$  values between canopy and understory leaves were not significantly different (Fig. 2a). Therefore, in this study, the concentration of  $\text{CO}_2$  originating from fossil fuel is shown to differ between study sites, while the  $\text{CO}_2$  was homogenized vertically at any single site. We made modifications to the equation related to diet age and  $\Delta^{14}\text{C}$  value (Eq. 7) to calibrate the variations of the  $\Delta^{14}\text{C}$  values of currently photosynthesized organic matter between study sites.

#### **Diet ages of prey**

Diet ages of aerial prey were always older than those of arboreal prey (Fig. 3). The



maximum diet ages of prey in each study site were 8 to 12 years and the prey were all Diptera (Appendix S3). Hyodo et al. (2006) showed older diet age (5 to 18 years) appeared only in detritivores (termites), whereas a younger diet age (0 to 4 years) appeared in herbivores (bees); thus diet age provides a tool in understanding both terrestrial grazing and detrital food webs as well as in understanding the interactions between these two. Old diet ages of aerial prey (Diptera) in our study indicate the dominant prey in aerial habitats were detritivores. Considering most Dipterans originate from soil (Frouz 1999) and soil ecosystems are a major part of the detrital food web, the old diet age of dominant aerial prey indicates the prey is subsidized from the belowground detrital food web.

These findings are consistent with the results of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of aerial prey, indicating they originated in the detrital food web. Decomposition with increased resident time tends to enrich  $^{13}\text{C}$  and  $^{15}\text{N}$  ratios of SOM. Hyodo et al. (2008) indicated a positive correlation exists between  $\delta^{15}\text{N}$  values and diet ages of detritivores (earthworms and termites), and also indicated humigated organic matter is enriched with  $^{15}\text{N}$ , confirming the  $^{15}\text{N}$  enrichment along a humification gradient (Tayasu 1998). Also,  $^{13}\text{C}$  in SOM is enriched with increasing depth (Michener and Lajtha 2007), which indicates  $^{13}\text{C}$  is also enriched during the decomposition process. High  $^{13}\text{C}$  and  $^{15}\text{N}$  ratios and old diet ages of aerial insects are all consistent with the hypothesis that their food sources are derived from detrital material.

Diet ages of some arboreal prey were negative (Fig. 3). This indicates arboreal prey consumed more recently photosynthesized fractions than whole plant leaves. A reasonable assumption is that a sizable percentage of the leaves consumed by the prey

species, that is, leaves made of structural substances such as cellulose and lignin (Kirschbaum and Paul 2002), is derived from older photosynthesized carbohydrates rather than labile fractions, such as glucose and lipids. Studies on resource allocation for growth and reproduction of perennial plants have shown plants have the ability to store previously photosynthesized organic matter (Sork et al. 1993), and such storage can be allocated to leaves especially during leaf formation. These results suggest extracting the labile fraction of plants for estimating the current  $\Delta^{14}\text{C}$  values may be more suitable, but selecting the "most appropriate" fraction that represents most recently-fixed carbon seems to be a topic for another study. Therefore, we concluded it was a practical option to assume  $\Delta^{14}\text{C}$  of whole plant leaves approximately represents the  $\Delta^{14}\text{C}$  values of organic matters of current production.

The maximum diet age of arthropods in each site was 8 to 12 years (Appendix S3). Such old diet ages suggest organic matter was retained in the detrital pool as biologically available fractions in every successional stage. This may be specific to areas undergoing secondary succession after clear-cutting, which release large amounts of dead organic matter into forest stands. Thus, an old diet age in a young timber stand indicates dead organic matter released by disturbance comprises important energy sources for food webs in early successional stages, such as a clear-cutting type of disturbance. Further studies will be required to reveal how quality and quantity of residuals will differentiate food web structures between differently disturbed ecosystems.

## Diet ages of spiders

The  $\Delta^{14}\text{C}$  values for almost all spiders were within the range of the  $\Delta^{14}\text{C}$  values of prey at each site (Fig. 3). This indicates our sampling covered potential food sources for spiders. The diet ages of spiders are positively correlated with the relative contribution rate of aerial prey, which have an older diet age than arboreal prey (Fig. 4). This indicates the diet age of spiders increases with an increasing the contribution rate from the predation of aerial insects, suggesting greater prey subsidies from belowground.

