Photosynthetic Activity as a Productive Potential in Fragmented Forest Ecosystems

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***Abstract* — The inertia of fragmented ecosystems allows the study of mechanisms that transform these at plant level, the objective of this research was to develop the methodology to evaluate the photosynthetic activity and the capacity of different plant species that are part of the ecosystem, to recover their productivity. The study was carried out in Acajete Puebla, Mexico, delimiting conserved, transitional and agricultural areas. Tests were carried out with two chlorophyll extraction techniques, and at the same time the CO_2 consumed by the plant was randomly measured. The technique with the highest recovery was maceration with 2.38 g of chlorophyll per gram of fresh material. On other hand, the productivity of the agricultural area is greater than the one in transition, which is attributed to the fact that agricultural area is more dynamic since most of the species are herbaceous and have a short life span, contributing to the rapid assimilation, storage and sequestration of carbon. This concludes that the herbaceous species *Lachemilla procumbens* (gross photosynthetic rate 0.19 ppm CO_2/s) and *Stevia subpubescens* (0.16 ppm CO_2/ s) are optimal to start the recovery of a degraded ecosystem.**

***Keywords* — Chlorophyll, Herbaceous, Maceration, Productivity, Soxhlet.**

I. Introduction

Infrared active gases such as carbon dioxide (CO_2) and carbon monoxide (CO), known as greenhouse gases, are responsible for global warming. These gases produce the so-called greenhouse effect by absorbing and re-emitting solar radiation, causing global warming on the earth's surface and the atmosphere. The levels of these gases have increased in recent years as nations have industrialized and forest and grassland soils have become agricultural land

These emitted CO_2 and other greenhouse gases in the atmosphere have become a priority issue in the worldwide political agenda [1]. Today forests annually sequester 2–4 PgC from the atmospheric [2], they play an important role in mitigation climate change and global warming by stabilizing CO_2 concentrations in the atmosphere by photosynthesis [3].

Different forest types store different amount of carbon and changes in forest cover can be monitored to understand potentiality of forests as carbon sink [4]. In the last few years, quantification of forest biomass and its carbon fixation has emerged as an important field of research [5]. The major carbon pools in a forest ecosystem are the living trees and the understory vegetation, litter and soil organic matter. Legumes and grasses are the most extensively used types of plant cover that tend to improve the soil properties and carbon storage within aggregate fractions during fallow.

Destructive and non-destructive methods are used to estimate forest biomass, and destructive sampling methods involve harvesting, drying and weighing. Though accurate, destructive methods are not preferred as they are expensive and cause damage to forest health [6].

Estimates of Net Primary Productivity (NPP) are necessary to understand the storage capacity of organic carbon in the ecosystem, they have been used as indicators of environmental stress and to analyze the consequences of biomass extraction above the maximum productivity limits

Therefore, evaluating the photosynthetic activity shows the potential of ecosystem species, through the carbon gain (in the form of CO_2) or oxygen production, which are measured with infrared gas analyzer (IRGA), with a high cost [7].

There are studies based on productivity and health of aquatic ecosystems evaluated by photosynthetic activity, in which the concentration of chlorophyll a is quantified to estimate the biomass of phytoplankton communities [8]. Even when the conditions of terrestrial and aquatic ecosystems are different, chlorophyll in terrestrial plants are responsible for producing oxidation-reduction reactions, being the main component of photosynthesis.

In crops with commercial value, the performance and health of these species are evaluated by the amount of chlorophyll. The most commonly used chlorophyll meter is the SPAD 502 portable meter [9] made comparisons to measure chlorophyll using this meter and the conventional Arnon method of extraction implemented in 1963 on tomato crop and found a correlation of r = 0.96 between both methods, which shows its efficiency.

The purpose of this work was to evaluate the productivity of different plant species in a fragmented forest ecosystem, through their photosynthetic activity, as well as soil respiration, they regulate the carbon cycle, facilitating the exchange of this element between the existing vegetation, the soil and the atmosphere, quantifying the photosynthetic activity allowed to identify the plant species with the greatest productive potential (photosynthetic activity) in the selected areas.

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II. Materials and Methods

The place of study was on "El Tintero" hill located in San Bartolo El Pinal, a municipality of Acajete, Puebla, Mexico. Located at the geographic coordinates: parallels 19º 00 '30 "and 19º 11' 06" north latitude and meridians 97º 53 '54 "and 98º 00' 00" western longitude. In it there are conserved, transitional and agricultural areas, a representative area of a fragmented forest ecosystem was selected, inhabited by pine-oak, being the Quercus genus (oaks) the one that predominates. This temperate forest ecosystem is distributed almost all over the country from places at sea level up to 3000 m of altitude [10] In addition, the characteristics of this region are similar to 10% of the state of Puebla, with a temperate subhumid climate and summer rainfalls.

