

A reference-based approach for estimating leaf area and cover in the forest herbaceous layer

Christopher A. Walter • Mark B. Burnham • Frank S. Gilliam • William T. Peterjohn

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Abstract Cover data are used to assess vegetative response to a variety of ecological factors. Estimating cover in the herbaceous layer of forests presents a problem because the communities are structurally complex and rich in species. The currently employed techniques for estimating cover are less than optimal for measuring such rich understories because they are inaccurate, slow, or impracticable. A reference-based approach to estimating cover is presented that compares the area of foliar surfaces to the area of an observer's hand. While this technique has been used to estimate cover in prior studies, its accuracy has not been tested. We tested this hand-area method at the individual plant, population, and community scales in a deciduous forest herbaceous layer, and in a separate farm experiment. The precision, accuracy, observer bias, and species bias of the method were tested by comparing the hand-estimated leaf area index values with actual leaf area index, measured using a leaf area meter. The hand-area method was very precise when regressed against actual leaf area index at the plant, population, and community scales (R^2 of 0.97, 0.93, and 0.87). Among the deciduous sites, the handarea method overestimated leaf area index consistently by 39.1 % at all scales. There was no observer bias

C. A. Walter (⊠) • M. B. Burnham • W. T. Peterjohn Department of Biology, West Virginia University, 35 Campus Drive, Morgantown, WV 26506, USA e-mail: cwalte12@mix.wvu.edu

F. S. Gilliam

Department of Biological Sciences, Marshall University, Huntington, WV 25755, USA detected at any scale, but plant overestimation bias was detected in one species at the population scale. The hand-area method is a rapid and reliable technique for estimating leaf area index or cover in the forest herbaceous layer and should be useful to field ecologists interested in answering questions at the plant, population, or community level.

Keywords Leaf area index · Plant cover · Herbaceous layer · Forest understory · Low-tech sampling

Introduction

Quantitative analysis of the forest herbaceous layer (all vascular plants one meter tall or less) relies on accurate estimates of the cover of plant species. Cover is broadly defined as the percent of ground area covered by individual plants, groups of plant species, or by the entire plant community. However, the term "cover" has many specific and specialized operational definitions (Wilson 2011). Regardless of which type of cover is being measured, cover data are essential in addressing several ecological phenomena including responses to experimental manipulations (Gilliam 2014), successional change (Ladwig and Meiners 2010), ecological restoration (D'Antonio and Meyerson 2002), comparisons of species diversity metrics (Thomas et al. 1999), and tracking the spread of invasive species (Didham et al. 2005).

Cover has been measured using a variety of methods. The more popular methods for estimating cover use visual estimation to assign cover-abundance classes to plants, species, or functional groups, e.g., the methods of Braun-Blanquet (1964), Daubenmire (1959), and Domin and Krajina (see Mueller-Dumbois and Ellenberg 1974). Visual estimations are done by one or more observers that determine the percentage of bare ground covered by individual plants, species, or entire communities. Although visual methods are quick, they rely on subjective classification, which can lead to errors in cover estimates as large as 20 % (Sykes et al. 1983; Kennedy and Addison 1987; Hatton et al. 1986; Tonteri 1991). Furthermore, errors in the repeatability of visual estimation methods are due to observer bias that cannot be overcome by observer training (Sykes et al. 1983; Kercher et al. 2003; but see Leps & Hadincova 1992).