Differences in diet ages between the two spider species were found (Fig. 2b), although diet age was difficult to measure repeatedly for all spiders because of the time and expense required to do  $\Delta^{14}\text{C}$  analysis. However, this study documents how species-specific differences in foraging behavior affected food utilization and, as a result, changed the dependency on detrital subsidies. The two species compared in this study were *L. subblanda*, an orb web spider, and *Y. striatipes*, a visual pursuit spider. The fact that diet age of an orb web spider species was older than a visual pursuit spider species suggests the orb web spider's diet depended more on the detrital food web than did the diet of visual pursuit spiders.

Notably, the difference in diet age among the study sites was relatively small, and no trend along the forest successional gradient was seen (Fig. 2b). GLMM analyses of the all spiders revealed diet ages of spiders were explained by their categories of feeding guilds, but stand age as a variable did not explain the diet age variation. This indicates the feeding guild of spiders affects the contribution rate of prey from the detrital food web, while environmental variations along successional lines only weakly influence the prey utilization of spiders. The results of the GLMM analysis also agrees

with the comparison of diet ages between two species (*L. subblanda* and *Y. striatipes*), in that feeding guilds of spiders, not the differences among study sites nor stand age of the sites, affected the contribution rates of the detrital and grazing food sources. This indicates arboreal spiders tend to specialize and consume a specific prey fraction to some degree and more than previously thought. That is, some preferred aerial prey, which mainly emerged from belowground, while others concentrated on arboreal prey, which mainly inhabited aboveground. This suggestion is surprising, considering that the availability of the two prey categories will increase during succession. Furthermore, orb web and crab spiders had a tendency to have an older diet age than nocturnal and visual pursuit spiders. The former two spiders take a sit-and-wait strategy, while the latter two spiders actively hunt. Considering both the major prey subsidy from detrital food webs and aquatic food webs were aerial insects, studies for prey subsidy from aquatic ecosystems are comparable with our results. Akamatsu et al. (2004) documented the tendency for cursorial spiders to have a lower subsidy of aquatic insects than web-building spiders. Henschel et al (2001) compared diets of an orb weaving spider species and a nocturnal species and showed that increases in aquatic prey availability affected differently on the two species. The relative contributions of aquatic insects were higher at a stream edge for both two spiders than at 30 m from the edge; the rate of increase was higher for the orb weaving spiders. These studies for aquatic prey subsidy, together with our results suggest the sit-and-wait spiders favor foraging active prey, the movement of which drive prey subsidy to spiders. Also, our results show the method of foraging of each spider feeding guild can have more influence than the differences in availabilities of subsidized prey which is determined by the surrounding environments.

These results indicate that feeding guild composition is an important factor affecting food sources for spider communities. Our study reveals the diet ages of spiders range from 0.11 to 0.68 (from minimum to maximum), including all individuals, and confirms a high level of variation exists in the relative contributions of Diptera, based on the MixSIR model. This variation indicates the amount of belowground subsidy assimilated by the shrub spider community can vary widely, based on one extreme condition, dominance of spiders that are least subsidized, and the other extreme condition, dominance of spiders that are most subsidized.

Previous studies have shown the importance of belowground subsidy in generating an abundance of spiders (Chen and Wise 1999; Rypstra and Marshall 2005) or for a specific feeding guild of spiders (for orb web spiders; Miyashita et al. 2003). However, the quantitative importance of the subsidy has never been compared between different environments across multiple feeding guilds because of the difficulty in quantifying food sources that originate belowground. Food web analyses using isotope measurements enabled us to compare the importance of each food source between different feeding guilds and to develop our understanding about each feeding guild's consumption of prey that originated in belowground environments. Shrub spiders segregate their food sources by feeding guild and showed a consistent tendency in the mixing rate of prey from belowground and aboveground within guilds. We also found a stronger effect was generated by the way spiders forage for their diets rather than by the availabilities of prey in surrounding environments. Results from this study suggest the relative abundance of sit-and-wait spiders during forest succession is one of the determinants of the subsidy rate for the shrub spider community. To our knowledge, this

is the first study to propose the importance of predator community composition in determining the rate of belowground subsidy.