In each area 2 sampling sites were delimited, in polygons of 100 m^2 (squared meters), fresh plant samples were selected by each identified species, then transported to the laboratory in paper bags to conserve moisture and to avoid direct light contact, because once cut, chlorophyll is easily degraded by radiation (photodegradation). Sampling was done with fresh plant samples for pigment extraction.

There are several methods and instruments that require sophisticated equipment for the measurement of photosynthetic activity, (manometric method, bell-by-titration, infrared absorption of CO_2, and portable chlorophyll meters, among others) [11]. In this project the method was selected based on the multiple measurements that were made, (one for each species found in the conserved, transitional, and agricultural areas) the one that most adjusted was the extraction of chlorophyll, with the help of acetone as a solvent, tests were performed with 2 different techniques for assessing its efficiency and choose the most appropriate:

i. *MACERATE*: 1g of clean plant material without veins was weighed and macerated in a mortar with 20 ml of acetone, immediately the extract was filtered by vacuum and adjusted to 50 ml with acetone, for its measurement in the UV Vis spectrophotometer. [12]
ii. *SOXHLET*: 1 g of clean plant material without veins was weighed and placed in a cartridge for the Soxhlet apparatus, adjusting a 250 ml flask, which contained 150 ml of acetone, with a heating grill the temperature was set to provide 20 cycles. Immediately after the cycles where completed, the extract obtained in the ball flask was emptied into a container with a lid to prevent the acetone from evaporating. The extract was protected from the light until its measurement.

The measurement was made immediately after obtaining the extract by any of the techniques, if not, they should be stored in refrigeration at 4° C avoiding exposure to light.

The chlorophyll concentration measurement was performed with UV-visible spectrophotometer, the calibration curve was previously made with concentrated chlorophyll, making 4 serial dilutions, in a water-acetone mixture as solvent: 1:10, 1:100, 1:1000, 1:10000, and a mixture of acetone-water as target, this with the objective of obtaining the coefficient of Absorptivity (ε) for chlorophyll, and thus to evaluate the concentration of the samples by Lambert-Beer Law.

At the same time with the portable CO_2 analyzer (model 2810 of the Bacharach brand), random measurements were made during sampling to 10 herbaceous species, whose atmosphere was isolated with a transparent chamber, with the objective of not modifying the radiation that the plant receives. The amount of CO_2 consumed by the plant was evaluated in parts per million (ppm) with a measurement range of 0 to 10,000 ppm.

The equipment was calibrated, and the measurements were taken in intervals of 30 seconds, for 5 minutes, first outside the chamber, in order to obtain a base value of CO_2 emission in the area and then inside the chamber with the plant and the meter inside.

This was done to observe CO_2 emissions as a result of the photosynthesis process. As the plant absorbs this gas, it is expected that the values obtained inside the chamber will be lower.

For the results of direct measurements of photosynthetic rate, the statistical test F was used, and included an analysis of variance (ANOVA) to evaluate if there was a significant difference between the data thrown in the measurement intervals.

A one-factor simple ANOVA was used, and the factor was (that is a qualitative variable was specified) the photosynthetic rate with which the groups or treatments to be compared are designated. Factor levels are the number of treatments or groups, for this case they are the time intervals considered. An ANOVA was carried out for each sample, it is expected that there will be a significant difference in the photosynthetic rate in each species, otherwise this would indicate that the amount of CO_2 used by the species is constant and that there are no peaks of consumption or respiration of the plant. For the comparison of means, the hypothesis set evaluates the statistical difference between the means of the data.

Due to the simplicity of the analysis, they can be done in Microsoft Excel, in Minitab or in Statistix and the results are the same.

III. Results and Discussion

Measurements of the dilutions and the extract of the samples (of the same species) were made in the spectrophotometer at a 640 nm wavelength, obtaining the following results (Table I).

