Institut für Nutzpflanzenwissenschaften und Ressourcenschutz
(INRES) Pflanzen- und Gartenbauwissenschaften
Universität Bonn, Germany

Influence of compost and humic substances on soil and fruit quality in Table Grape under intensive management in Chile

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DEDICATION

To Simon and Nico,
to sow more tomatoes and see more frogs this spring,
and especially to bring us more love between us, for others
and for the planet….

mami

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To God and my lucky stars to guide my steps.
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ERKLARUNG

Ich versichere, dass ich diese arbeit selbstandig verfasst habe, keine anderen Quellen und Hilfsmittel als die angegebenen benutz und die Stellen der Arbeit, die anderen Werken dem Wortlauf oder dem Sinn nach entnommen sind, kenntlich gemacht habe.
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Bonn, den 29.02.12

SUMMARY

The objective of this study was to evaluate the effects of the application of organic amendments (compost and humic extracts) on some soil quality indicators, agronomic variables, and the exportable yield in table grape (Vitis vinifera, var.Thompson seedless) grown in an inceptisol soil in the Limari valley in Chile Experimental research was performed in three stages: 1) production of compost from grape pomace and the extraction of humic substances from it, 2) evaluation of the compost and humic extract as organic amendments in pots, and 3) evaluation of humic extract under field conditions. Compost was prepared using grape pomace byproduct from the production of pisco and goat manure in different proportions (9 treatments). The co-composting process was monitored during a 220-day period. The optimal treatment was defined according to Chilean National Standard and Compost Council USA standards for compost, including: high humification ratio (humic acids/fulvic acids: HA/FA), low concentration of heavy metals and microbial pathogens, high germination percentage (%G), and the incorporation of a considerable proportion of grape pomace. The compost produced under optimal treatments was used to obtain humic extract (liquid humus) by alkaline extraction (extraction ratio compost/ extractant: 1:10 p/v). In the second phase of the research compost from grape pomace, liquid humus, a commercial microbial inoculant, and chemical NPK fertilizers were assessed under experimental conditions. Four C-rates were evaluated for each organic amendment: Compost—0, 500, 1000 and 2000 kg C/ha, and liquid humus—0,100, 200 and 400 kg C/ha; and both organic materials at their maximum C-rates were also evaluated in the absence of chemical fertilization. Medium chemical fertilization levels were used. The field phase of the experiment was conducted in a 1-year old table grape orchard under drip irrigation. Using a factorial experimental design, 16 treatments were evaluated during two seasons using liquid humus at four C-rates (0, 100, 200 and 400 kg C/ha) and chemical fertilizer with nitrification inhibitor at four N-rates (0, 30, 60 and 120 kg/ha). Chemical, biochemical, and microbiological soil properties, as well as fruit quality and exportable yield were determined each season and plant tissue was analyzed. A methodology to select a minimum data set size for establishing compost, soil, and fruit quality indices was developed using regression and frequency analysis. In each case treatments were considered, as populations and changes in different properties were evaluated over time. Three ecosystems exhibiting different soil types were used as a base line: 1) a xerophytic forest on a mountain slope (Mountain baseline, BLM), 2) a riparian vegetation site on the Rio Claro (River baseline, BLR), and 3) a site with uncultivated soil in the same grape field (AES).

The results indicated strong root development in plants treated with compost and inoculant application ($p<0.029$), obtaining more root dry matter than the control treatment; probably due to the production of indole acetic acid (IAA) and continuous mineralization of organic matter which increased nutrient availability. All compost treatments exhibited significant increases in the enzymatic activities of β-glucosidase ($p<0,0001$), acid phosphatase ($p<0,001$), and alkaline phosphatase ($p<0,0001$), that were significantly higher than the liquid humus treatments (56,6>13.8 UBG, 228,1>103,0 acid UP and 327,9>100,6 alkaline UP, respectively). This can be explained by the fact that compost increased total C, N and P concentrations, which stimulated enzymatic activity. In terms of organic matter content and enzymatic activity, significant differences ($p<0,05$) were found among the three baselines considered: BLR>BLM>AES. The enzymatic activity of alkaline phosphatase and β glusocidase, and the content of humic substances (HS: humic + fulvic acids) were selected from the minimum set of variables to explain changes in the soil where table grape was grown under field conditions. The application of liquid humus resulted in significant ($p<0,01$) increases of: exportable harvest mass (from 13 T ha-1 to 16 T ha-1), water-soluble carbon, and humic substances. None of the traditional fruit quality parameters exhibited changes, the still content of total chlorophyll and polyphenoloxidase were proposed as potential indicators of fruit quality under the conditions found in this experiment.

Key words: organic amendment, liquid humus, soil and fruit quality indicators, table grape

ZUSAMMENFASSUNG

Ziel dieser Untersuchung war es, die Auswirkungen von organischen Zusätzen (Kompost und Humusextrakte) auf einige Indikatoren der Bodenqualität, der agronomischen Variablen und dem exportierbaren Ertrag von Tafelweintrauben (*Vitis vinifera, var.*Thompson seedless) zu untersuchen, die in einem Inceptisol-Boden in Limari Tal in Chile angebaut wurden. Eine wissenschaftliche Untersuchung in drei Phasen wurde durchgeführt 1.) Herstellung von Kompost aus Traubentrester und den daraus extrahierten Huminstoffen, 2.) die Bewertung von Kompost und Humusextrakten als organische Zusätze in einem Topfexperiment und 3.) die Auswirkungen von Humusextrakten im Freilandversuch. Der Kompost wurde aus Traubentrester der Pisco Industrie und aus Ziegendung in unterschiedlichen Verhältnissen hergestellt (9 Behandlungen). Der Prozess der Co-Kompostierung wurde über einen Zeitraum von 220 Tagen überwacht. Die beste Behandlung wurde nach Vorgaben der nationalen chilenischen Standards und den Richtlinien des Compost Counsil USA festgelegt. Diese beinhalten: eine hohe Humifizierungsrate (Huminsäuren/Fulvinsäuren: HA/FA), eine niedrige Konzentration an Schwermetallen und mikrobiellen Krankheitserregern, ein hoher Prozentsatz an Keimfähigkeit (%G) sowie die Verwendung eines beträchtlichen Anteils Traubentrester. Der aus den besten Behandlungen entstandene Kompost, wurde zur Gewinnung von Huminextrakt (flüssigen Humus) durch alkalische Extraktion (Extraktionsquotient Kompost/Extraktionsmittel: 1:10p/v) verwendet. In der zweiten Phase wurde Kompost aus Traubentrester, flüssigem Humus, einem kommerziellen mikrobiellen Inokulanten und chemischem NPK Dünger für das Topfexperiment ausgewählt. Für jeden organischen Zusatz wurden jeweils 4 C-Raten getestet. Diese waren beim Kompost: 0, 500, 1000 y 2000 kg C ha-1 und beim flüssigen Humus: 0,100, 200 und 400 kg C ha-1; beide organischen Substanzen mit ihrer jeweils höchsten C-Rate wurden ebenfalls ohne den Zusatz eines chemischen Düngemittels untersucht. Gedüngt wurde im mittleren Bereich. Ein Freilandversuch wurde auf einer einjährigen Tafeltraubenplantage durchgeführt, wobei die Wasserversorgung mittels Tropfbewässerung gewährleistet wurde. Innerhalb eines faktoriellen Experiments wurden 16 Behandlungen über einen Zeitraum von 2 Anbaujahren durchgeführt. Flüssiger Humus wurde dabei in 4 C-Raten (0, 100, 200 und 400 kg C ha-1) und der chemische Dünger mit Nitrifikationshemmer in 4 N-Raten (0, 30, 60 und 120 kg ha-1) verwendet. In jedem Anbaujahr wurden neben der Analyse des Pflanzengewebes, die chemischen, biochemischen und mikrobiologischen Bodeneigenschaften sowie Fruchtqualitätseigenschaften und der exportierbare Ertrag bestimmt. Eine Methodik zur Auswahl eines Mindestdatensatzes wurde angewendet, um Indizes für die Qualität von Kompost, Boden und der Frucht mittels Regressions- und Frequenzanalyse zu erstellen. Dabei wurden die jeweiligen Behandlungen berücksichtigt und demnach die Populationen und Veränderungen der verschiedenen Eigenschaften über die Zeit bewertet. Drei Ökosysteme mit unterschiedlichen Böden dienten als Ausgangsbasis: 1.) Xerophtenwald im Gebirge (Baseline Gebirge/BLM), 2.) Ufer nahe Vegetation am Rio Claro Fluss (Baseline Fluss, BLR), und 3.) unkultivierter Boden (AES).

Die Ergebnisse zeigten eine starke Wurzelentwicklung bei den Pflanzen, die mit Kompost und Inokulanten ($p<0,029$) behandelt worden sind. Es konnte eine höhere Wurzeltrockenmasse als in der Kontrollbehandlung festgestellt werden. Dies ist womöglich auf die Produktion von Indolessigsäure (IAA), der kontinuierlichen Mineralisierung von organischem Material und der dadurch verbesserten Nährstoffverfügbarkeit für die Pflanze zurückzuführen. Das konnte durch die Zunahme der enzymatischen Aktivitäten von β-Glucosidase ($p<0,0001$), saurer Phosphatase ($p<0,001$), und alkalischer Phosphatase ($p<0,0001$) in allen Kompost-Behandlungen belegt werden. Sie lagen deutlich höher als bei den Behandlungen mit flüssigem Humus (56,6>13,8 UBG, 228,1>103,0 saures UP bzw. 327,9>100,6 alkalisches UP). Dies kann dadurch erklärt werden, dass durch den Kompost die Gehalte des Gesamt-C, -N und -P gestiegen sind, wodurch die enzymatische Aktivität angeregt wurde. Bezüglich des Gehaltes an organischem Material und der enzymatischen Aktivität konnten signifikante Unterschiede zwischen den drei in Betracht gezogenen Ökosystemen festgestellt werden: BLR>BLM>AES.

Unter der Berücksichtigung einer Mindestanzahl von Variablen wurden die enzymatischen Aktivität der Alkalischen Phosphatase und der β-Glucosidase sowie der Huminstoffgehalt (HS: Huminsäure und Fulvinsäure) bestimmt, um anhand dieser Veränderungen des Bodens bestimmen zu können auf dem die Tafelweintrauben unter Freilandbedingungen angebaut worden sind. Die Anwendung von flüssigem Humus führte zu einer signifikanten Zunahme des gesamten exportierbaren Ertrags (von 13 T ha^{-1} auf 16 T ha^{-1}), sowie zu einer Zunahme von organischem Kohlenstoff und Huminstoffen im Boden. Keine der herkömmlichen Parameter zur Bestimmung der Fruchtqualität wies Veränderungen auf, dennoch wurden unter diesen Versuchsbedingungen der Total Chlorophyll - und Polyphenoloxidasegehalt als potentielle Indikatoren zur Bestimmung der Fruchtqualität vorgeschlagen.

Schlüsselbegriffe: organischer Zusatz, flüssiger Humus, Indikatoren für Bodenqualität und Fruchtqualität, Tafeltraube

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ABBREVIATIONS

C	Total C
N	Total N
AcP	Acid phosphatase
Acty	Actinomycetes
AES	Base line Agro ecosystem uncultivated soil
AGS	grape agroecosystem
AlkP	Alkaline phosphatase
Amyl	Amylolytic microorganisms
ANOVA	Analysis of variance
Ant	Anthocyanin
ATP	Adenosine triphosphate
Avail N	Available Nitrogen
B	Boron
Bact	Bacteria
β Glu	β-glucosidases
BLM	Mountain baseline
BLR	Riparian vegetation –RiverBase line
Ca	Calcium
CCQC	Compost Council Quality of California
CEC	Cation exchange capacity
Cellul	Cellulolytic bacteria
CQI	Compost quality indices
Cu	Copper
CV	Coefficient of variation
Chl a	Chlorophyll a
Chl b	Chlorophyll b
DDGE	Denaturation Gradient Gel Electrophoresis
DM	Dry matter
DW	Dry weight
DOC	Dissolved organic carbon
Dumas	Combustion method
EC	Electrical conductivity
EGM	Exhausted grape marc
ER	Erosion factor
F&Y	Fungi and yeast
FA	Fulvic acids
FO	Field operation factor
FQI	Fruit quality index
GM	Goat manure
GP	Grape pomace
GS	Grape stalks
HA	Humic acids
HE	Humic extract
HM	Horse manure
HS	Humic Substances
ISCSA	Increment of shoot cross sectional area
K	Potassium
LSD	Least significant difference
MDS	Minimum data set
MetN	Metabolized nitrogen
N	Nitrogen
MPN	Must Probable Number
$N\text{-}NH_4$	Ammonium nitrogen
$N\text{-}NO_3$	Nitrate Nitrogen
NRed	Nitrate reductase
NTCh	National Technical Standard
OlsP	Olsen Phosphorus
OM	Organic matter
OS	Oat straw
P	Phosphorus
P sol	Phosphate Solubilizing Bacteria
PCA	Principal Component Analysis
PDA	Potato dextrose Agar
PR	Pruning residues
PPO	Polyphenoloxidase
Proteo	Proteolytic bacteria
qCO_2	Respiration Quotient
RD	Root density
SCI	Soil Conditioning Index
SCSA	Shoot Cross Sectional Area
SOC	Soil organic carbon
SOM	soil organic matter
SPAD	Chlorophyll by Spad
SQ	Soil Quality
SQI	Soil quality index
TA	Total Acidity

TChl	Total Chlorophyll
TD	Trunk diameter
TN	Total Nitrogen
U	Ureases
UβGLU	β GLU Units: µg p-nitro phenol*g*h^{-1}
UP	Phosphatase Units: µg p-nitro phenol*g*h-1
UPPO	Polyphenoloxidase Units
UU	Urease Units: µg NH_4*g^{-1}*h^{-1}
UV-VIS	Ultra violet-visible
WHC	Water holding capacity
WSC	Water soluble carbon
YFR	Yeast and fermentation residues
Zn	Zinc

SYNOPSIS OF THE STUDY

Chapter 1: Introduction

This chapter provides a brief background leading to the development of this research. Emphasis was made on the use of solid wastes from pisco industry in Chile as a source of raw material to obtain high-quality compost and organic amendments as liquid humic acids. A review on the selection of quality indices for the evaluation of the effects of the application of organic materials in soil and quality of table grapes is presented.