One question that still needs to be answered is whether changes in spider community composition during succession will alter a food web in terms of the amount of input from belowground. Our study implies conditions in late successional forest favor sit-and-wait predators, through the enhancement of subsidy from belowground. Several studies documented changes in guild compositions of spiders during forest succession (Bultman and Uetz 1982) and the existence of relationships between guild compositions and surrounding environments (Uetz et al. 1999), although impacts of the compositions on their use of subsidy were not known. To answer this question, both the dynamics of the spider community and the food sources for each species need to be determined. Further study using both isotope measurements and spider community analyses will reveal what changes in predator community composition during succession affect their function as a driver of food web coupling.

## **Conclusions**

This study reveals the extent of prey subsidy for predators in forest shrubs during succession. Diet ages indicate that old carbons were subsidized from belowground via soil dipterans. Different spider feeding guilds segregated their food sources and diet ages differently. Also, each spider feeding guild had a consistent tendency in their diet age that did not show an increasing nor decreasing trend during forest succession. These results indicate each predator group has consistently used either grazing or detrital prey regardless of forest age. Therefore, arboreal predators may alter their assemblages based

on changes in the availability of grazing/detrital prey during forest succession, which is ultimately determined by the energy flow from grazing on detrital food chains. This study highlights the importance of incorporating changes in predator community composition to elucidate the dynamics of food web coupling during succession.

## **Acknowledgments**

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## Figure legends

**Fig. 1** Carbon and nitrogen stable isotope biplots of arboreal prey categories (Collembola, Hemiptera and larval Lepidoptera), aerial prey (Diptera) and arboreal spiders in (a) 1-, (b) 7-, (c) 11-, (d) 24-, and (e) 105-year-old sites. Isotope ratios of prey are shown with the mean  $\pm$  standard deviation, whereas those of spiders were offset by the trophic enrichment factor (0.5 ‰ for  $\delta^{13}\text{C}$  and 2.3 ‰ for  $\delta^{15}\text{N}$ ) and shown individually. Data sets shown in the figure were applied to the MixSIR source mixing model (see materials and methods).

**Fig. 2** (a)  $\Delta^{14}\text{C}$  values of leaves of dominant tree species (see S1 for sample composition) and (b) diet ages of the two spider species (*Yaginumaella striatipes* and *Leucauge subblanda*) with calibration of foliar  $\Delta^{14}\text{C}$  means at every study site. Labels on the x axis indicate stand ages of study sites in which the samples were collected. Bars indicate means  $\pm$  standard deviations.

**Fig. 3** Diet ages of arboreal prey categories (Collembola, Hemiptera and larval Lepidoptera), aerial prey (Diptera) and arboreal spiders. Labels of the lateral axis indicate stand ages of study sites at which the samples were collected. Bars indicate means  $\pm$  standard deviations.

**Fig. 4.** Relationships between diet ages of spiders and (a) the relative contribution of aerial prey (b)  $\delta^{13}\text{C}$  (c)  $\delta^{15}\text{N}$ . Lines in (a) to (c) indicate statistically significant linear regression (n = 51).