More accurate methods for estimating cover exist, but they also have limitations that make them less than ideal for use in the forest herbaceous layer. Allometric relationships between leaf dimensions and leaf area (Wargo 1978) can be more accurate than visual estimations of cover, but this method requires both a priori knowledge of the allometry and extensive time measuring one or more dimensions of individual leaves. Line-intercept sampling (Tansley and Chipp 1926; Kent and Coker 1992) can be an accurate technique to measure cover that employs a transect line stretched in a random direction across an area. An observer records, for each species, the length of the line that intercepts that species. The percent cover of a species is then calculated as the distance of the line that was intercepted by that species divided by the total distance of the line and multiplied by 100. Likewise, point-intercept sampling (Drew 1944; Levy and Madden 1933; Goodall 1953) is another technique that can be more accurate than visual estimation. In point-intercept sampling, a gridded frame is placed above the sampling area and a pin is placed vertically from each grid point to the ground. The percent cover of a species is then calculated as the number of pins which intersect the species divided by the total number of pins and multiplied by 100. However, lineintercept sampling is most appropriate for more sparsely vegetated areas like shrublands (Spellman 2011), whereas the point-intercept method is subject to weather-related (e.g., wind and rain) errors in measurement, in addition to being time-consuming when carried out in plant communities with intricate architecture and high species richness (Fenner 1997; Stampfli 1991).

Finally, using ground-based, nadir-facing photography to measure cover is a relatively new method that is at least as accurate as visual methods (Dietz and Steinlein 1996; Macfarlane and Ogden 2012). Photographic methods are done by extending a tripod or frame above the sampling area and attaching a downward-facing camera. Photographs of the sampling area are taken and the area of plants, species, or the entire community is determined using image processing software. The distinct drawback of the photographic method is that it only measures the uppermost level of vegetation (Dietz and Steinlein 1996; Vanha-Majamaa et al. 2000). While this layer of vegetation-known as "top cover" (Wilson 2011)-can be a useful metric, it is not as robust a measurement for comparing species abundances, determining species richness and diversity, nor measuring vegetation close to the ground. Thus, the photographic method would fail to accurately measure the cover of dense, rich, and structurally complex communities, such as those in the temperate deciduous forest understory.

A simple approach to measure cover is presented, whereby an observer compares the area of their hand to the area of foliar surfaces. This approach has been used successfully to measure herbaceous layer cover in contrasting forest ecosystem types and experimental manipulations. Gilliam and Christensen (1986) used this method to assess effects of varying season and frequency of prescribed burning on the herbaceous layer of a Coastal Plain pine flatwoods. It was used by Gilliam and Turrill (1993) and Gilliam et al. (1995) to quantify effects of forest harvesting on herbaceous layer communities of central Appalachian deciduous forests, with Gilliam and Turrill (1993) further combining visual estimates along with subsampling of aboveground biomass to allow for extensive non-destructive estimates of herbaceous layer biomass. A more recent focus at this deciduous forest site has been on assessing the effects of experimental additions of nitrogen (Gilliam et al. 2006). However, despite these published applications, this method has yet to be assessed quantitatively with respect to its precision and accuracy.

Accordingly, the objective of this research was to test the precision, accuracy, and potential observer bias of the hand-area method as a rapid and reliable estimator of leaf area and cover in the forest herbaceous layer at three scales: (1) individual plants, (2) individual populations in small plots, and (3) the entire plant community in small plots.

Materials and methods

Study area and species selection

The cover of individual plants, individual populations, and the entire plant community within small plots was estimated, and measured, in the West Virginia University Core Arboretum-a 36.8-ha deciduous forest preserve in the north-central Appalachian region of West Virginia, USA (39.6460° N, 79.9801° W). The Core Arboretum supports predominantly mixed mesophytic forest stands of variable age ranging from early successional to old growth. The dominant tree species include white oak (Quercus alba), red oak (Quercus rubra), shagbark hickory (Carya ovata), pignut hickory (Carya glabra), American beech (Fagus grandifolia), sugar maple (Acer saccharum), black cherry (Prunus serotina), and white ash (Fraxinus americana). Similar to other mesophytic forests, the herbaceous layer at the Core Arboretum is diverse, with over 300 non-woody vascular plants present. Vegetation in the herbaceous layer is primarily a mixture of annual, perennial, and biennial forbs, and woody tree seedlings. Rubus allegheniensis plants that were being grown at a nearby experimental farm were also included in this study. R. allegheniensis plants were grown and measured at the West Virginia University Agronomy Farm (39.6595° N, 79.9028° W), located four miles east of the Core Arboretum. The ability to accurately estimate the cover of R. allegheniensis is of particular importance because this species has become increasingly dominant in the herbaceous layer of Appalachian forests following enhanced nitrogen inputs (Gilliam 2014).