Chapter 2
Objectives and hypothesis

This chapter considers the investigation questions as hypothesis and the objectives proposed to demonstrate them.

Chapter 3
Literature Review

It considers generalities of the table grape crop, its nutritional requirements, climatic conditions, and agronomical characteristics. This chapter includes a review about organic amendments, uses in table grape orchards, composting process, compost quality indicators, and some previous experiences about the use of compost as amendment in table grape.

Chapter 4
Methodology

This chapter includes the optimization of composting process from grape marc, the humic substances extraction (humic and fulvic acid, HFa). It also includes the methods for the evaluation of compost and humic extract in table grape, under controlled conditions and the evaluation of humic extract as C source in table grape under field conditions for two seasons. Finally, it describes the method to select chemical, physical, microbiological and biochemical properties as a minum data set to be part of a quality index for compost, soil, and fruit in table grape.

Chapter 5
Results and Discussion

This chapter was organized in four parts. The first includes the process, extraction, and quality parameters of compost and liquid humic acid; the second includes the results of the pot experiment. The third part describes the results of the 2-year field experiment; the fourth and last part, describes the results of the methodology proposed to select a minimum data set to explain soil and fruit quality, based on regression and frequency analysis.

Chapter VI
General Conclusions

This chapter includes the general conclusions of the investigation as well as the suggestions for future research related to the definition of compost and soil quality indicators in table grape.

References

CHAPTER I

INTRODUCTION

Table grape, is one of the main exporting crops in Chile, ranking third worldwide, being a major producer along with Mexico and South Africa. The crop is grown from the third to the seventh regions of Chile, covering a long territory with several grape varieties produced (ASOEX, 2010). The grape varieties grown in Chile are mostly Thompson Seedless, Red Globe and Crimson Seedless. For Thompson seedless, the optimum yield range is between 19 and 32 ton ha^{-1} with an average of 27 ton ha^{-1}, while the normal yield range is between 15 - 28 ton ha^{-1} with an average of 21 ton ha^{-1} (ASOEX, 2010).

The variety Thompson Seedless has approximately 15.971 hectares planted out of 62.411 ha of table grapes (Ministerio de Agricultura de Chile, 2009,Agricultural Census, 2007). Coquimbo region (IV region in Geographical distribution of Chile), is a major grape producer, including wine, table grape, and Muscatel grape used for Pisco, the national spirit.

Pisco is a distilled spirit and in its process, a large amount of solid residues are produced. These residues include grape marc, exhausted marc, and grape raquises, all of which are materials with low pH and high content of phenolic compounds, which may inhibit microbial degradation activity in composting process. Compost is applied to grape crops with the idea of improving yield and quality, but normally grape marc is usually disposed on the roads, particularly in dry seasons for dust control. Grape marc has very good components in terms of nutrients, organic matter content (OM) and has shown very good effects as organic amendment (as compost or in co-composting process). In Chile, some vineyards have implemented the composting process from grape marc and bunch raquises, producing about 800 tons of compost per season, with a percentage of organic matter that ranges between 69 and 80%. The compost is used for land reclamation, at rates of approximately 10 ton ha^{-1} (Undurraga, 2003). Compost, humic acid, and biofertilizers (yeast) have been used to reduce nitrogen fertilizer in Thompson seedless grapes. In this respect, it was found that the humic acid lowered leaf nitrogen levels, when in combination with biofertilizer, but the presence of humic acids in combination with residues increased the level of nitrogen in leaves (Eman ***et al.***, 2008).

Paradelo ***et al.*** (2009) demonstrated the effect of humic acid application on root and humification process in soil; they studied the following rates: of 4%, 8% and 16% compost, in volume, respectively. Compost at the highest rates, increased biological activity and nutrient availability, and in turn increased microbial biomass and enzyme activity. On the other hand, Fernandez ***et al.*** (2008), demonstrated carbon degradation during composting with organic wastes from distillery industry, yielding as a result the so called “exhausted grape marc” (EGM). This exhausted grape marc is very rich in nitrogen whose compactness can be adjusted mechanically by mixing during the co-EGM composting with other organic material with higher carbon content. The final product is a compost which include all solid wastes from distillery, bunch stems (grape stalks-GS), with proper pH, porosity, C/N which allows for optimum composting.

Compost could be used to obtain other C fractions as humic substances, through an alkaline extraction process. This liquid amendment improves different fractions of C, including humic and fulvic acids, water soluble fractions, and C reserves as humic substances for soil microorganisms.

Changes in soil could be measured and quantified using different visual, chemical, biochemical and biological soil properties as indicators of soil quality. Soil quality indicators have been

defined from the ecological, economic and social development standpoints; they usually take into account soil properties or associated crops that can be used to evaluate the dynamic changes in agro ecosystems. These indicators are not well defined, and must be determined for every type of soil and local conditions (Bouma, 2002). Changes in soil quality may be measured through quality indices including different indicators, to make more objective the soil quality determination, compared with a given base line.

Soil quality indices have been developed to try to explain soil quality as a principal target for ensuring the sustainability of the environment and the biosphere. Literature shows a great number of soil quality indices for both, agro-ecosystems and natural or contaminated soils, and the most straightforward index used in the literature is the metabolic quotient (qCO_2) (respiration to microbial biomass ratio). This index is widely used to evaluate ecosystem development, disturbance or system maturity; however this integrates only two parameters and provides insufficient information about soil quality or degradation; most indices are only defined according to chemical, biological and biochemical soil properties but not according to crop yield or fruit quality. For this, lately there has been a wide development of multiparametric indices. This tool clearly establish differences among management systems, integrating different parameters (soil, harvest and agronomical characteristics), looking for parameters with high sensitivity, and which are easy to define such as pH, organic matter, microbial biomass C, respiration or enzymatic activity (Ortega and Santibañez, 2007).

The major part of multiparametric indices has been established based on either, expert opinion (subjective), or using mathematical–statistics methods (objective). Biochemical indicators are very sensitive to changes in soil management and have not been widely used yet to establish soil or harvest quality indices. Some of these methods can provide information about what the roles of specific microorganisms and their enzymes are in key processes related to soil functionality and help producers define the fruit quality earlier than with traditional physico-chemical analysis.

With this background, I intended to assess, in a table grape crop Thompson seedless variety, the effect of different organic amendments, on soil properties, agronomic productivity, and fruit quality and to determine a minimum set of data that can give information regarding the quality of soil and fruit. The objectives and hypothesis are presented in the next chapter.

CHAPTER II

OBJECTIVES AND HYPOTHESIS

Four research hypotheses were proposed for the present study:

- It is possible to define compost maturity, in terms of humic substances content, using functional groups of hydrolytic microorganisms, hydrolytic enzymes, and C/N characteristics.
- Applying liquid humus can be equivalent or better than using compost in terms of its effects on soil and fruit properties.
- Changes in soil quality indicators take place at different speeds and directions depending on organic matter application levels and compared with base line soils.
- It is possible to define a minimum data set to create composed quality indices based on a linear combination of several compost, soil, and fruit properties.

To prove these hypotheses, the following objectives were proposed:

General

To determine the effects of compost and humic substances on soil and fruit quality in Table Grape under intensive management.

Specific

1. Organic Matter:
   1.1 To evaluate the enzymatic activity of β – glucosidase, acid and alkaline phosphatases, and urease, as potential biochemical indicators of organic matter quality.
   1.2 To define the best extraction procedure of humic and fulvic acids from compost.
   1.3 To select a set of variables that could be part of a maturity index for compost.

2. Soil:
   2.1 To evaluate the effect of OM type and rate on soil quality in table grape.
   2.2 To define, quantify, rank, and compare different soil properties that could be used as soil quality indicators, compared with a base line.

3. Agro ecosystem
   3.1 To define a set of variables that could be part of quality indices for soil and fruit.

CHAPTER III

LITERATURE REVIEW

3.1. Table grape

The vine is a species of the Ampelidaceae family. *Vitis vinifera* L. has its origin at the southern regions of the Caspian Sea in Europe (Armenia), as wild vines in forests of the Caucasus and Sardinia (NRC, 2011)

Table grape, is one of the main export crops from Chile, ranking third worldwide and being a major player along with Mexico and South Africa. It is grown from the third to the seventh region Chile, covering a vast territory and variety of grapes produced (Association of Exporters of Chile ASOEX, 2010). The most common varieties found in Chile are Thompson Seedless, Crimson Seedless, Red Globe, Flame Seedless, Superior Seedless, Autumn Royal, Ribier and Princess (ASOEX, 2010); the first with the highest rate of export with 27% of the total, followed by Red Globe (22%) and Crimson Seedless (18%). Thompson seedless has between 15.971 and 17.898 hectares planted out of a total of 62.411 ha of table grapes (Agriculture Census, 2007); approximately 85% of them are in production (Ministerio de Agricultura, Chile, 2009).

3.1.1. Table grape in Chile

The first vines were introduced into the Captaincy General of Chile between 1541 and 1554 (Chilevinos, 2011) and the first plantings and harvest were made in the city of La Serena (capital of Coquimbo Region) in 1548- 1551.

The table grape production in Chile is about 1.250.000 tons, over the 893,758 tons of the United States, which is one of the leading competitors. Thompson Seedless, Red Globe, and Crimson Seedless are the most exported varieties with 27, 22, and 18%, respectively (ASOEX, 2010). The average productivity of table grapes is 14,4 ton ha^{-1} (study area: Atacama, Coquimbo, Santiago and O'Higgins). In 2010, table grapes together with apple, accounted for 67% of the country's fruit exports, with table grapes representing 36,1% of the total 847.680 tons exported during the period 2008-2009 (ASOEX Chile 2010).

The grape varieties grown in Chile are mostly Thompson Seedless, Red Globe and Crimson Seedless (table 1). With regards to yield, the optimum range is between 19,3 and 31,7 ton ha^{-1} with an average of 27 ton ha^{-1}, while the normal yield is in the range of 15 to 28 ton ha^{-1} with a average of 20,8 ton ha^{-1} (Costabal, 2010).

Table 3.1. Grape Varieties Production in Chile, Season 2010

Variety	Production (Nr. of Ha)
Thompson seedless	15971
Crimpson seedless	8070
Red Globe	10704
Flame seedless	9108
Superior seedless	3839
Autumn royal	1127
Ribier	469
Princess	495
Subtotal	49783
Others	4143
Total	53926

(Costabal, 2010)

3.1.2. Climatic conditions and phenologycal stages

Environmental conditions are very important for plant growth. Grapevine root system shows different patterns depending on age, cultivar, climate and environmental stresses. Ruiz (2000) defined two growth peaks for roots of cv. Thompson Seedless, the first occurring between 3 to 10 weeks after budding and decreasing in intensity with berry growth (McArtney and Ferree, 1999). During the year, the roots grow, presenting minimal values in July and reaching similar growth levels during flowering and harvest (Callejas *et al.*, 2009). In spring, the root growth is superficial with abundant fine rootlets; this corresponds to one of the growth peaks and its intensity depends on the level of reserves of the roots (Terence *et al.*, 2002). The second peak, which is less intense than the first, occurs after harvesting.

The changes in root morphology including differences in root length, dry matter and rooting are associated with changes in soil temperature (McMichael and Burke, 1998).

3.1.3. Vitis vinifera Thompson seedless, agronomical and cultural labors

Thompson seedless is a variety produced in several environments and countries, including Chile, Australia, Brazil, Egypt, India, Israel, Mexico and South Africa. In Chile Table grape production, is along the north-south axis, from arid to mediterranean climate; it is vulnerable different pests and pathogens (Table 3.2) like *Phytophthora* sp., and *Botrytis cinerea* that cause major problems in the root and fruit respectively. *Botrytis cinerea* is the most aggressive pathogen during cold storage, where research has focused describing biological indicators that estimate the prevalence of mold and splits during the storage (Zoffoli *et al.,* 2009).