Figures

Fig. 1

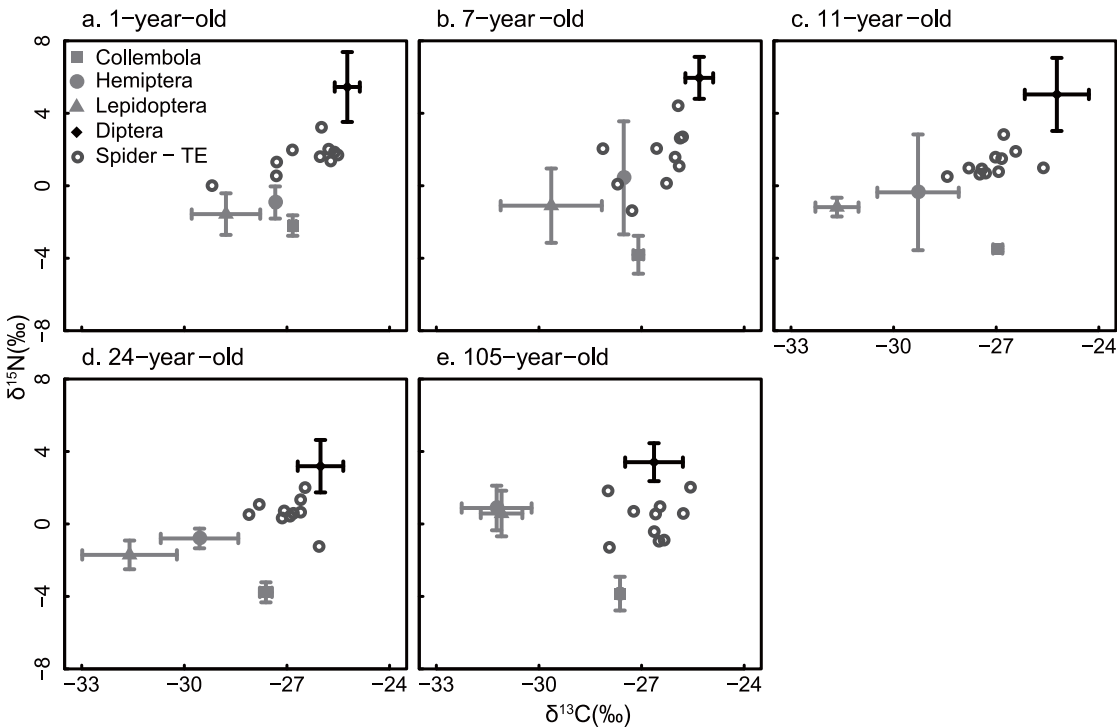


Fig. 2

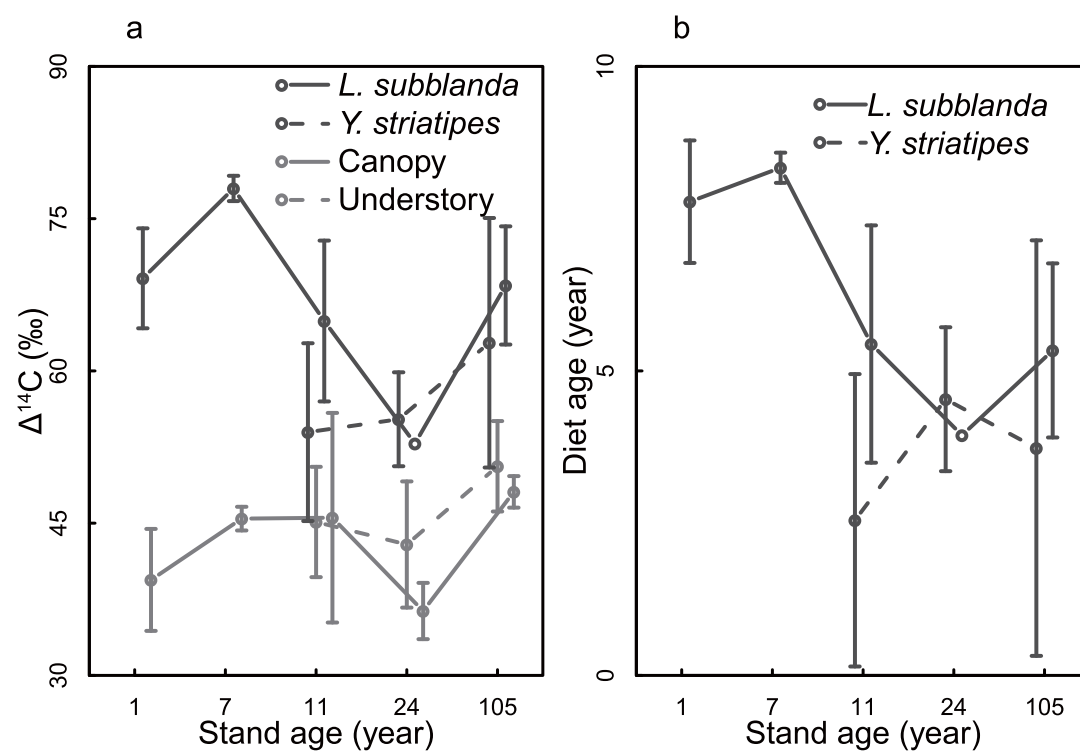


Fig. 3

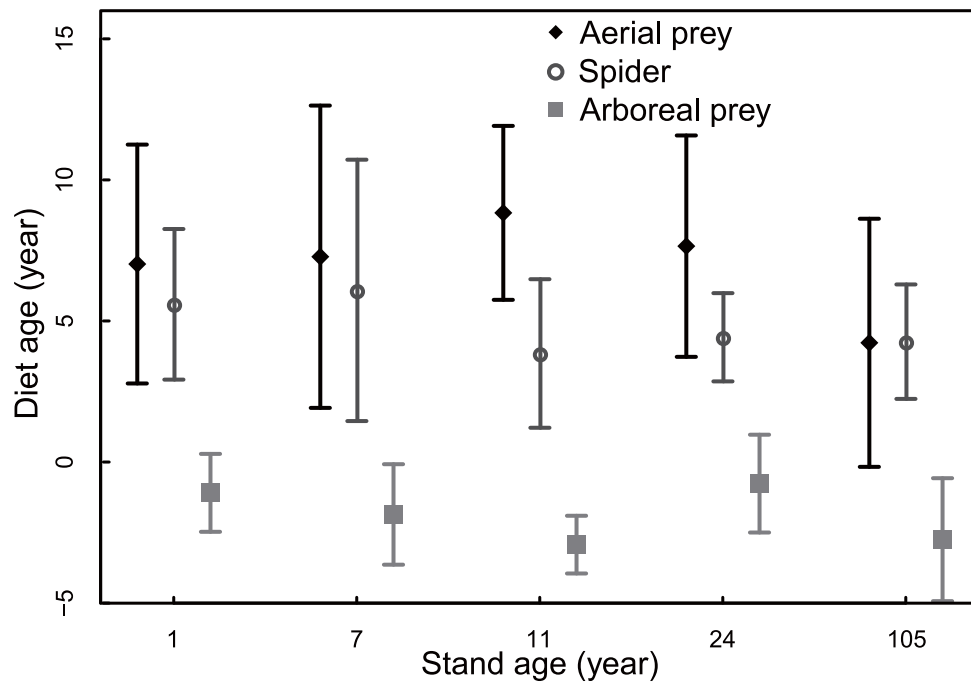
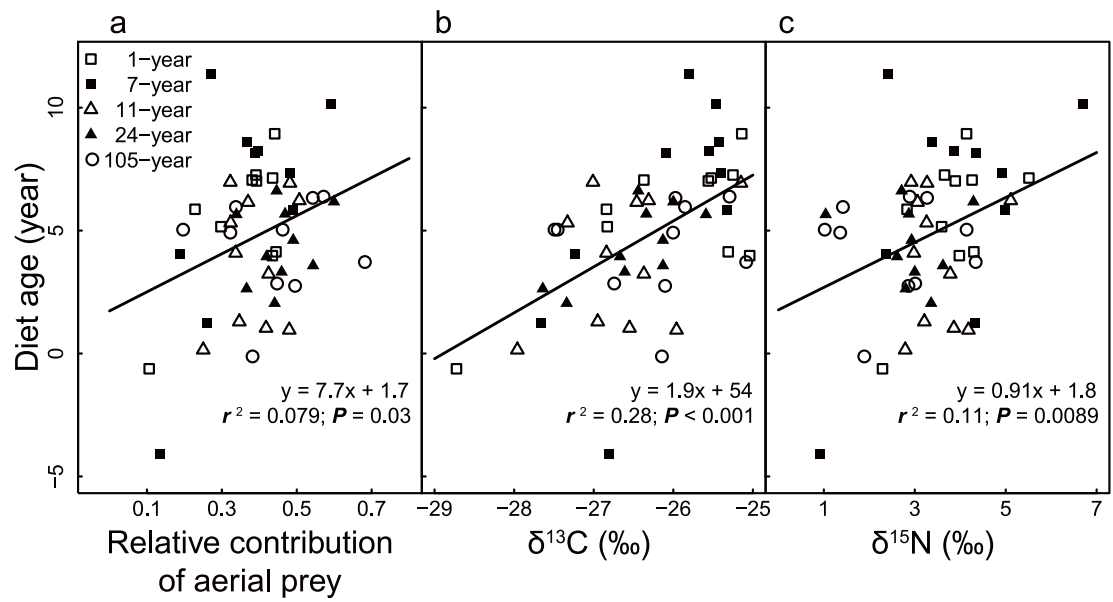




Fig. 4



# Contributions of detrital subsidies to aboveground spiders during secondary succession, revealed by radiocarbon and stable isotope signatures

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**Electronic supplementary material (ESM)**

Appendix S1. Measured values and attributes of isotope samples of fresh leaves.