Experimental design

In order to examine the accuracy and precision of the hand-area method (HA), herbaceous layer cover estimates were compared to measurements made using a leaf area meter (LI-3100, LI-COR, Nebraska, USA). Since cover is typically defined as the proportion of the ground covered by a particular species (i.e., leaves that overlap are not measured separately), measuring each leaf of that species on a leaf area meter would overestimate cover. To work around this potential for overestimation, we measured a particular type of cover using the hand-area method—leaf area index (LAI) which is the leaf area of a plant, population, or community per unit ground area (Wilson 2011). Herbaceous

layer LAI was estimated in situ using HA at four randomly chosen sites along a transect within the Core Arboretum and at the West Virginia University Agronomy Farm. We defined the herbaceous layer as all vascular plants one meter tall or less (Gilliam and Roberts 2003). Once in situ LAI estimates were completed using the hand-area method, the plants were clipped at the base and placed in paper bags for transport to the leaf area meter. The plants were then removed from the bags and the leaves were removed from each plant and passed through the meter to obtain measurements of true LAI. Thus, at the plant, population, and community scale, we had both an estimate of LAI from the hand-area method (LAI_E) and the measurement of actual LAI (LAI_A) from the leaf area meter.

Within each arboretum site, four randomly selected 1-m² circular plots were surveyed. Two sites were chosen to estimate the LAI_E of each plant of a randomly selected species in order to test the accuracy of our method at the scale of individual plants. This resulted in the use of 21 plants from four species (Solidago spp., Acer rubra, P. serotina, and C. glabra). At the same two sites, we also estimated the total LAI_E of each species found in every plot in order to assess the accuracy of our method at the scale of individual populations. A total of 20 different species were used at the population scale, 7 tree species, 2 woody vine species, and 11 herbaceous species. In the other two sites, we estimated only the total LAIE of all plants regardless of species in order to assess the accuracy of our method at the scale of the entire plant community found in the small plots. Finally, to strengthen our assessment of this method for estimating the leaf area of individual plants, we used hand-area method to estimate the LAIE of 42 R. allegheniensis plants that were being grown in pots at the West Virginia University Agronomy Farm under a variety of light and fertilizer treatment combinations. At each scale, the plants were harvested and analyzed with a leaf area meter to measure LAI_A.

Hand-area method

The HA method compares the area of a hand with the area of the individual leaves of a plant, a species, or a community. An observer places a hand, palm-down and fingers closed, directly above the foliar surface of a plant. The observer then determines the size of leaf surfaces in relation to their hand (Fig. 1), either individual leaves or clusters of smaller leaves in increments as small as 0.5



Fig. 1 Diagram illustrating the hand-area method for measuring the leaf area index of a *Smilax rotundifolia*, approximately 0.5 hand; b *Dennstaedtia punctilobula*, approximately one hand; and c *Acer pensylvanicum*, approximately two hands. Plant images from Britton and Brown (1913)

hands. After the total leaf area for each plant was estimated using HA, an observer should have touched all leaves of that plant, comparing their hand area to the leaf area. Likewise when estimating the LAI_E of a population, the observer would have touched all of the leaves of that species and all of the leaves in the entire plot when estimating the LAI_E of a plant community. To improve both the precision and accuracy of the method, two observers made hand-area estimates separately (either at the plant, population, or community scale), then compared their estimates and recorded the average of the two estimates-a process known as active feedback (Wintle et al. 2013). Observers used only their dominant hand for all measurements and traced the outline of their hands on paper and analyzed them using the leaf area meter to determine the actual area of their hands.

Statistical analysis

To assess both the precision and accuracy of the handarea method, LAI_E was regressed against LAI_A at the individual plant, population, and community scales. The precision of HA was evaluated using the coefficient of determination (R^2) from regression models, with higher R^2 values indicating a greater precision because LAI_E explained more of the variance in LAI_A .