Table 3.2. Main diseases affecting table grapes in Chile.

Pathogen/Pest	Damage	Source
Phytophthora cinnamomi, P. cryptogea	Root	Chilevinos, 2011
Phomopsis viticola (Sacc.)	*Phomopsis* cane and leaf blight*	Australian Government report 2005
Plasmopora viticola	Downy mildew	NRC Grapes, India
Uncinula necator	Powdery Mildew	NRC Grapes,India
Xanthomonas campestris pv. *viticola*	Bacterial canker	
Thysanoptera (thrips)		
Frankliniella australis Morgan [Thysanoptera: Thripidae]	Chilean flower thrips	
Frankliniella occidentalis (Pergande) [Thysanoptera: Thripidae]	Western flower thrips	Australian Government report 2005
Brevipalpus chilensis Baker [Acari: Tenuipalpidae]	Chilean false red mite	

A particular feature of seedless varieties is that the berry does not grow naturally, which has resulted in the need of gibberellic acid application to increase the size of the berries (Williams and Ayars, 2004). Another management practice studied is the irrigation that this species should receive; several irrigation times were tested (6,12 and 18 hours), with the most effective being the last, which had higher pruning weight and size of berries at harvest (Selles *et al.*, 2003).

The cycle of grape describes five distinct phases: the first stage from bud break to the beginning of flowering, where the plant requires high nitrogen levels and where 90% of the required nutrients are supplied by the previously accumulated reserves; in this phase, the excess N and K deficiency should be avoided to control an excess of putrescine. The second phase covers the period from the beginning of flowering to fruit veraison, which defines the level of production; during this stage, the levels of potassium (K), boron (B) and zinc (Zn), should be reviewed; a foliar analysis should be performed and the root growth evaluated. The third phase includes from veraison to berry ripening (harvest); during this phase the berry gains caliber and should have a rapid maturation; calcium requirements are high, therefore it should be directly sprayed to the bunch. The fourth includes the second root growth peak, which requires phosphorus (P), and potassium (K); the potential deficiencies of zinc (Zn) and boron (B) should be assessed. Finally, the fifth phase corresponds to recess, where pruning is performed, and plants accumulate the hours of cold necessary to stimulate sprouting (Palma, 2006).

3.1.4. Soil characteristics

Chemical parameters such as pH and electrical conductivity represent plant growth balance. The pH range for optimal growth is between 2,5 to 8,5, but at pH> 6,5 the nutrients like Fe, Zn, Mn, Cu, P, Bo, and are less available and a pH <5,5, the molybdenum is not available. These changes in nutrient availability show the importance of pH in maintaining a balance of essential nutrients and thus optimizing sound quality phenology development and productivity; the conductivity of soil extract must be less than 1,5 dS m^{-1} (Palma, 2006).

3.1.5. Nutrient requeriments

Table grape var. Thompson seedless, exhibits different nutrient requirements according to phenologic cycle. Elements such as N, P, K, Mg and Ca are considered very important to obtain harvests between 7-25 Ton ha^{-1} (Table 3.3) Nutritional requirements affect performance and

quality of fruit. Calcium (Ca) is essential for cell wall and thus for protection against pathogens such as *Botrytis*, improving rooting and quality of berry; Magnesium (Mg) acts on the chlorophyll and phosphorus (P) aids in the division and transfer of energy at the cellular level. In terms of mineral nitrogen (NH_4, NO_3) it is estimated that more than 80 ppm in the soil is sufficient for a high yield; the C / N is used for the characterization of nitrogen and its relationship with organic matter, where an adequate ratio is between 12 and 15 on the other hand, from 11 to 25 ppm of phosphorus is adequate (Palma, 2006).

Table 3.3. Nutrient requeriments for Thompson seedless in Chile

	NUTRIENTS (kg ha^{-1})									
Harvest Ton/ha	N[1]	P_2O_5[1]	K_2O[1]	MgO[1]	CaO[1]	Fe[2]	Mn[2]	Zn[2]	Cu[2]	B[2]
7-25*	22-90	5-35	41-48	6-25	28-204	300-1000	50-700	100-500	60-900	37-200
18-22**	80-120	30-60	110-60	30-60	60-120					

*Christensen and Bianchi, 1994

**Extraction curves in Thompson seedless.Palma 2006, INIA Intihuasi, Vicuña, Chile

[1]: Kg/ha; [2]:g/ha

According to Chilean National standard (INN, 1991) and international market requirements (USDA, 1971, 1999), Table grape quality includes different parameters in the field, such as age, maturity, firmness, no splitted or crushed, yellowish-green color, no sunburn, stems free from mold and decay , and size (diameter of at least 75% of the fruit shall be between 10/16 of an inch, corresponding to 16mm. These physical properties correlate with some chemical properties like total chlorophyll, Chlorophyll a and b, ° Brix, total acidity, anthocyanin, total polyphenols concentration and polyphenoloxidase activity.

3.2. Organic amendments

The use of organic matter (OM) in agriculture is of great importance for plant nutrition, soil aggregation, structural stability, root penetration and water retention capacity. In relation to chemical properties OM application increases nutrient storage and buffer capacity and enhances the action and absorption of nutrients by rootlets. Regarding to biological properties, OM application promotes metabolic activity in the rhizosphere, encouraging and maintaining an appropriate level of microbial growth. In sum, the use of OM as an agricultural practice strongly influences different parameters in soil having effects even on pesticide applications as these are adsorbed by organic matter or contribute to degrade fumigants (Magdof and Weil, 2004).

Plant quality in vine is determined by adequate level of nutrients, irrigation water, root stock variety, fertilizer sources, environmental conditions, root development and soil management conditions that, at the same time, make part of agricultural sustainability. The concept of agricultural sustainability has been given great significance. It is defined as an agroecosystem with the ability to: stay productive even under stress, maintain the quality of the environment, provide food and fiber necessary for human beings, be economically viable and improve the quality of life for farmers and society (FAO, 1994).

Soil organic matter (SOM) is a matrix of heterogeneous compounds with carbon base; formed by the accumulation of materials of animal, plant and microorganisms in a constant state of decomposition and synthesized substances of all living and dead organisms (Manalay *et al.*, 2007) .

Organic matter (OM) in soil is very homogeneus and represents 95 to 99% of the dry weight of living things; however, is the non living component of organic matter, and contains three C pools: root exhudates and rapid decomposed components of litter, called “active” pool; the

stabilized and persistent organic matter for thousands of years, called “passive pool” and the low stablilized that persists years to centuries, called “slow or intermediate C pool” (Kleber and Johnson, 2010). The content of organic matter in soils varies greatly. There are references in ranks going from 2 g kg^{-1} in deserts (Magdoff and Weil, 2004) to 700 g k^{-1} in some Histosols (Pereira *et al.*, 2006). Organic matter is the most important fraction in soil in order to improve productivity and soil fertility by supplying organic carbon to microbial activity (Ferreira and Alarcon, 2001).

In table grape, the practice in Chile has been the application of organic amendments in order to “give back” to the soil and it has become the basis of organic agriculture. Mustin (1987), indicated that the presence of OM is generally poor, forming only around 5% of total nitrogen, together with some elements which are essential for plants such as phosphorus, magnesium, calcium, sulfur and micronutrients.

Applications of organic matter in the grape crop have been a very popular cultural practice in Spain. This practice responds to a recycling policy where organic material from urban and agricultural waste (olive-oil processing residues, winery and distillery wastes) are considered as amendments for soil, preserving organic matter content, and improving nutrient content (Barral *et al*., 2009). Thus, the use of this material in soil results in an environmentally friendly method to reduce the organic wastes in land fields.

Other organic amendments used for table grape production include animal manures (poultry, horse or goat), crop residues, wastes from food processing that can be used fresh or transformed. Among them is the application of vermicompost which increases the level of soil organic matter and thus the enzymatic and microbiological activity. Bertrán *et al.* (2004), determined that sludge and grape pomace can be used as organic amendments in vineyards with low SOM using a 1:2 ratio (sludge: grape pomace), humidity of 55%, temperature of 65ºC and 10% oxygen.

Organic amendments supply C, N, P and energy for microorganisms in soil (Tabatabai and Dick, 2002), and activate all soil functions associated with enzymatic hydrolysis, production of biologically active substances, and rooting, but application of unstable and/or immature organic amendments may induce several adverse effects on soil properties, plant growth and water quality. Fresh materials increase the mineralization rate of native soil organic C through improved microbial oxidation activity, and the inmobilization of available N by microorganisms substracting O_2 from root respiration and reducing nitrification process with formation of nitrites and sulphides (Senesi and Plaza, 2007).

For all these reasons soil quality can be measured in terms of organic matter which can affect chemical, physical and biological properties. Today there is a holistic approach to the study of soils aiming to understand the contribution of different properties and their relationship with the plant and its productivity.

3.2.1. Compost as organic amendment

The composting process is defined as a biological transformation of organic matter under aerobic thermophilic and mesophilic conditions by which native microorganisms produce a material which is stable, sanitized, safe and with an important concentration of humic substances (Marhuenda –Egea *et al.*, 2007). Compost amendments are an attractive way to incorporate organic matter in the soil as it has beneficial properties, including mobilization of mineral phosphates (Wickramatilake., 2010).

The production of compost is an alternative to burning agricultural and forestry waste, and therefore an option to reduce air pollution and loss of organic matter in soils by calcination. This agricultural practice reduces the volume of waste sent to landfills, as well as odors and vector attraction. In addition and particularly in Chile, the use of compost of grape pomace

has the potential to discourage the practice of collecting “Tierra de hojas” or “soil of leaves” which basically refers to the collection of soil and leaf litter around native trees in mountainous areas with high environmental impact reducing the cycling of organic matter in native forests. (CONAMA, 2008).

Overall, around the world the quality of compost is defined in terms of their chemical, physical and biological properties, with particular emphasis on the elements to guarantee the protection of the environment and health (of humans and animals).EU standards, as well as the U.S., Australia, Switzerland, Austria, leading the latter to be the most demanding in terms of quality, are defined on the basis of prevention, so that it can protect soil quality . Another aspect to be considered in almost all countries is the presence of human pathogens which in turn is related to the rules of the process of “temperature-time”), physical impurities, the presence of weeds and stability and phytotoxicity. (Hogg et al., 2002).

The Chilean National Standard, classifies compost and organic amendments as Class A and B according to their physical, chemical and microbiological characteristics (Table 3.4). In general, compost moisture must be between 30-45% (wet basis), without unpleasant odors (ammonia sulphide, mercaptans, reduced sulfur). It must have an earthy odor and a dark brown to black color (EPA, 1995). Other characteristics include organic matter (OM)> 20%, pH between 5 to 8,5, and with a maximum presence of viable seeds and weed propagules of maximum 2 g^{-1} of compost when placed in a growth chamber (INN, 2004).

Table 3.4. Heavy Metals Contents of Raw materials and Compost*

	Raw Material Mx. Concentration (mg kg^{-1}) dry base	Chilean Standard NTCh 2880/04		EU**	
		Type A	Type B	Type A	Type B
Arsenic (As)		15	20	-	-
Cadmium (Cd)	10	2	8	0,7	1,5
Copper (Cu)	1500	100	1000	100	600
Chromium (Cr)	1000	120	600	100	600
Mercury (Hg)	10	1	4	0,5	1,25
Nickel (Ni)	200	20	80	50	150
Lead (Pb)	800	100	300	50	120
Zinc (Zn)	3000	200	2000	200	1500

*Total contents **European Union Standard (2008)

Table 3.5. Microbiological parameters for compost - Chilean Standard

Type of microorganism	**Limit of Tolerance**		
	NCh 2880 & EPA		EU
	A	B	
Fecal coliforms (MPN $100ml^{-1}$)	<100	<1000	<1000
Salmonella spp.	Absent	Absent	Absent
Viability of Helminth / *Ascaris* sp.	<1 viable egg		1 viable egg

Compost maturity cannot be described with a single property but it is best assessed by measuring two or more parameters. Maturity is related to the stability of the material and also includes the potential impact of other chemical properties of the compost on plant development.

Immature compost may contain high amounts of free ammonia, certain organic acids or other water-soluble compounds which can limit seed germination and root development. Any use of compost requires it to be mature and free of any potentially phytotoxic components (Bernal *et al.*, 2009).

Laboratory tests must be easy, rapid and reliable for proper evaluation of composts. These tests include the carbon:nitrogen ratio (C:N); ammonium-N:nitrate-N ration; analysis of humic substances; microbial biomass, cation exchange capacity (CEC); water extract analysis, germination percentage and reheating tests. All of these can provide additional information on material characteristics but have limitations when applied to the interpretation of the diversity of compost products. For example, an assumed ideal C:N ratio for a mature compost may be 10 (Compost Council Quality of California-CCQC, 2001).