Sampling sites were named for the stand age (years after clear-cutting).

Sampling site	Species	Canopy/understory	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\Delta^{14}\text{C}$ (‰)
1-year	<i>Quercus serrata</i>	canopy	-28.4	0.1	42.3
1-year	<i>Quercus serrata</i>	canopy	-28.7	1.3	33.6
1-year	<i>Quercus serrata</i>	canopy	-28.9	1.6	42.3
7-year	<i>Clethra barvinervis</i>	canopy	-29.4	-0.9	46.3
7-year	<i>Clethra barvinervis</i>	canopy	-29.8	-0.9	44.1
7-year	<i>Clethra barvinervis</i>	canopy	-30.4	-1.0	46.0
11-year	<i>Castanea crenata</i>	canopy	-31.0	-1.8	47.9
11-year	<i>Castanea crenata</i>	canopy	-31.2	-1.5	34.3
11-year	<i>Castanea crenata</i>	canopy	-28.1	-1.7	54.5
11-year	<i>Prunus grayana</i>	understory	-29.7	-3.1	44.2
11-year	<i>Prunus grayana</i>	understory	-30.9	-2.6	51.0
11-year	<i>Prunus grayana</i>	understory	-30.0	-1.7	40.2
24-year	<i>Castanea crenata</i>	canopy	-29.9	-1.6	34.0
24-year	<i>Castanea crenata</i>	canopy	-30.7	-1.1	35.6
24-year	<i>Castanea crenata</i>	canopy	-30.7	-1.0	39.4
24-year	<i>Hydrangea paniculata</i>	understory	-30.2	-1.6	38.1
24-year	<i>Hydrangea paniculata</i>	understory	-30.4	-1.0	40.6
24-year	<i>Hydrangea paniculata</i>	understory	-31.7	-2.0	49.9
105-year	<i>Quercus serrata</i>	canopy	-30.7	-2.4	46.4
105-year	<i>Quercus serrata</i>	canopy	-29.5	-2.3	48.4
105-year	<i>Quercus serrata</i>	canopy	-28.5	-0.6	49.5
105-year	<i>Fagus crenata</i>	understory	-31.8	-1.8	46.0
105-year	<i>Fagus crenata</i>	understory	-32.2	-0.3	50.9
105-year	<i>Fagus crenata</i>	understory	-32.6	-0.1	54.9

Appendix S2. Measured values and attributes of isotope samples of spiders. Sampling sites were named for the stand age (years after clear-cutting). Sampling site, species and feeding guild shown in the table were used as explanatory variables for the analyses of isotopic values and diet ages.