The accuracy of the hand-area method was assessed by comparing the slopes of regression lines to the 1:1 line using two-tailed, one-sample t tests. We determined the 1:1 line using the weighted average of the measured hand areas of all observers. The weighted average was used because some pairs of observers measured more plants or plots than others. Slopes significantly lower than the slope of the 1:1 line indicate that HA overestimated LAI_A, and slopes significantly higher than the 1:1 line indicated HA underestimated LAIA. To test if the accuracies were equal across the plant, population, and community scales, the slopes of regression lines were compared to each other in a pairwise fashion using multiple analysis of covariance tests (ANCOVA; model effects: LAI_E and LAI_E×scale) without an α -level correction for family-wise error. An α -level correction was not used because it inflates the type-II error rate and increases the likelihood of reporting falsely that HA is equally accurate across all scales (Saville 1990).

In addition to testing the precision and accuracy of our method, we also tested for any species-related and observer biases. We tested for a species-related bias at the individual plant and population scales by comparing the residuals of the LAI_A vs. LAI_E regression line in a oneway analysis of variance (ANOVA; model effect: species). Species with significant negative mean residual distances indicated that HA overestimated LAIA relative to the regression, and species with significant positive residual distances indicated HA underestimated LAIA. Species that were only observed once were not included in residual analysis because ANOVA requires a sample size of at least two for each species comparison. A post hoc Tukey's honest significant difference (THSD) test was used to compare the mean residual distance among species to determine pairwise differences.

A preliminary test of the effect of leaf morphology on the accuracy of HA was also made at the population scale. The LAI_A was regressed against LAI_E for species with three or more occurrences at the population scale a total of six species—and the slopes of the lines (i.e., the accuracies) were compared using an ANCOVA. We consider this test to be an initial assessment because we had only 22 observations that could be used to create regression lines (LAI_A vs. LAI_E) for six species. The leaf length-to-width ratio was used as an index of leaf morphology for each species. Leaf length was defined as the length of the axis from leaf petiole to leaf tip, and leaf width was defined as the length of the longest perpendicular axis. We determined the mean ratio for 10 leaves of each species using plants growing in the Core Arboretum or using specimens from the West Virginia University Herbarium. To determine if leaf morphology had an effect on the accuracy of HA, the slopes of the regression lines of LAIA vs. LAIE were regressed against the leaf length-to-width ratios, and that relationship was assessed using R^2 and a one-sample t test to determine if the slope was different from zero.

To test for observer bias, an ANCOVA (model effects: LAI_E and LAI_E×observer pair) was used to determine if the accuracy of HA depended on the observer pair at each scale. If any significant effects of the $LAI_E \times$ observer pair term were found, then they would indicate a bias in HA for at least one observer pair. Two groups of distinct observer pairs were compared at the individual plant scale, three at the community scale, and two at the population scale. Due to the limited degrees of freedom and the complexity of the model, the ANCOVA test at the plot scale could only be applied at seven of the eight plots where all plants were measured together. Furthermore, observer bias could not be tested for leaf area estimates of individual R. allegheniensis plants at the West Virginia University Agronomy Farm because the same observer pair measured all of the plants. Two individual plants from the arboretum were identified as outliers using a jackknife distance test based on the multivariate mean of LAIE and LAI_E, and they were removed from all analyses. All statistical analyses were performed using SAS JMP (SAS Institute 2013), and transformations were applied when appropriate to normalize residuals and meet parametric test assumptions.

Results

Hand-area precision

Regression of LAI_A vs. LAI_E at the individual plant, population, and plant community scales in the Core

Arboretum produced R^2 values of 0.97, 0.93, and 0.87, respectively (Fig. 2). At the scale of the entire plant community, the leaf area in eight plots was determined by estimating the cover of all plants regardless of species, and the community-scale leaf area of the remaining eight plots was determined by adding the values for the constituent populations. An ANCOVA of LAIA vs. LAIE for entire plant communities revealed that the effect of HA on LAIA did not depend on whether the leaf area of the plants in the plots were estimated together or calculated by adding the estimates obtained for individual populations (one line for both cases in Fig. 2d). However, the regression of LAI_A vs. LAI_E in the eight plots where the leaf area of the plants was estimated together had an R^2 of 0.80, and in the eight plots where the total leaf area was estimated by adding the values for the constituent populations, the R^2 was 0.95. For individual R. allegheniensis plants grown at the agronomy farm, the R^2 was 0.94.