Compost producers and users should realize that the presently accepted methods to evaluate stability and maturity may not completely or accurately address the most important characteristics. All of the test procedures provide indirect interpretations for the potential impact on plant growth but can be used by farmers to take appropriate decisions.

Complementary tests are recommended by the Compost Council Quality of California, and required (one from each group A and B) by the Chilean National standard (INN,2004). These tests include in group A: carbon dioxide evolution or respiration, oxygen demand and self heating, and in Group B: ammonium: nitrate ratio, ammonia concentration, volatile organic acids concentration and plant tests . With these complementary tests three rating categories are suggested in the CCQC Compost Maturity Index, including very mature, mature and immature (Table 3.6).

Table 3.6. Maturity Index Proposed by CCQC

VERY MATURE	**MATURE**	**IMMATURE**
Well cured compost	Cured compost	Uncured compost
No continued decomposition	Odor production not likely	Odors likely
No odors		
No potential toxicity	Limited toxicity potential	High toxicity potential
Minimal impacts on soil N		Significant impact on soil N

Method	**Very Mature**	**Mature**	**Immature**
OUR Test O_2 / unit TS / hr	< 0,4	0,4 - 1,3	> 1.3
SOUR Test O_2 / unit BVS / hr	< 0,5	0,5 - 1,5	> 1,5
CO_2 Test C / unit VS / day	< 2	2 - 8	>8
SCL CO_2 C / unit VS / day	< 2	2 - 8	> 8
WERL CO_2 C / unit VS / day	< 5	5 - 14	< 14
Dewar Temp. rise (°C)	< 10	10 - 20	> 20
Solvita¨ Index value	7 - 8	5 - 6	< 5

SCL = Soil Control Laboratory
WERL = Woods End Research Laboratory

The alcohol distilleries for the production of spirits such as whisky, gin, brandy or pisco, generate large amounts of solid and liquid organic waste which needs to be treated in order to reduce its polluting organic load. The environmental problems associated are low pH or presence of toxic substances for plants, such as polyphenols (Bustamante *et al.*, 2008) that inhibit germination (Zucconi *et al.*, 1981) and immobilization of N in soil. Different methods

have been proposed to use solid waste, for example yeast production (Lo Curto and Tripodo, 2001), extraction and recovery of phenolic compounds (Louili *et al.*, 2004), pesticide activity (Corrales *et al.*, 2010) or mulching in autumn (CONAMA, 2008).

Waste of pisco industry derived from fermentation of Muscat grapes and additional phenolic compounds, has the same characteristics of Grape marc: low pH (3,8 to 4,4) (Bustamante *et al.*, 2008) and EC between 3 and 4, high concentrations of P, K, organic matter and micronutrients (Bustamante et al., 2007) which can potentially be used in agricultural soil.

However, the safe use of compost depends on characteristics such as stability and maturity. Some parameters to take into account for the quality of compost are stable temperature, dark brown-black ash, no odor, alkaline pH, C / N> =20. Some authors, also include low activity of hidrolytic enyzmes, low ATP content and reduction of 35% total sugars, as stability indicators of compost obtained from grape marc (Bustamante *et al.*, 2008).

3.2.2. Humic substances as organic amendments

With the intensification in agricultural production and the incorporation of irrigation systems in the plantations, the application of compost or manure is practically limited to planting or surface applications. Surface applications besides being costly are not very efficient, because many of the nutrients get lost by run-off effect and in addition, it is necessary to incorporate the compost or manure in perennial crops where active roots may be several feet below the surface. However, when this material is applied and incorporated into the soil, different beneficial effects are provided such as aireation, maintaining moisture and stimulation of rhizosphere-effect.

Commercial alternatives of extracted humic substances applied as a supplement of chemical fertilization and solid organic amendments, has been observed to reduce the loss of organic carbon in soil. Carboxylic acids, amino acids, extracts of manure and humic acids in liquid form, suggest interesting products that could increase the organic C content (Paustian *et al.*, 1992).

Humic substances (HS) are natural organic products. They are found in aquatic and terrestrial ecosystems, in sediments, lignites, brown coal, compost and other deposits (Grinhut *et al.*, 2007). In the environment, HS are a mixture of associated OM, including complex molecules and small and simpler organic structures that interact with particles and minerals (Sutton and Sposito, 2005). Humic substances are formed during humification process, a secondary synthesis reaction during the transformation of organic matter. Early characterization analysis of this compounds indicated that HS are based on three fractions according to their solubility under acid or alkaline conditions. humin, the insoluble fraction of humic substances, the humic acid (HA), the soluble fraction under alkaline conditions (but not pH< 2) and fulvic acids (FA) the fraction soluble under all pH conditions, but does not indicate the existence of three different types of organic molecules (Hayes *et al.*, 1989). The studies by Stevenson (1994) indicate that HS are comprised mainly of aromatic, aliphatic, phenolic, quinolic and N-derived components (Fig 3.1), bound through C-C, C-O-C and N-C bonds, that support the complex polymer model of HS structure (Cameron *et al.*, 1972) and extreme stability to microbial attack (Picolo, 2001).

However, in last decade, different analytical approaches using spectroscopic, pyrolisis, and isotopic analysis, show the humic substances in soil, as a complex of macromolecules with high activity and influenced by soil mineral (Sulton and Sposito,2005). The origin of HS are in discution; soil biochemistry studies indicate that these molecules are a complex of polymeric organic acids with a wide range of molecular weights, including aromatic, aliphatic, phenolic and quinolic functional groups with different properties depending on the origin and age of the material (Gu *et al.*, 1985, Chin *et al.*, 1998).

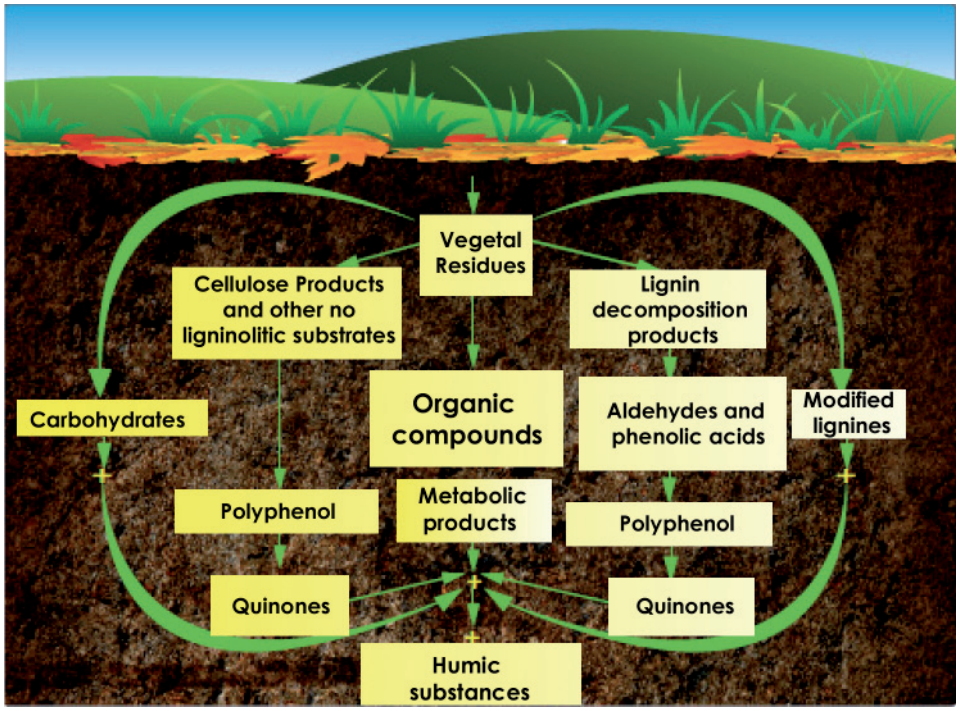


Figure 3.1. Humification process from plant residues. Polymeric Model of HS (Grinhut *et al*., 2007, Stevenson, 1994)

Polyphenols are considered, by many authors, as humic acid precursors, because of their reactive sites suffered further transformations as condensation (Burdon, 2001). However, as was mentioned before, because of heterogenicity, humic substances, should be consider a system in soil, created by associations of components present during humification process, including N derivatives (amino acids), C derives (lignin, pectins and carbohydrates that inteact by intermolecular forces. The HS formation process depends on geographical situation, temperature, plant exhudates influence, microorganisms, biochemical and physical factors (Grinhut *et al.*, 2007, Tiquia, 2005).

Humic substances have different effects on plants. Vaughan and Malcolm (1985) and Chen *et al.* (2004), showed evidence of stimulation on plant growth by humic substances and consequently increased yield by acting on mechanisms involved in: cell respiration, photosynthesis, protein synthesis, water, and nutrient uptake, enzyme activities. Results have been demonstrated to be C rate dependent and particularly effective at low concentration (Chen and Aviad, 1990). Optimal concentrations capable to affect and stimulate plant growth have been generally found in the range of 50-300 mg L^{-1}, but positive effects have been also seen with lower concentrations (Chen *et al.*, 2004).

3.3. Soil quality

3.3.1. Definition

The concept of soil quality should be associated with production and fertility. Doran and Parkin (1994) had defined soil quality as “the soil’s ability to operate within environmental limits, to sustain biological productivity while maintaining environmental quality and promoting the health of the flora and fauna”. Soil quality is determined through dynamic properties of soil such as organic matter and diversity of organisms or microorganisms. These properties change and their function is measured through promoting productivity of the system without losing their properties, reduce environmental pollutants and pathogens and promote the health of plants, animals and humans.

The concept of sustainability and resilience of soil was described by Blum and Santelises (1994), including six ecological and human functions; soil as biomass producer; soil as filtering

matrix and reactor of polluting compounds; soil as buffer and carrier of material to protect the environment, groundwater and food chain from contamination; soil as habitat for countless species or as a biological and genetic reserve; soil as a physical environment and; soil as natural resource and cultural heritage. These concepts suggested by Warketin (1996), served as the basis of the current concept of soil quality, proposed by the Soil Science Society of America, "The capacity of soil to function within ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant and animal health" (Karlen *et al.*, 1997). By 2008, the definition of soil quality focused mainly on its function and changes through time are measured with quality indices which are defined for crops taking into account specific conditions (Karlen *et al.*, 2008).

Once soil quality has been established for a specific situation it is possible to define policies and actions such as agricultural practices which allow to maintain or improve this quality. Therefore in order to give an accurate assessment of soil quality, parameters must meet three main aspects: first, choose appropriate indicators to complete a minimum data set, second transform indicators into scores, and finally, report the scores as indices. Statistical techniques such as principal component analysis, factor analysis or regression and coefficient of variation analysis have been useful to identify the critical parameters as indicators of quality (Masto *et al.*, 2008, Velasquez *et al.*, 2007; Janvier *et al.*, 2007; Arshad and Martin, 2002, Ortega and Santibáñez, 2007).

3.3.2. Soil quality indicators

Indicators of soil quality have been defined, from the ecological, economic and social development points of view; they usually take into account soil properties or associated crops that can be used to answer the dynamic changes in agro ecosystems. These indicators are not well defined, nor are there accepted or approved parameters to characterize or to define soil quality (Bouma, 2002). Changes in soil quality can be measured through indicators, which include physical, chemical, biological, and biochemical processes and characteristics so it is necessary to provide quality indices including different indicators, to make more objective the soil quality determination.

According to the USDA-NRCS (2006), indicators of soil quality are classified into four categories that include visual, physical, chemical, and biological indicators (Table 3.7). Visual indicators can be obtained through field visits, perception of farmers and local knowledge. These indicators are identified through observation or photographic interpretation, subsoil exposure, erosion, presence of weeds, color, type of coverage and by comparison with native systems. All these parameters give a clear idea whether soil quality, has been positively or negatively affected. Mairura *et al.* (2007) reported in Kenya, the integration between scientist and farmer´s evaluations show how the use of local knowledge as indicator is valid for consistent classification of soil quality.

Table 3.7. Minimum set of physical, chemical and biological properties for soil quality definition.

Indicators of soil condition	Relation and function of the soil condition
	Physical Parameters
Texture	Water holding capacity and chemical in solution transport
Soil and root deep	Productivity potential and erosion
Infiltration and density	Leaching potential, productivity and erosion
	Chemical parameters
Organic matter	Soil fertility, stability and erosion
pH	Biological and chemical activity
Electrical conductivity	Microbial and plant activity
N, P y K extractable	Potential of N mineralization and nutrient availability
	Biological Parameters
C and N Microbial biomass	Catalytic potential
Enzymatic activity	Biochemical reactions in soil and nutrient cycling.
Organisms (colembola, worms, ants)	Biodiversity and biological activity

Doran and Parkin, 1996; Gutierrez, 2009

In addition, these indicators should be sensitive enough to detect changes, measureable, easy to interpret and affordable to many users. In this sense, they constitute an effective tool to show important changes in soil properties (Benintende *et al.*, 2008; Janvier *et al.*, 2007).