Sampling site	Species	Feeding guild	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\Delta^{14}\text{C}$ (‰)	Diet age (year)
1-year	<i>Araneus macacus</i>	Orb web	-26.4	4.3	65.6	7.0
1-year	<i>Argiope bruennichi</i>	Orb web	-25.0	4.0	52.8	4.0
1-year	<i>Carrhotus xanthogramma</i>	Visual pursuit	-26.8	3.6	57.4	5.1
1-year	<i>Cheiracanthium japonicum</i>	Nocturnal	-28.7	2.3	37.6	-0.6
1-year	<i>Diaea subdola</i>	Crab	-26.8	2.8	60.4	5.8
1-year	<i>Leucauge subblanda</i>	Orb web	-25.5	5.5	66.0	7.1
1-year	<i>Leucauge subblanda</i>	Orb web	-25.1	4.1	74.8	8.9
1-year	<i>Leucauge subblanda</i>	Orb web	-25.3	3.7	66.6	7.2
1-year	<i>Leucauge subgemmea</i>	Orb web	-25.6	3.9	65.4	7.0
1-year	<i>Neoscona adianta</i>	Orb web	-25.3	4.3	53.4	4.1
7-year	<i>Araneus stella</i>	Orb web	-27.7	4.3	49.2	1.2
7-year	<i>Carrhotus xanthogramma</i>	Visual pursuit	-27.2	2.4	59.1	4.0
7-year	<i>Cyclosa hamulata</i>	Orb web	-25.5	6.7	87.7	10.1
7-year	<i>Leucauge subblanda</i>	Orb web	-25.4	3.4	79.4	8.6
7-year	<i>Leucauge subblanda</i>	Orb web	-25.5	3.9	77.5	8.2
7-year	<i>Leucauge subblanda</i>	Orb web	-26.1	4.3	77.1	8.1
7-year	<i>Leucauge subgemmea</i>	Orb web	-25.8	2.4	94.7	11.4
7-year	<i>Oxytate striatipes</i>	Crab	-25.4	4.9	73.1	7.3
7-year	<i>Tetragnatha yesoensis</i>	Orb web	-25.3	5.0	66.4	5.8
7-year	<i>Trachelas japonicus</i>	Nocturnal	-26.8	0.9	34.7	-4.1
11-year	<i>Clubiona rostrata</i>	Nocturnal	-26.8	3.0	59.2	4.1
11-year	<i>Leucauge subblanda</i>	Orb web	-26.5	3.1	67.6	6.1
11-year	<i>Leucauge subblanda</i>	Orb web	-26.4	3.8	56.0	3.2
11-year	<i>Leucauge subblanda</i>	Orb web	-25.2	3.3	71.1	6.9
11-year	<i>Menosira ornata</i>	Orb web	-28.0	2.8	45.8	0.1
11-year	<i>Oxytate striatipes</i>	Crab	-26.3	5.1	67.9	6.2
11-year	<i>Plexippides doenitzi</i>	Visual pursuit	-26.0	4.2	48.3	1.0
11-year	<i>Tetragnatha yesoensis</i>	Orb web	-27.0	2.9	71.3	7.0
11-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.5	3.9	48.5	1.0
11-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-27.3	3.3	64.1	5.3
11-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.9	3.2	49.3	1.3

Sampling site	Species	Feeding guild	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\Delta^{14}\text{C}$ (‰)	Diet age (year)
24-year	<i>Araneus stella</i>	Orb web	-27.3	3.4	46.1	2.0
24-year	<i>Clubiona japonica</i>	Nocturnal	-25.6	1.0	59.7	5.6
24-year	<i>Leucauge subblanda</i>	Orb web	-26.7	2.6	52.8	3.9
24-year	<i>Menosira ornata</i>	Orb web	-27.6	2.8	48.1	2.6
24-year	<i>Oxytate striatipes</i>	Crab	-26.0	4.3	61.9	6.1
24-year	<i>Plexippides doenitzi</i>	Visual pursuit	-26.1	3.6	51.5	3.6
24-year	<i>Tetragnatha yesoensis</i>	Orb web	-26.4	2.7	63.8	6.6
24-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.1	2.9	55.4	4.6
24-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.3	2.9	59.8	5.6
24-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.6	3.0	50.5	3.3
105-year	<i>Clubiona rostrata</i>	Nocturnal	-26.8	3.0	58.6	2.8
105-year	<i>Leucauge subblanda</i>	Orb web	-25.9	1.4	70.8	6.0
105-year	<i>Leucauge subblanda</i>	Orb web	-25.3	2.9	72.7	6.4
105-year	<i>Leucauge subblanda</i>	Orb web	-25.1	4.3	61.8	3.7
105-year	<i>Neoscona scylla</i>	Orb web	-27.5	4.1	66.9	5.0
105-year	<i>Oxytate striatipes</i>	Crab	-26.1	2.8	58.2	2.7
105-year	<i>Tetragnatha yesoensis</i>	Orb web	-26.0	1.3	66.5	4.9
105-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.2	1.9	48.9	-0.1
105-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-27.5	1.0	66.9	5.0
105-year	<i>Yaginumaella striatipes</i>	Visual pursuit	-26.0	3.2	72.4	6.3

Appendix S3. Measured values and attributes of isotope samples of prey. Diptera samples were divided by body length; the “size fraction” column gives the size ranges. Sampling sites were named for the stand age (years after clear-cutting). "-" indicates the item was not measured.