Hand-area accuracy

For individual plants, populations, and entire plant communities, one-sample t tests confirmed that the slopes of the regression lines of LAI_A vs. LAI_E were all lower than the 1:1 line that was calculated using the weighted mean hand-area of observer pairs (i.e., LAI_E overestimated LAI_A; Fig. 2). For individual plants in the Core Arboretum, the slope was 39.4 % lower than the 1:1 line (t=20.438, p<0.0001). However, for the individual R. allegheniensis plants at the agronomy farm, the slope was only 16.5 % lower (t=3.914, p < 0.0001). At the population scale, the slope of the regression line of LAIA vs. LAIE was 41.8 % lower than the 1:1 line (t=20.981, p<0.0001), and at the community scale, it was 36 % lower (t=13.188, p<0.0001). Pairwise ANCOVA tests revealed that the slopes of the regression lines at the plant, population, and community scales in the Core Arboretum were not different from one another, and the mean difference between the 1:1 line and realized slopes was a decrease of 39.1 %.

Species-related bias

At the plant scale, an ANOVA determined that there were no differences among species in mean deviation from the regression line of $LAI_A vs. LAI_E$ —and thus no detectable species-related bias. However, at the population scale, there was an effect of species on residual



Fig. 2 Comparisons of leaf area index measured via leaf area meter (actual LAI) vs. leaf area index estimated using the handarea method (estimated LAI) for: **a** individual plants of *R. allegheniensis*; **b** individual plants of four different species (*Solidago* spp., *A. rubra*, *P. serotina*, and *C. glabra*); **c** populations of plants (20 different species) within 1-m² plots; and **d** the entire plant community within 1-m² plots. *Dashed lines* are 1:1 lines,

distance (F=2.838, p=0.0117; Fig. 3), and thus a species-related bias. Specifically, the post hoc THSD revealed that the species *Stellaria pubera* had a residual distance that was lower than *A. rubra* (p=0.0029), *C. glabra* (p=0.0192), and *A. saccharum* (p=0.0339). At the population scale, an ANCOVA determined that there was a difference among species in the slopes (i.e., accuracies) of LAI_A vs. LAI_E (F=4.262 p=0.0245; Fig. 4) and a further regression revealed a negative trend between the species slopes (from the regression of LAI_A vs. LAI_E) and leaf length-to-width ratio (t=5.23, p=0.0871; $R^2=0.56$; Fig. 4 inset).

Observer pair bias

The ANCOVA models testing observer pair bias found no effect of observer pair on the relationship between LAI_A and LAI_E at the scale of the individual plant, population, or entire plant community. Individual hand areas ranged from 115.7–159.9 cm², and mean hand areas of observer pairs ranged from 122.3–124.2 cm².

obtained using the weighted average hand area of observer pairs. *Open circles* in graph **d** indicate where the all plants in 1-m^2 plots were measured together and *closed circles* are the sum of the populations within 1-m^2 plots. The slope of the 1:1 line equals the weighted mean area (in square centimeter) of the hands used to make the estimates

Discussion

The hand-area method of estimating herbaceous layer LAI in a deciduous forest was found to be very precise at the scale of individual plants, plant populations, and entire plant communities. As a result, this method should be very useful for quickly assessing the relative differences in leaf area index and cover that can occur through time, space, or in response to experimental treatments. For studies requiring accurate estimates of leaf area index and cover, this method should also be useful. We found that HA overestimated LAI_A at each scale, but the degree of overestimation was consistently ~39.1 % across the scales we examined at the Core Arboretum (Fig. 2b-d). Thus, at this site, accurate estimates of leaf area can be obtained by simply subtracting 39.1 % from each LAI_E value in the dataset—or, equivalently by multiplying each LAI_{F} value by 0.609. For other investigators, and sites, it is recommended that a simple calibration be performed by harvesting a subset of the plants surveyed and measuring the actual leaf area as was done in this investigation.