Physical indicators are those that reflect the way the soil accepts, holds and transmits water to the plants, as well as how it responds to stress situations. Parameters include infiltration and bulk density, aggregate stability, water holding capacity and soil conductivity (Mon *et al.*, 2007).

Aggregation and aggregate stability are physical properties that respond to any kind of changes that happen to the soil. An aggregate is a group of soil particles that cohere more strongly to each other than to other adjoining particles. The aggregates in soil form clusters and these define the soil structure. Biotic and abiotic factors play important roles in the formation of soil aggregates; roots disrupt and promote granulation. Organic matter binds the soil particles and promote the growth of microorganisms which can contribute to form the water-stable aggregates, through the production of extracellular polysaccharides, glomalin, mycelium and cementant substances (Cabria *et al*, 2002 and Silvya *et al.*, 2005).

Chemical indicators include pH, salinity, organic matter, phosphorus concentration, cation exchange capacity, nutrient cycling, and the presence of contaminants such as heavy metals, organic compounds, radioactive substances, etc. These indicators determine the soil-plant-related organisms, nutrient and water availability for plants and other organisms and the mobility of contaminants. Chemical fertility and soil quality indicators, in conventional and organic crops, have an intimate relationship with soil biology. Garcia-Ruiz *et al*. (2009), described differences in the total carbon exchange capacity of Ca^{+2} and K^{+}, which are present in high levels in soils with organic management, but no significant differences in the exchange of sodium carbonate, total nitrogen, pH and CEC. Chemical indicators consist of a set of parameters which include

organic matter content (% OM), total and oxidized carbon, total nitrogen, $N\text{-}NO_3$ and $N\text{-}NH_4$, pH, electrical conductivity and the percentage of humic and fulvic acids.

Biological indicators is another group which includes properties associated to biological activity on organic matter such as microbial biomass (Suman *et al.*, 2006) and soil respiration (Janssens *et al.*, 2006; Marriot and Wander 2006). Other biological indicators are abundance, diversity, food chains, stability of communities (Doran and Zeiss, 2000), organisms associated to mesofauna such as earthworms (Andersen, 2008), nematodes (Blair, 1996) and arthropods. Biological activities such as enzyme activity (Liu *et al.,* 2008), and potentially mineralized nitrogen or CO_2 production are associated to this group of biological indicators (Doran and Zeiss 2000, Tejada *et al.*, 2006).

3.3.2.1. Biological indicators

Concentration or population of earthworms, nematodes, termites, ants, as well as microbial biomass, fungi, actinomycetes, or lichens are used as biological indicators, because of their role in soil development and conservation, nutrient cycling and fertility (Anderson, 2003).

Soil organisms are sensitive indicators, and reflect the influence of human management and climate changes. Similarly, soil organisms are indicators of quality and health because their diversity and abundance may be related to different functions such as decomposition of organic matter, plant and root development (competition). Soil organisms are also related to sequestration and detoxification of heavy metals (Nakatsu *et al.*, 2005), pesticides and other pollutants, disease-suppressive soil and presence of pathogens in soil and plant (Schroth and Hancock, 1982; Gao *et al.*, 2008; Scherwinsky *et al.*, 2007; Hartmann and Widmer, 2006; Del Val *et al.*, 1999).

Metabolic processes such as microbial respiration are used to detect microbial activity in soil. A common used index: the metabolic quotient (qCO_2), defined as the ratio between respiration and microbial biomass, is associated to mineralization of organic substrate per unit of microbial biomass (Bastida *et al.*, 2008). Enzymes such as cellulases, arylsulfatase, ammonium monooxigenase and phosphatases are considered biological indicators, and relate to specific functions of substrate degradation or mineralization of organic S, N or P. Soil enzyme activity is a potential indicator of ecosystem health and can be operationally practical and sensitive. Enzyme activity can offer an holistic “biological fingerprints” of soil management in the past, including information related to soil tillage and structure (Dick 2000). Determination of decomposition rates of plant debris in bags or measurements of the number of weed seeds, or the presence and quantification of pathogenic organisms’ populations can also serve as biological indicators of soil quality (Janssens, 2006).

3.3.2.2. Microbial indicators

Soil contains a large variety of microbial taxa with a wide diversity of metabolic activities (Parkinson and Coleman, 1991) and microorganisms play a leading role in soil development and preservation specially associated to decomposition of dead organisms and incorporation to biogeochemical cycles (Six *et al.*, 1998). Soil microbial biomass is a sensitive indicator influenced by different ecological factors like plant diversity, root exudates, soil organic matter level, moisture, and climate changes. Microorganisms in soil play a key role in nutrient cycling and energy flow (Li and Chen, 2004) and gives information about the impact of intercropping, incorporation of organic matter, management practices (Shannon *et al.*, 2002) and tillage activities, because all of these affect microbial activity at the plant rhizosphere, and processes such as N mineralization (Suman *et al.*, 2006).

Microbial communities respond to environmental stress or ecosystem disturbance, because of changes in the availability of energetic compounds that support microbial populations (Marinari *et al.*, 2007) but it is very difficult to evaluate concentration, number of species and

frequencies and these values can be only estimated. For this reason it is impossible to define diversity of species using a single soil analysis (Anderson, 2003). It is important to observe the interdependency between biodiversity and functional parameters and to define the sensitivity groups respect to functions in soil. According to Domsch *et al.* (1983) in Anderson (2003), *Rhizobium* species, actinomycetes, nitrifying bacteria, and microorganisms associated to organic matter decomposition show higher degree of sensitivity if compared to total bacteria, total fungi, and denitrification or ammonification process.

Any environmental impact that affects members of a microbial community should be detectable at the community level by a change of a particular total microbial activity (qCO_2, Vmax, Km). Similarly, microorganisms respond more rapidly to environmental stress in comparison to higher organisms, because of their direct contact with the surrounding medium given their surface:volume ratio. In most cases, changes in microbial population or activity precede changes in physical and chemical properties of soil, providing early indications of improvement or deterioration of the soil. In general, microbial indicators should be selected based on easiness of measurement, reproducibility and sensitivity to the variables that control the quality and soil health. Table 3.8 presents a set of data of microbial indicators (Nielsen and Winding, 2002).

Table 3.8. Microbial indicators used to determine soil quality.

PARAMETER	MICROBIAL INDICATORS	METHOD
Biodiversity	Genetic diversity	PCR – DGGE – T-RFLP
	Functional diversity	BIOLOG™
	Lipids	PLFA
Carbon Cycle	Respiration	CO_2 Production or O_2 Consumption
	Decomposition of Organic Matter	Enzymatic activity
	Enzymatic activity	Enzymes– cultura media– DGGE
	Methanotrophic	FISH – PLFA
Nitrogen Cycle	N mineralization	NH_4^+ accumulation
	Nitrification	NH_4^+ oxidation
	Desnitrification	Acetylen assay
	N fixation	MPN – nitrogenase activity– PCR
Biomass	Fungi, yeast	PLF_A – Ergosterol
	Protozoan	MPN
	Relation fungi- bacteria	PLF_A
Microbial activity	Bacteriophage	Host-specific assay
	RNA determination	RT-PCR – FISH
	Microbial physiology	CO_2 production or O_2 consumption
Key species	Mycorhizal	Microscopy – PCR
	Human pathogens	Selective media– PCR

3.3.2.2.1. Metabolic substances

- **Ergosterol**

Ergosterol, is the main endogenous sterol of fungi, actinomycetes and some microalgae. Its concentration is an important indicator of fungal growth on organic compounds and mineralization associated to fungic activity (Battilani *et al.* 1996). Ergosterol is a stable compound in soil and can support different stress conditions. Barajas-Aceves *et al.* (2002)

demonstrated that heavy metals (Cu 80 ppm, Zn 50 ppm or Cd 10 ppm) and fungicides (thiram 3 ppm or pentachlorophenol 1.5 ppm) reduced the metabolic activity between 18% and 53% (pollutant-stressed cultures) but did not affect the ergosterol content, while the fungicide Zineb (25 ppm) reduced significantly ergosterol content in biomass basis.

Molope *et al.* (1987) working with pastures and arable soils, found correlation between fungi hyphae and ergosterol quantity and soil aggregates stability demonstrating by electronic microscopy the importance of fungi, on physical process involving rearrangement of the clay micelles, in soil.

Also Puglisi *et al.* (2003), analyzed the content of cholesterol, sitosterol and ergosterol in agricultural soils of Italy, determining that the rotation does not affect the presence of these sterols in soil.

- **Enzymes**

Enzymes in soil refer to a product of microbial, animal (worms), or plant metabolism. Their role in relation to organic matter focuses on mineralization processes. Enzymes have been studied as indicators of soil quality since the 80's (Karlen *et al.*, 2008).

Soil organic mineralization involves metabolic processes and enzymes are catalytic substances in these biological reactions. Soil enzymes have been reported to be a key factor in the availability of essential nutrients in the soil (N and P). Their activity depends on the availability of the substrate and product, as well as the presence of inhibitors such as humic substances, clay or CO_2 (Ebersberger *et al.*, 2003) and various soil biotic and abiotic components such as mineral colloids and weather conditions (Kandeler *et al.*, 2006).

Enzymes play a key role as indicators of the effect of agricultural practices, soil management (De la Paz- Jimenez *et al.*, 2002) or soil fertility status (Dick *et al.*, 1988; Masciandaro *et al.*, 1999). Enzymes may also indicate processes of degradation or desertification of soils (Garcia *et al.*, 1997). Enzymes are more sensitive than total C concentration in response to vegetation disturbance (Cadwell *et al.*, 1999) and to agricultural management practices in tropics or semiarid conditions (Caravaca *et al.*, 2002). Enzymes are directly related to the portion of soluble organic matter and show the effects of organic amendments applied to the soil (Gutiérrez *et al.*, 2008.) The most studied enzymes in soil are those related to SOM mineralization, diversity and availability of nutrients such as N, P, and S.

Enzymes are related with biogeochemical cycles, the degradation of organic matter and soil remediation processes, so that they can determine, together with other physical or chemical properties, the quality of a given soil (Gelsomino *et al.*, 2006). Enzymes are associated with living cells, in non-proliferating cells such as fungal spores or cysts, attached to dead cells or cell debris though can also be found free (Fig. 3.2). The enzymes not associated with living cells are called abiontics, and are present in soil solution or associated to clay, mineral particles and humic acids where they are immobilized (Burns, 1982). This immobilized condition can remain stable and protect the enzymes of denaturalization by proteolysis and heat (Rao *et al.* 2000).

Few enzymes have potential as indicators of soil quality. Dehydrogenases, have a long history as biological indicators because they are generally closely related to the average activity of living microorganisms. Dehydrogenases only exist as an integral part of viable microorganisms. However, dehydrogenases could be not such good indicators when detecting long-term changes or history of soil quality. They depend on the microorganism being alive and can change only if changes in soil management or climatic conditions are significant and affect the microbial population. For this reason another group of enzymes, hydrolases, that are related to organic matter content, can be permanent indicators of quality. They are extracellular enzymes and are probably protected and coupled to complex clays or humic acids (Dick *et al.*, 1996).

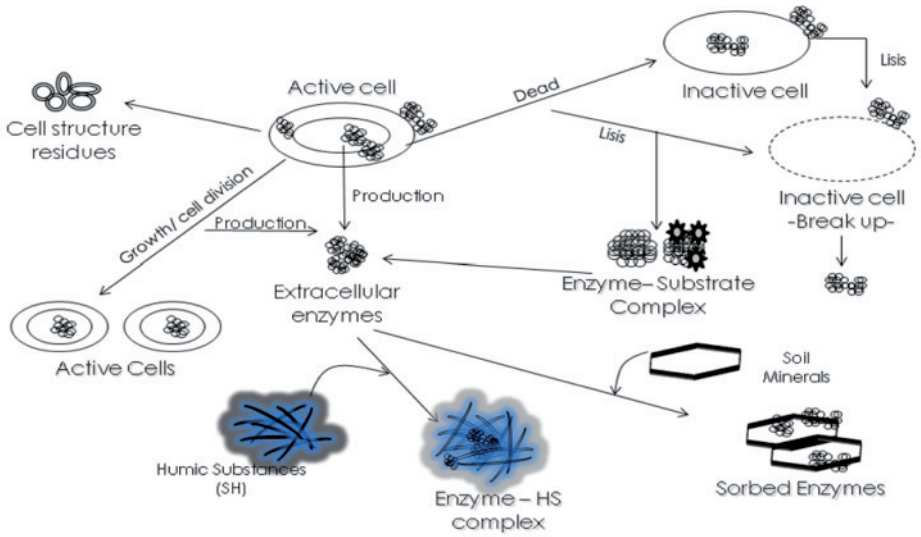


Fig 3.2. Dinamics of enzymes in soil
Burns, 1992, adapted by Gutierrez (2008).

In general, hydrolytic enzymes are a good choice as indicators because organic waste decomposing organisms are probably the biggest contributors of soil enzymatic activity. Organic matter has different organic compounds and the hydrolysis of those are key in the biogeochemical cycles of C, N, P and S. Most of the studies made with hydrolytic enzymes, such as β-glucosidase, urease, phosphatases and sulphatase are associated with the mineralization of nutrients important in plant nutrition.