Sampling site	Prey category	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\Delta^{14}\text{C}$ (‰)	Diet age (year)
1-year	Collembola	-26.8	-2.8	35.5	-1.4
1-year	Collembola	-26.8	-2.0	-	-
1-year	Collembola	-26.6	-1.8	-	-
1-year	Diptera 0- 5 mm	-25.4	6.3	56.0	4.8
1-year	Diptera 5-10 mm	-25.3	6.8	54.2	4.3
1-year	Diptera 10- mm	-24.7	3.2	91.6	11.8
1-year	Hemiptera	-27.3	-1.9	40.6	0.4
1-year	Hemiptera	-27.2	-0.3	-	-
1-year	Hemiptera	-27.4	-0.5	-	-
1-year	Lepidoptera larvae	-30.0	-0.3	33.1	-2.3
1-year	Lepidoptera larvae	-28.1	-2.4	-	-
1-year	Lepidoptera larvae	-28.2	-2.0	-	-
7-year	Collembola	-26.9	-3.6	40.3	-1.8
7-year	Collembola	-27.2	-5.0	-	-
7-year	Collembola	-27.0	-2.9	-	-
7-year	Diptera 0- 5 mm	-25.1	5.5	93.5	11.2
7-year	Diptera 5-10 mm	-25.6	5.1	49.1	1.2
7-year	Diptera 10- mm	-24.8	7.3	84	9.5
7-year	Hemiptera	-27.5	-1.8	35.7	-3.6
7-year	Hemiptera	-27.4	2.6	-	-
7-year	Lepidoptera larvae	-31.4	1.3	45.2	-0.1
7-year	Lepidoptera larvae	-28.5	-2.2	-	-
7-year	Lepidoptera larvae	-29.1	-2.4	-	-
11-year	Collembola	-27.0	-3.4	38.6	-2.4
11-year	Collembola	-26.8	-3.6	-	-
11-year	Diptera 0- 5 mm	-25.8	3.9	72.4	7.2
11-year	Diptera 5-10 mm	-25.5	3.8	71.0	6.9
11-year	Diptera 10- mm	-24.0	7.4	100.9	12.4
11-year	Hemiptera	-30.7	-2.8	34.6	-4.1
11-year	Hemiptera	-28.4	-1.6	-	-
11-year	Hemiptera	-28.9	3.3	-	-
11-year	Lepidoptera larvae	-31.0	-1.5	39.1	-2.2
11-year	Lepidoptera larvae	-32.3	-0.6	-	-
11-year	Lepidoptera larvae	-32.0	-1.4	-	-

# Appendix S3 continued

Sampling site	Prey category	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	$\Delta^{14}\text{C}$ (‰)	Diet age (year)
24-year	Collembola	-27.8	-4.4	41.7	0.7
24-year	Collembola	-27.5	-3.4	-	-
24-year	Collembola	-27.4	-3.5	-	-
24-year	Diptera 0- 5 mm	-26.2	4.9	67.6	7.4
24-year	Diptera 5-10 mm	-26.5	2.8	52.5	3.8
24-year	Diptera 10- mm	-26.1	1.4	90.5	11.6
24-year	Hemiptera	-30.5	-1.3	38.7	-0.3
24-year	Hemiptera	-28.3	-0.2	-	-
24-year	Hemiptera	-30.0	-0.8	-	-
24-year	Lepidoptera larvae	-31.9	-1.3	32.3	-2.7
24-year	Lepidoptera larvae	-33.1	-2.6	-	-
24-year	Lepidoptera larvae	-30.2	-1.2	-	-
105-year	Collembola	-27.7	-4.5	38.1	-4.3
105-year	Collembola	-27.5	-3.2	-	-
105-year	Diptera 0- 5 mm	-25.6	3.7	78.0	7.5
105-year	Diptera 5-10 mm	-26.8	4.3	47.1	-0.8
105-year	Diptera 10- mm	-27.3	2.3	70.8	5.9
105-year	Hemiptera	-30.2	0.1	48.6	-0.3
105-year	Hemiptera	-31.5	0.2	-	-
105-year	Hemiptera	-32.4	2.3	-	-
105-year	Lepidoptera larvae	-30.6	1.6	39.6	-3.7
105-year	Lepidoptera larvae	-31.2	-0.8	-	-
105-year	Lepidoptera larvae	-31.8	0.9	-	-