Fig. 3 The mean residual distance (and SE) by species for regressions of actual leaf area index (measured via leaf area meter) vs. estimated leaf area index (estimated using the hand-area method).

Species are presented in ascending order from *left to right* according to the average leaf area per plant and *dissimilar letters* indicate significant differences (p < 0.05)



Fig. 4 Regression lines comparing leaf area index measured via leaf area meter vs. leaf area index estimated using the hand-area method for six separate species at the population scale. The

numbers in parentheses indicate the leaf length to leaf width ratio. *Inset:* The slope of the actual LAI vs. estimated LAI vs. the leaf length-to-width ratio for the same six species at the population scale

Our results also suggest that greater accuracy might be achieved when the reference area (a hand in this case) more closely matches the size and shape of the leaves being measured. For example, at the population scale, the leaf area of S. pubera was overestimated relative to estimates obtained for the three tree species in the residual analysis (Fig. 3), and it was the most over-estimated when compared to five other species in the leaf morphology analysis (Fig. 4). The leaves of S. pubera are typically less than 7.6 cm long and 3.2 cm wide, grow in opposite arrangement around a central stem, and are lanceolate in shape and sessile at the leaf base. By comparison, the leaves of tree and vine seedlings are typically more than twice as long and three times as wide, are more ovate or pinnate, and are more distinct from stems because they grow from petioles. Thus, the morphological characteristics of the trees and vines more closely resemble those of a hand and should improve the accuracy of the hand-area method. In fact, the average length to width ratio of the observer's hands in this study was 1.79-equal to the leaf length-to-width ratio of the most accurately estimated plant at the population scale, Parthenocissus quinquefolia (Fig. 4). The idea that leaf morphology affects the accuracy of estimation techniques is also supported by Sykes et al. (1983), who found that observer error using visual estimation techniques was highest among plants with smaller and thinner leaves. Leaf morphology is also the most likely reason why greater accuracy was achieved for R. allegheniensis plants (Fig. 2a). R. allegheniensis leaves are typically palmately compound with larger terminal leaflets and smaller lateral leaflets, and the leaflet configuration is very similar to the shape of a hand. Thus, the use of multiple reference areas for different types of leaves might be warranted but the additional effort would be unnecessary if, as in this study, a simple calibration (subtracting 39.1 % from each LAI_E observation) results in a robust correction factor.

The effect of leaf morphology on estimation accuracy is not unique to HA. Visual estimation techniques attempt to minimize this error by selecting areas in which to place plots with *a priori* knowledge of species composition (Mueller-Dumbois and Ellenberg 1974). The logic behind this practice is that errors created due to particular leaf morphology will be repeated in subsequent plots. However, practitioners of HA have the potential to disregard the practice of picking plots *a priori*, and quantitatively correct for differences in morphology by using the relationship between the slope of LAI_A vs. LAI_E and a measure of leaf morphology of that species (Fig. 4 inset) to estimate a species-specific correction factor—instead of applying the simpler calibration factor, mentioned above, to all species at once.

In addition to the precision and potential accuracy of this method, it is noteworthy that there was no over- or underestimation bias among observer pairs despite differences among the observers in both their experience and hand area. We believe the lack of an observer bias using the hand-area method may be due, in part, to the fact that it employed active feedback which is known to improve measurement accuracy (Wintle et al. 2013). The fact that some observer pairs were trained immediately prior to sampling, while others were experienced practitioners, is an indication that this method is not only robust with respect to its accuracy and precision, but also that it is easy to learn.

The results of this study indicate HA is a precise, accurate (when calibrated), easily learned, and convenient way to assess LAI and cover in the forest herbaceous layer. Therefore, HA should be useful to ecologists who are examining questions relevant to individual plants, plant populations, and entire plant communities in either field or experimental settings.

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