These data were plotted (concentration Vs absorbance) and adjusted by the linear regression method, obtaining (1).

$$y = 5.5486x + 0.0243 \quad (1)$$

Where the value 5.5486 represents the slope (m) of the line that the data models, and consequently this value corresponds to the coefficient of Absorptivity (ε = 5.5486 L/cm * Mol). With this value the concentration of the samples by both methods is evaluated, according to Beer's Law given in (2).

$$A = \varepsilon \cdot c \cdot l \quad (2)$$

TABLE I: Results For The Calibration Curve Of Chlorophyll

Serial dilutions	Concentration (Mol/L)	Absorbance (640 nm)
Blank	0	0
1:10	0.113006047	0.646
1:100	0.011300605	0.141
1:1000	0.00113006	0.022
1:10000	0.000113006	0.009

A. With the Macerate Technique

The Absorptivity (A) obtained from the reading in the sample was A = 0.296, so the concentration was calculated as (3).

$$c = \frac{A}{\varepsilon \cdot l}$$

$$c = \frac{0.296}{\left(5.5486 \frac{L}{cm \cdot Mol}\right) \cdot 1\ cm} = \left[\frac{0.053\ Mol}{L}\right] \cdot \frac{893\ g}{Mol} = 47.63\ g/L \qquad (3)$$

Expressed in g of chlorophyll per gram of fresh vegetation given in (4).

$$= \frac{47.63 \frac{g}{L} * 0.050 L}{0.9998 g} = 2.38 \frac{g\ de\ chlorophyll}{g\ de\ material\ fresco} \qquad (4)$$

Since the extraction techniques were performed on different days, 2 calibration curves were needed. For this second measurement the same calculations were made but this time the slope value was equal to 16,115, so the coefficient of absorptivity equals to ε = 16,115 L / cm * Mol.

B. With the Soxhlet Apparatus

The Absorptivity (A) obtained in the sample was A = 0.0765, so the concentration was calculated as (5).

$$c = \frac{A}{\varepsilon \cdot l} = \frac{0.0765}{\left(16.115 \frac{L}{cm \cdot Mol}\right) \cdot 1\ cm} = \left[\frac{0.0047\ Mol}{L}\right] \cdot \frac{893\ g}{Mol} = 4.23\ g/L \qquad (5)$$

Expressed in g of chlorophyll per gram of fresh vegetation (6).

$$= \frac{4.23 \frac{g}{L} * 0.150 L}{0.9998 g} = 0.63 \frac{g\ de\ clorofila}{g\ de\ material\ fresco} \qquad (6)$$

The Soxhlet extraction technique obtained a lower percentage of chlorophyll, which could be because after the fourth cycle there was no apparent recovery, so the technique was suspended after the sixth cycle.

Sampling was carried out in the months of February, March and April of 2016 and were found: 16 plant species in conserved area, 23 in transition area and 30 in an agricultural area. Since it´s a forest ecosystem there are repeated species in the 3 areas and a total of 63 species were quantified but only 38 were identified by name. Eleven of the totals were arboreal although the analysis only included shrub and herbaceous species, due the objective of this study that is to identify productive species that reproduce in short term.

The rate of recovery of chlorophyll extracted by other authors in shrubs such as tomatoes varies between 0.00326 - 0.0025 per gram of plant [9] and in arboreal, as pine goes from 0.88 to 1.06 g [13] or even lower in orange and tangerine [14] so it is possible to say that the amount of chlorophyll extracted by the mashing method is significantly high.

The following values are the average of CO_2 measurements, divided by the total measurement time, to have a gross photosynthesis rate per species (Table II). The CO_2 fixation rates in leaves of different unidentified species, vary from 0.01-1 ppm / s, which provides a valid CO_2 measurement method for the purposes of the experiment [15].

There hasn't been found any bibliography related to the quantitative measurement of herbaceous species productivity, so it isn´t possible to affirm that the species found are the most efficient to improve the productivity of agroforestry systems. However, it can be stated that the content of chlorophyll extracted from the herbaceous is higher than other shrub and tree species, and that the gross photosynthetic rate of the analyzed samples are within the limit of detection obtained in plants with similar conditions.

Describing the results, the species that have the highest photosynthetic rate, have been chosen as species with competitive advantage they are listed in Table II, out of a total of 23 species, 8 weren´t identified taxonomically, but they are mostly herbaceous.

TABLE II: GROSS PHOTOSYNTHETIC RATE OF THE MOST PRODUCTIVE HERBACEOUS SPECIES

Sample (Specie)	Gross photosynthetic rate (ppm CO_2/s)
Lachemilla procumbens	0.188
AAM5*	0.542
AAM2*	0.188
ATM11*	0.212
ATM16*	0.073
Stevia subpubescens	0.158

IV. CONCLUSION

The macerate method shows greater efficiency for the recovery of photosynthetic pigments, surpassing the Soxhlet method by 1.75g of chlorophyll per g of fresh material, it´s also a fast technique, less solvent is used (the difference is about 80 ml) and is lost by evaporation. This allowed us to choose it for the extraction of chlorophyll and determination of photosynthetic activity in plant species characteristic of a fragmented forest ecosystem).