♦ β glucosidases

This group of enzymes, hydrolyse carbohydrates with β-D-glucoside bonds, such as maltose and cellobiose (Klose and Tabatabai, 2002) producing glucose as a C and energy source for microorganisms (Fig 3.3). Among the most studied is the β glucosidase, an enzyme associated with the biodegradation of cellulose and cellobiose which is directly related to the content of glucose, C content and microbial biomass in soil (Esen, 1993). This enzyme plays an important role in making energy available in the soil which relates directly to the content of labile C and with the ability to stabilize soil organic matter, showing low seasonal variability (Knight and Dick, 2004). β Glucosidase is sensitive to soil management practices in short periods of time, i.e. less than two years (Dick *et al*., 1996; Sotres *et al*., 2005).

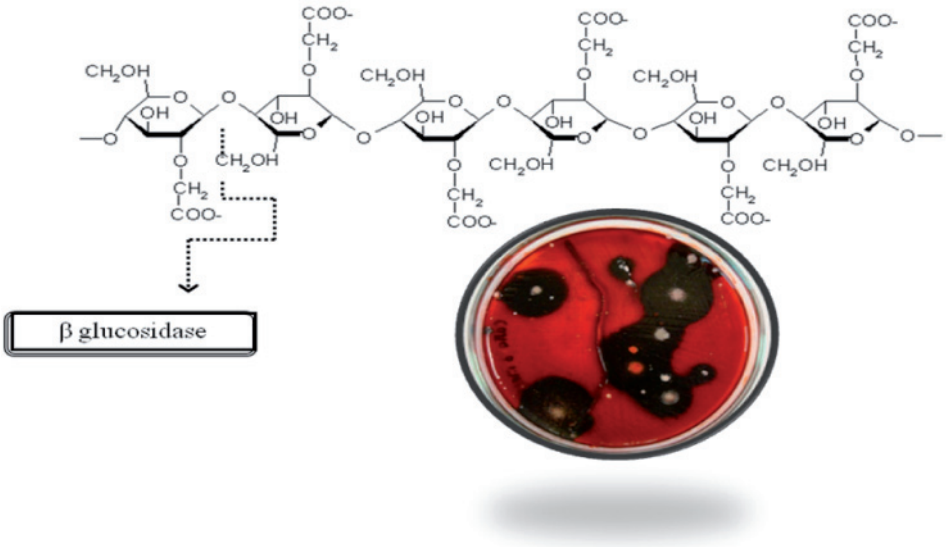


Fig 3.3. β Glucosidase activity a) on cellulose molecule. b) celullolitic bacteria in selective media. Photo by Gutierrez (2008).

The enzymatic activity is sensitive to soil change due to tillage (Acosta – Martinez and Tabatabai, 2001) or commercial forest effects on soil (Chaer and Totola, 2007).

♦ Phosphatases

Phosphatases are ubiquitous enzymes in soil and play a key role in phosphorous mineralization and P cycling. Phosphatases catalyze the hydrolysis of organic phosphomonoester to inorganic phosphorous (Fig. 3.4), making it available for plants to take (Tabatabai, 1994). According to their optimum pH they are classified in acid (orthophosphoric monoester phosphohydrolase, pH: 6,5) and alkaline (orthophosphoric monoester phosphohydrolase, pH:11) (Verchot and Borelly, 2005). Together with urease, phosphatase activity is significantly affected by concentration of metals such as Cu and Zn, which are used as indicators of effects of materials such as biosolids used in agriculture and leacheates. These enzymes could be present and active on the surface of roots, produced by plants and soil microorganisms, favoring the hydrolysis and metabolism of complex phosphates (Sukhada, 1992).

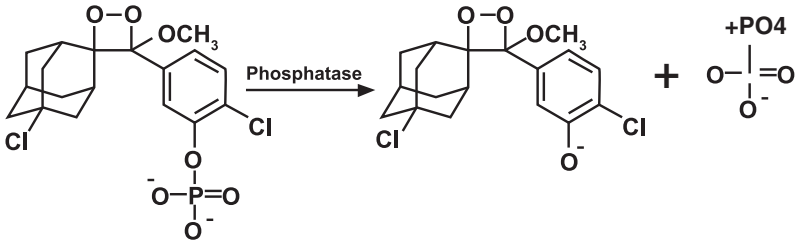


Fig 3.4 Phospahatases activity, liberation of phosphate group.

• Ureases

These enzymes are responsible for the hydrolysis of N-urea in the soil. Urease works by breaking the C-N bonds of the molecule resulting in the formation of ammonia, under appropriate environmental conditions (Ecuation 1). This corresponds to an amidohydrolase enzyme that acts on peptide bonds are not linear amides such as urea and others.

$$(NH_2)_2CO + H_2O \xrightarrow{\text{Urease}} CO_2 + 2NH_2 \quad [1]$$

Urease activity has been used as an indicator in different areas, for example in the application of compost in soil (Garcia-Gil *et al*., 2000).

3.3.2.2.2. Functional Groups of microorganisms as soil indicators

The study of soil microorganisms has acquired great importance, not only in order to know what kind of organisms inhabit in the soil but also to understand soil quality, as evidenced by its state of recovery or degradation. Through the microbial analysis, it is possible to obtain general parameters such as the determination of C and N microbial biomass, nitrogen mineralization, soil respiration, ATP and level of some enzymes that allow the detection of a specific substrate (Hernandez *et al*., 2007).

Some organisms like actinomycetes, amylolytic, heterotrophic, proteolytic, yeasts and fungi are associated with development, nutrition and plant health, including phytostimulation, biofertilization, biological control, and bioremediation.

- **Celullolytic**

Cellulolitic microorganisms as its name describes, have the ability to hydrolyze cellulose. The molecule of cellulose is an unbranched polymer of 1000 to 1 million D-glucose units, linked together with beta-1,4 glycosidic bonds. Cellulose can be obtained from different sources, but differ in the crystalline structures and bindings from other organic compounds. There are two types of hydrogen bonds in cellulose molecules: C_3 OH group and C_6 OH group; these bonds in one molecule are bounded to the oxygen of the glucosidic bond of the other molecule. Normally, the beta-1,4 glycosidic bonds are not too difficult to break, but in the cellulose molecule, the presence of these hydrogen bonds, gives to cellulose the possibility to form very tightly packed crystals. These crystals are broken by exogluconases, a subgroup of cellulase enzyme that includes β glucosidase, which acts upon the terminal glucosidic bond. This breaking of bonds continues in amorphous cellulose allowing the penetration of endogluconase, another subgroup of cellulase that catalyzes the hydrolysis of internal bonds (Lynd *et al*., 2002).

- **Yeast**

Colonies of this group of fungi are characterized by single-celled organisms and multiply by budding or fission. Its morphological characteristics are important for identification, and can be ascomycetes or basidiomycetes (Botha, 2011).

Yeasts are of great ecological importance because they act with biotic and abiotic factors of the system, and they have the ability to influence microbial and plant growth and, in addition, participate in the formation of soil aggregates. Yeasts play an important role by contributing to mineralization of organic matter. Many of the yeast species have shown no intraspecific diversity maintaining its functionality in soil. The function is related to the assimilation of different sources of carbon and nitrogen (Kurtzman and Fell, 1998, cited by Botha, 2011).

The yeasts found in soil, are able to use L-arabinose, D-xylose and cellobiose aerobically (Botha, 2006), derived from the enzymatic hydrolysis of hemicellulose by bacteria and molds acting on plant debri. There are yeasts also able to assimilate intermediates in the degradation of lignin (Sláviková, *et al*., 2002).

It is also recognized that soil yeast have the ability to promote plant growth directly or indirectly (El-Tarabily and Sivasithamparam, 2006), by antagonism and competition (Botha, 2011).

• Proteolytic microorganisms

These soil microorganisms are associated with protein degradation and their regulation at the ecosystem level. Proteolysis refers to protease activity and is found in different organisms in plants, animals and microorganisms. Proteases of microorganisms are the major protein-degrading source in soil.

These proteolytic enzymes are classified in two types: neutral metalloproteases and serine proteases. Some organisms such as *Bacillus subtilis*, *B. amyloliquefaciens*, *Pseudomonas* sp., *Lysobacter enzymogenes* and *Escherichia coli* have both types as extracellular enzymes and the level of expression is based on genetic information. The method by which these organisms regulate the expression of these enzymes is subject to physical and chemical factors in the environment (Mrkonjic *et al.*, 2008).

Mrkonjic *et al.* (2008), evaluated the effects of N and organic carbon on proteolytic enzymes, and found a variation in the number of 16 rRNA copies depending on soil management practices. Extracellular proteases have been detected in different culture media and could be used as indicators of soil disturbation (Kaiser *et al.*, 2010).

• Phosphate solubilizing bacteria

Phosphorus solubilization by microorganisms is one of the most important processes in the soil. This element is usually found in large amounts in soil, but their availability is relatively low, and depends specially on soil pH. In the presence of carbohydrates, microorganisms, produce organic acids (Fig. 3.5) and thus changing the pH around, or they produce acid or alkaline phosphatases which break the phosphates groups in organic matter (Mikanova *et al.*, 2002).

Rodríguez and Fraga (1999), described the use of phosphate solubilizing microorganisms as inoculum to get better absorption of phosphorus by the plant, thus increasing crop yield. The genera *Pseudomonas*, *Bacillus* and *Rhizobium* sp. have as primary mechanism the production of organic acids and acid phosphatase for the mineralization of organic phosphorus in soil (Caballero *et al.*, 2007). Based on the multifunctionality of these groups of microorganisms many biofertilizers have been commercially developed and some have been isolated from thermo-resistant strains of microorganisms for composting processes (Chang and Yang, 2009); and others have been used to remove phosphorus from contaminated sediments (Kim *et al*., 2005).

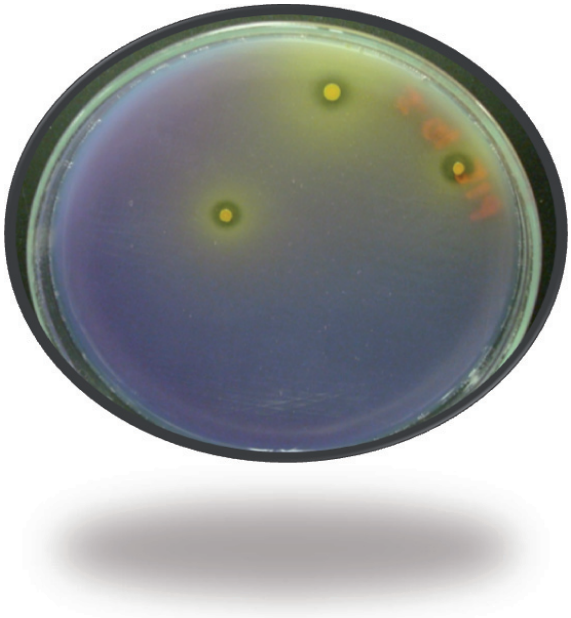


Fig. 3.5. Phosphate solubilizer bacteria, in selective culture media.
Gutiérrez (2008) (with permission)

- **Heterotrophic Bacteria**

These organisms are related to the decomposition and mineralization processes of organic matter. In general, nutrient competition generates microbial interaction, depending on the metabolism of plants and different populations of microorganisms that promote plant growth. This relationship depends on the environment in the rhizosphere, type of plant exudates, genetic response of the microorganism species and chemoattractants in the rhizosphere (Reyes *et al*., 2008). Root exudates consist of sugars, mucigel, organic acids and amino acids which can form 40% of plant photosynthates. Exudates create an environment favorable for the activity of plant growth promoting bacteria. The exudates are the main element of the released soluble organic compounds (Peña and Reyes, 2007).

3.3.3. Selection of indicators

Soil quality is estimated by observing or measuring different properties or processes, and several of these indicators can be used to determine soil quality indices.

According to Doran and Zeiss (2000) and Cantu *et al.* (2007), indicators should be limited and manageable in number by different types of users, simple and easy to measure, covering the largest possible situations (soil types), including temporal variation and have high sensitivity to environmental changes and soil management.

The selection of indicators for specific functions depends on the soil being assessed. These functions include support for the development of living organisms, water and nutrient flows, diversity and productivity of plants and animals, elimination or detoxification of organic and inorganic contaminants. Likewise, the selection depends on the sensitivity of these properties to soil management or changes in climate, as well as the accessibility and usefulness to producers, scientists, conservationists and policy makers (Doran and Parkin, 1996, Rezaei *et al.*, 2006).

The selection of indicators implies to know the target of the research and the capacity to interpret the indicator. The sensitivity of the soil property depends on the relationship between the indicator and the soil function that is being evaluated, the facility and reliability of measurement, the variation in times (crop rotation, effect of seasons) or cultural management as application of organic matter (Rezaei *et al.*, 2006).