The productivity of the agricultural area (0.179 ppm CO_2/s) is greater than that of the area in transition (0.157 ppm CO_2/s), which is attributed to the fact that although in the latter the three vegetable strata still exist, the agricultural area is in the recovery process. In addition, the conserved area (0.218 ppm CO_2/s) is more productive than agriculture, but in this case, this productivity is not accompanied by the richness of an ecosystem that has been "dismantled" and that is in the process of regeneration, as dictated by the succession because in this process the ecosystem always seeks to establish optimal conditions for its own restoration.

An agricultural ecosystem is more dynamic than an ecosystem in conservation or one in transition, since most species present in the place are herbaceous and have a short life span, contributing to the fixation, storage, and sequestration of carbon with greater speed than shrub and tree species, who, when they die, take more time to decompose and contribute carbon to the soil. Based on the previous observations, it is concluded that herbaceous species are optimal to initiate the recovery of a degraded ecosystem, as well as promoting diversity. And since 23 species with photosynthetic activity above the average (from 0.02 to 0.54 ppm CO_2/s) have been listed, it is concluded that the objectives set out in this research are met.

REFERENCES

[1] Edenhofer O, Pichs-Madruga R, Sokona Y, Farahani E, Kadner S, Seyboth K, Adler A, *et al.* Climate Change 2014: Mitigation of Climate Change. Contribution of Working Group III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. *IPCC*, 2014.

[2] Qureshi A, Pariva, Badola R, Hussain SA. A review of protocols used for assessment of carbon stock in forested landscapes. *SciVerse ScienceDirect*, 2011: 81-87.

[3] Robert M. *Septième Réunion Du Sous-comité Ouest Et Centre Africain de Corrélation des Sols Pour la Mise en Valeur Des Terres: Ouagadougou, Burkina Faso, 10-17 Novembre 1985*. Food and Agricultural Organization of the United Nations, 2001.

[4] Dixon RK, Solomon AM, Brown S, Houghton RA, Trexier MC, Wisniewski J. Carbon pools and flux of global forest ecosystems. *Science*, 1994; 14(263): 185–190.

[5] Usuga JC, Toro JAR, Alzate MVR, Tapias AJ. Estimation of biomass and carbon stock in plants, soil and forest floor in different tropical forests. *Forest Ecology and Management*, 2010; 260(10): 1906-1913.

[6] Nordh NE, Verwijst T. Above-ground biomass assessments and first cutting cycle production in willow (Salix sp.) coppice-a comparison between destructive and non-destructive methods. *Biomass and Bioenergy*, 2004; 27(1): pp. 1-8.

[7] Víctor HL. Métodos para medir fotosíntesis. Cátedra de Fisiología Vegetal. Argentina, Facultad de Ciencias Agropecuarias, Universidad Nacional de Entre Ríos. 2008.

[8] Rivera C, Zapata A, Pinilla G, Donato J, Chaparro B, Jiménez P. Comparación de la estimación de la clorofila-a mediante los métodos espectrofotométrico y fluorométrico. *Acta Biológica Colombiana*, 2005; 10(2): 95-103.

[9] Rodríguez M, Alcántar G, Aguilar A, Etchevers J, Santizó J. Estimación de la concentración de nitrógeno y clorofila en tomate mediante un medidor portátil de clorofila. *Terra*. 1998; 16(2): 135-141.

[10] Instituto Nacional de Ecología y Cambio Climático (INECC). Los Ecosistemas de México. Instituto Nacional de Ecología y Cambio Climático, SEMARNAT. 2009.

[11] Schlegel B. Estimaciones de biomasa y carbono en bosques del tipo forestal siempreverde. *Simposio Internacional Medición y Monitoreo de la Captura de Carbono en Ecosistemas Forestales*. 18-20 October, 2001: Valdivia, Chile.

[12] Lichtenthaler K, Welburn AR. Determination of Total Carotenoids and Chlorophylls A and B of Leaf Extracts in Different Solvents. *Biochemical Society Transactions*. 1983; 11(5): 591-592. http://dx.doi.org/10.1042/bst0110591.

[13] Golfari CBY, Pinus Y. Obtenidos a partir del follaje de Pinus Caribea. *Morelet Var*, 2004; 23(2).

[14] Reyes M, Villegas Á, Colinas M, Calderón G. Peso específico, contenido de proteína y de clorofila en hojas de naranjo y tangerino. *Agrociencia*, 2000; 34(1): 49-55.

[15] McDermit DK, Norman JM, Davis JT, Ball TM, Roerner SR, Norman JM, Welles JM. CO_2 response curves can be measured with a field-portable closed-loop photosynthesis system. *Annals of Forest Science*, 1989; 46(Supplement): 416–420.



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