Moreover, many soil ecosystem functions are difficult to infer directly and, consequently, other measurable properties are necessary to define soil quality especially in time (Magdoff and Weil, 2004). Some indicators may change faster than other ones; thus, not only the changes detected must be real but also sufficiently sensitive within short periods. In this way, thanks to the indicator a quick action on the agro ecosystem can be implemented to correct problems before undesirable situations or irreversible loss of soil quality occurs. General properties such as aggregate stability, density, pH, salinity, cation exchange capacity, microbial biomass, enzymatic activity, and basal respiration are used as indicators of soil quality (Magdoff and Weil, 2004).

In fact, some authors suggest that a soil quality indicator is not adequate if it is not directly related to the target user. If the goal is a soil quality index for crop production, then soil organic matter, infiltration, soil aggregation, pH, microbial biomass, N forms, density, electrical conductivity or salinity, and removable nutrients, represent a group of indicators that can be used to describe most of soil basic functions. In addition with these functions, the ability to accept, hold and release water to plants, maintain productivity and respond to management and erosion processes could be included (Rezaei *et al.*, 2006).

In the same way, for a better interpretation of soil quality indicators, Segnestam (2002) suggested the need of using a baseline to compare and determine positive or negative impacts on environment. Variations in time and rates of change of the property used as indicator, as well as local conditions should be determined to define potential models for larger scales but could

be necessary to adjust for each situation (Cantú *et al.*, 2008). For this reason the indicators associated to organic matter are considered to determine soil quality. They can correlate with high-sensitivity properties, and can offer to stakeholders, policy or research institutions results in short time so that decisions for a given agro ecosystem can be made on a timely manner.

3.3.4. Role of soil organic matter and associated indicators

Soil organic carbon (SOC) is a soil property considered as an important indicator of soil quality (Haynes, 2005). It is directly related to the maintenance of soil structure, water holding capacity, presence of different groups of microorganisms, mineralization of organic matter and nutrient availability (Goulding *et al.*, 2000).

Soil properties associated with soil organic matter (SOM) have been recognized as key indicators (Doran and Parkin, 1994) and to have an effect on other properties (Fig 3.6) Soil organic matter defines the energy supply to microorganisms, availability and quality of substrates, and the biodiversity necessary to sustain many soil functions. However, SOM varies with changes in climate, soil and crop management, being higher in places with larger average annual precipitation (Burke and Cole, 1995), lower mean annual temperature and higher clay content (Nichols, 1984). Similarly, the content of SOM is affected by intermediate grazing intensity (Allen 2010), incorporation of crop residues or the addition of organic matter fractions (Franzluebbers *et al.*, 1998) and by soil management practices such as minimum or conservation tillage (Acosta-Martinez and Tabatabai, 2001).

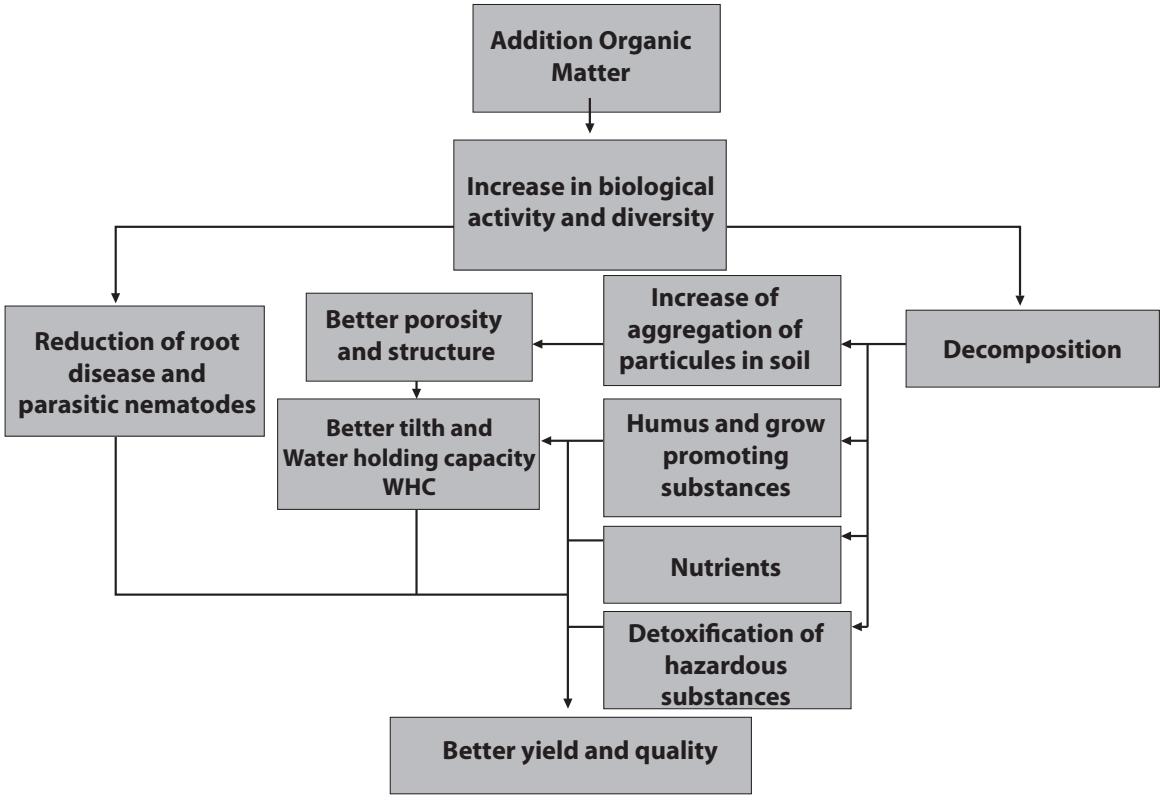


Fig. 3.6. Role of Organic Matter on soil properties
Margdof and Weil (2004).

Regarding SOM decomposition, there are factors such as N, P, or polysaccharide content that affects its decay, altering soil properties associated with soil quality. Some fractions, like starch or protein, are easily metabolized. Humic substances are more resistant to decay (Tate, 1987) and participate in nutrient exchange processes as well as in the formation of aggregates between organic substances and mineral particles, and in the immobilization of toxic materials (Ceccanti and Garcia, 1994).

Content and composition of SOM is affected by soil management, particularly when comparing organic with conventional soil management. However, changes in total SOC content from land use changes may be difficult to detect. Water soluble organic carbon (WSC) is a property which is more sensitive to change (Haynesand Beare, 1996), because are directly related to mineralization process in aqueous medium. Chan *et al.* (2002), found that particulate organic matter related to aggregate stability and nitrogen mineralization is more responsive to changes in management practices, than the total organic carbon.

Once organic matter, in different stages of decomposition, such as compost, manure or humic extracts, is applied to the soil, microbial communities are able to degrade it by mineralization, which occurs, depending on the local climate (Vargas-García and Suárez-Estrella, 2008). The mineralization is the transformation through enzymatic activity, of organic matter that can reach the total destruction of organic compounds resulting in simple products such as CO_2, NH_3, H_2O, etc. It is an important factor in recycling of N, P, S and CO_2 (Leon *et al.*, 2006) and results in the improvement of soil fertility by the accumulation of nitrogen, phosphorus, and other plant nutrients in the soil solution, effects that cannot be sustained over time. After mineralization, humification, a new process, starts. Humification is a process of chemical changes in organic matter, increasing molecular complexity and resistance to biodegradation. This process is also called stabilization or accumulation and refers to the inclusion or polymerization of organic molecules (Tate, 1987). Dick and McCoy (1993) indicated that mineralization and humification processes take place slowly in temperate zones, offering chemical, physical, and biological benefits for several years by increasing the levels of soluble organic carbon (WSC). Moreover, in tropical areas, there is an active mineralization rate leading to rapid depletion of stocks of organic and mineral nutrient, depending on the type of soil and climate (Busby *et al.*, 2007).

Mineralization and humification, are opposite processes that result from chemical and biological reactions that normally take place in each soil system. To understand the dynamics of soil organic matter, it is necessary to know the balance that occurs between degradation and synthesis. Soil is naturally in equilibrium when it is not under antropogenic intervention. However, in intervened soils the equilibrium tends to be altered either by increased mineralization, with the consequent reduction of organic status, or mineralization decreases in aerobic conditions (Heredia *et al.*, 2007).

Regarding the processes of mineralization and humification, the products may vary over time, depending on environmental conditions and on the presence of available nitrogen. Authors such as Weber *et al.* (2007) found a relative increment of HA (in relation to FA) in urban soils amended with compost, for some time, while Stride *et al.* (2004) found a depolymerization of HA to FA over time in soils where compost from municipal solid waste was applied. Almendros *et al.*, (1989, 2004) described, progressive degradation, accumulation of humic-like substances and microbial products after compost application to soil.

Likewise, differences in the concentration of HA during the humification at different temperatures (Bertoncini *et al.*, 2007, Contreras *et al.*, 2004) with smaller increases during winter are described in the literature (Madrid *et al.* 2004).

3.3.5. Soil indicators and environmental changes

Agricultural production results in changes in the ecosystem, not only because of the application of fertilizers or organic amendments, but also because of the production of organic wastes, or the intensification of land use with lasting, even permanent consequences, such as reduced fertility, productivity and biotic community structure (Stevenson, 1994), resulting in changes in soil quality.

When soil quality (soil properties) change, there is an effect on, water and air quality, which has an impact in terms of environmental and agricultural sustainability (Fig.3.7) (Doran and Zeiss, 2000; Andrews *et al.*, 2002).

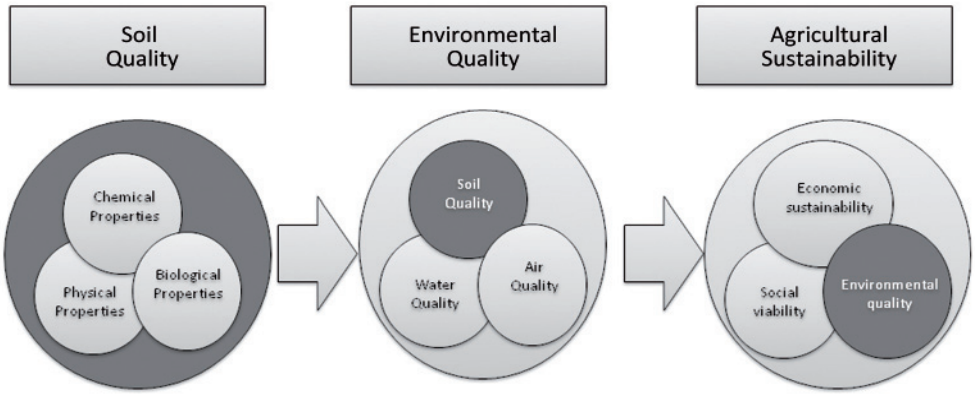


Fig. 3.7. Soil quality and agricultural sustainability. Andrews *et al.* (2001), USDA (2008).

The determination of the changes locally, regionally, nationally and globally, could help to better assess environmental problems, identify and evaluate the results of the implementation of International conventions and national and trade association regulations or standards. These changes could be measured using environmental indicators.

An indicator is a variable that summarizes and simplifies important information about a phenomenon or condition making it visible. An indicator should be measured, quantified and communicated in a comprehensible manner by different stakeholders such as producers, trade associations, scientific community or agricultural or environmental policy institutions. Indicators can include qualitative or nominal variables, ordinal or ranks, especially when there is no quantitative information available or when the geting such information may be costly. An indicator is a tool for the analysis of changes and should be simple, limited in number (high degree of aggregation), interdisciplinary, sensitive to temporal and spatial variations, climatic, environmental and anthropogenic changes (Doran and Zeiss, 2000).

Indicators are superior data as an analytical tool for several reasons. Firstly, they can work as a basis for assessment by providing information on conditions and trends of sustainable development. Secondly, as a basis of such assessments, indicators can provide input to policy formulation processes. Thirdly, by presenting several data in one number that is commonly simpler to interpret than complex statistics, they can facilitate the communication among different groups, for example between experts and non-experts. When two or more indicators, alternatively several data, are combined an index is created. Indices are commonly used at more aggregated analytical levels such as at the national or regional level. At these levels, it may not be easy to analyze the causal links using individual indicators since the relationships among different indicators become more and more complex the more aggregated the analytical level is. However, there are problems with computing indices as well. For example, sustainable development indices are extremely complex to create, and indices that cover issues from one and the same sector, or aspect, are thus more common (Segneststam, 2002).

The World Bank in its document “Indicators of Environmental and Sustainable Development”, points out the importance of establishing a baseline of activity that can impact the system positively or negatively. This base line is used to monitor or follow up on the negative impacts and to define a threshold limit or goals and objectives to assess whether the positive impact of response persists over the time (Segneststam, 2002).

These indicators and indices can be developed for soils under different scenarios (Doran and Parkin, 1994) or regional or local situations (Cantú *et al.*, 2002, Lilburne *et al.*, 2004, Cantú *et al.*, 2007). The construction of an index depends on the availability of adequate data and processing (Barbiroli *et al.*, 2004). Several soil quality indices have been proposed, associated with the effect of organic matter (Andrews *et al.*, 2002; Wander and Bollero 1999) on disturbed soils (Chaer *et al.*, 2009) or the impact of the quality of irrigation water on soil (Mandal *et al.* 2008).

Soil classification and interpretation are traditionally based on factors such as climate, parent material, time, topography and vegetation, and inherent qualities. This is the reason why single values to describe the quality of soil for all land or land uses cannot be used. The dynamics of soil that occur between 20-30 cm depth descibes the status of specific soil conditions, and may show relatively recent changes in land use or crop management (Karlen *et al.*, 2003) offering the possibility of relating better with improvement or degradation processes of soil (Fig. 3.8).

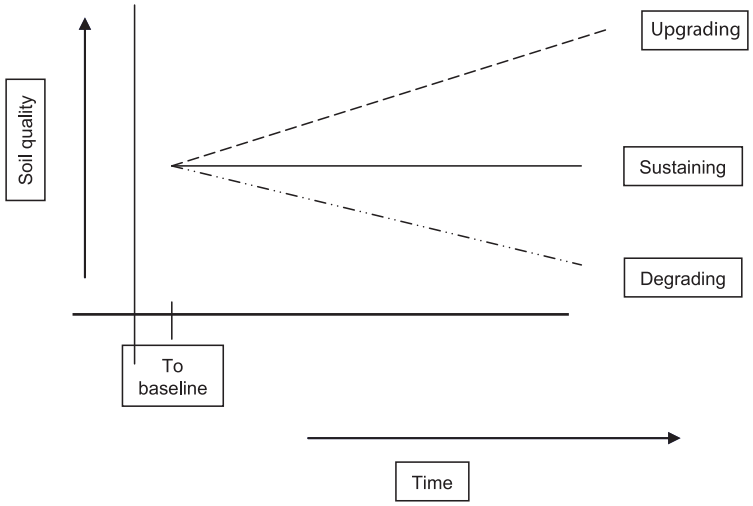


Fig. 3.8. Possible temporal trends in dynamic soil quality assessments. Karlen *et al.*, (2003).

3.3.6. Soil quality index

The concept of integrated indicators for soil proposed by Larson and Pierce (1991); applies concepts of soil ecology to assess the sustainability of the soil and ecosystem management, combining a variety of information that uses multi-objective analysis (Andrews and Carroll, 2001).

Internationally, there are kits of semiquantitative indicators, for 0-7,6 cm soil depth that include bulk density, infiltration rate, water holding capacity, electrical conductivity, pH, NO_3 and soil respiration, as developed by Soil Quality Institute (Doran, 1994, USDA-NRCS, 1998). There is also the Visual Soil Assessment protocol developed for local conditions in New Zealand and includes soil structure, porosity, color, earthworm number, evidence of a tillage pan and apparent susceptibility to wind, and water erosion. This index also correlated quality characteristics of the plant as uniform emergence, height, root development, disease incidence and costs associated with the handling properties of soil. In this protocol there are combined characteristics of pedogenesis with plant response as key indicators (Shepherd, 2000).

Four main steps are usually followed in order to determine a soil quality index or SQI: (i) to define the management goal(s); (ii) to select a minimum data set (MDS) of indicators that best represent soil functions as determined by the specific management goals; (iii) to score the MDS indicators based on the performance of the soil functions; and (iv) to integrate the indicator score into an index of soil quality (Karlen *et al.*,2003; Mandal *et al.*,2008).

Crop yield can be an important indicator of soil quality because it serves as a plant bioassay of the interacting soil characteristics. However, productivity alone may not always be the main criterion by which sustainability of an agricultural system should be judged.

Hussain *et al.* (1999), adapted this SQI in order to assess the effect of three management systems (no-till, chisel plow and moldboard plow) on soil quality under maize and soybean crops. They concluded that the methodology was sensitive to detect problems on soil quality

caused by soil management. Glover *et al.* (2000) used this methodology to assess soil quality in apple orchards and observed higher SQI in integrated management than in conventional or organic managements. In Brazil, Melo Filho *et al.* (2007) used this methodology to assess the SQI of soils under natural forest, observing a low value of SQI mostly because they oriented its SQI to crop production (Acosta, 2011).

The Natural Resources Conservation Service USDA-NRCS, proposed in 2003 the Soil Conditioning Index (SCI) in conservation planning. This index was developed to estimate the effect of conservation practices in the maintenance or increase of organic matter levels in soil, assuming that the organic matter content in soil is an indicator of quality (USDA-NRCS, 2003). The choice of appropriate attributes or properties should allow for inclusion in the index, showing properties not only effects on the specified function, but also on production and sustainability of the crop (Granatstein and Bezdicek, 1992). On this way, the values of indicators can be combined in a Soil Quality Index (SQI) that is measurable through a flexible model. Moreover, a valid SQI could also: (i) provide an early indication of soil degradation and the need for taking remedial measures, and (ii) show changes in soil properties, according to base line (Mandal *et al.*, 2008).

In other cases, the applied methodology standardizes the indicators. Cantu *et al.* (2007), in mollisols soils of low to moderate development in Argentina, determined an index of soil quality, obtaining a single value for each parameter. Indicators were according to the proportion that each operation had in the total area. Afterwards, the indicators were standardized using a 0-1 scale representing, respectively, the worst and best condition from the point of view of quality, regardless of the absolute values measured for each indicator. The authors concluded that indicators of soil resources are not universal and must be chosen depending on the environment and soil of the region.

CHAPTER IV

MATERIALS AND METHODS

4.1. Location

The study was performed within Empresas Bauzá, located in the Ovalle Comune at Limarí Valley (29°54′28″S, 71°15′15″W), in the Coquimbo Region of Chile (Fig. 4.1). The climate is classified as steppe according to Köppen Climate Classification System (Pidwirny and Irving, 2009 on line), with a thermal amplitude of 20°C, an average annual precipitation of 100 mm and a mean minimum and maximum temperatures of 6.3 and 28.5 ºC, respectively.

Fig.4.1. Location of Bauzá Table Grape Production

The study included four phases: 1) compost production based on grape marc, 2) extraction of humic substances from compost, 3) evaluation of compost and humic substances in a pot experiment, and 4) evaluation of humic extracts and nitrogen under field conditions.

4.2. Composting Process

The compost was elaborated based on the raw materials available, and these materials were collected in October 2008 to April 2009. Nine compost piles (18 m length x 2 m width x 1,5 m height) were composted from May to November 2009 (winter-spring season) and kept in maturity until January 2010 (spring-summer season). The piles were turned mechanically every 10 days, to provide oxygen and to give homogeneity to the system. Moisture content of each pile was adjusted according to a manual test, by adding water to maintain optimum composting conditions (approximately 60% moisture). Pile temperatures were measured using thermocouples connected to loggers, and analogue thermometers at top, middle, and bottom locations of each pile. Treatments consisted on different proportions of residues from the Pisco industry, grape pomace, pruning materials, and manure (Table 4.1). The materials were

analyzed in terms of their chemical composition, physical characteristics as well as microbial activity, every 60 days, until 220 day from establishment.

Table 4.1. Composition of evaluated treatments

Treatment	HM	GM	GP-Fresh	GP-Aged	YFR	PR+OS	Total
	----------%----------						
1	1	0	89	0	0	10	100
2	9	7	82	0	0	2	100
3	0	0	91	0	0	5	100
4	0	50	50	0	0	0	100
5	0	63	33	0	0	4	100
6	22	25	53	0	0	0	100
7	21	26	21	28	0	4	100
8	42	20	33	0	0	5	100
9	0	66	34	0	0	0	100

HM=horse manure; GM=goat manure; GP=grape pomace; YFR=yeast and fermentation residues; PR=pruning residues; OS=oat straw.

Fig. 4.2. Raw material used in co-composting process. a) Goat manure b) Grape pomace “orujo” (Pisco industry) c) Grape pomace “escobajo”

4.2.1. Compost sampling

Samples for microbiological and chemical analysis were collected, when the compost was turned, from three equidistant cross sections to a 60-cm depth from the pile surface, using a 5-cm diameter soil probe (Rodríguez *et al*., 2007). Ten subsamples were randomly collected and mixed to form a composite sample; two replications per treatment were collected.

4.2.2. Analytical determinations

Immediately after sampling, samples were homogenized by hand. Subsamples were taken for immediate analysis of microbiological parameters, moisture, pH, electrical conductivity and water-soluble nutrients, and for drying at 60°C up to constant weight. The rest of each sample was cooled and stored for later use.

4.2.2.1. Microbiological analysis

Bacterial (Bact) populations were determined using the micro drop method on nutritive agar (NA) (Merck). Fungi and yeast (F&Y), cellulolytic (Cellul), amylolytic (Amyl), and phosphate solubilizing microorganisms (Psol) were determined by surface plate count method in specific media: potato dextrose agar (PDA Merck media) yeast peptone dextrose agar (YPD, Merck)

cellulose agar, starch agar, milk agar and SMRS1 agar, SRSM (Sundara Rao and Sinha, 1963) respectively (Pedroza *et al.,* 2003; Caballero *et al.,* 2007); for the cases of the amilolytic and cellulolytic groups, it was necessary to reveal the activity by the addition of lugol (Merck) and congo red 1% (m/v), respectively (Pedroza *et al*., 2003). Microbial colonies were counted as colony forming units per fresh gram (cfu g^{-1}).

4.2.2.2. Enzymatic activity

The acid (AcP) and alkaline phosphatases (AlkP) were determined using *p*-nitrophenyl phosphate (Sigma) as substrate, and measuring the product *p*-nitro phenol to determine the phosphatase units (1 phosphatase unit is defined as UP = µg *p*-nitrophenol released per gram of dry sample per hour) (Dick *et al.,* 1996). The group of β-glucosidases (β Glu) were evaluated by also measuring the *p*-nitrophenol released, but in this case, from the substrate *p*-β-D-glucopyranoside (Sigma), expressing the results in terms of β-glucosidase units (UBG = µg *p*-nitro phenol released per gram of dry sample per hour) (Dick *et al.,* 1996). Ureases (U) were extracted following the method of Nannipieri *et al.* (1980) and their activity measured by the indophenol blue method, it was expressed in terms of urease units (UU = µg NH_4 released per gram of dry sample per hour). The following is a summary of the units used: Acid phosphatase (UP: µg *p*-nitrophenol g^{-1} h^{-1}). Alkaline phosphatase (UP: µg *p*-nitrophenol g^{-1} h^{-1}). β-glucosidase (UBG: µg *p*-nitrophenol g^{-1} h^{-1}). Urease (UU: µg NH_4 g^{-1} h^{-1}).

4.2.2.3. Chemical and maturity properties

Electrical conductivity (dS m^{-1}) and pH were analyzed in 1:5 (w/v) water-soluble extract (Thompson *et al*., 2001: TMECC 04.10; TMECC 04.11), total carbon (TC%) and total nitrogen (TN) were determined by the dry combustion method (Dumas method) in a LECO analyzer (TMECC 04.02-D).

The extraction ratio effect on humic substances content and concentration was determined evaluating different compost:extractant ratios, including: 1:7,5, 1:10, 1:15 and 1:30, respectively. Extractant used was 0,1M KOH.

Fulvic acids (FA) content were determined, extracting the compost material with 0.5 M NaOH and after 18h of agitation and centrifugation (9000 rpm). Precipitation of humic acids (HA), was made by acidifying the extract below pH 2,0 (Schinitzer, 1982; Anderson and Schoenau, 1993). Both fractions were dried and weighed. HA and FA substances were calculated based on mg HA or FA per g of compost. Optical densities of the HS solutions were measured at 465 (E465) and 645 (E645) wavelengths on a HACH UV-Visible spectrophotometer, and these values were used to calculate de E_4/E_6 ratio (Chen *et al*., 1977). The NH_4-N and NO_3-N contents were determined according with the TMECC 05.02-C method. The phytotoxicity of the compost samples was determined following the method of Zucconi et al., (1981) using radish seeds (*Raphanus sativus* L.) and the germination percentage (% Germination), based on a control with distilled water, was determined using the method TMECC 05.05-A. All determinations were made in triplicate. Fecal coliforms and *E. coli* were determined using MPN technique in LMX-MUG broth. The results were expressed as most probable number (MPN) g^{-1} (TMECC 07.01-B). *Salmonella* spp. detection and quantification was made according to the most probable number (MPN), expressed as MPN 4 g^{-1} (TMECC 07.02-B). Heavy metals were determined using digestion in nitric acid and microwave (TMECC 04.12-A) and atomic absorption spectrophotometry (TMECC 04.13-B) (Thompson *et al*., 2001).

4.2.3. Statistical Analysis

The experiment was treated as a factorial on a completely random design. Factors were compost piles and sampling dates. Main effects and interactions were evaluated by analysis of variance and protected LSD ($p<0.05$) in SAS (SAS Institute, 2000). Since one of the objectives of compost application is to add humic substances to the soil, a mathematical linear model including those

properties that can explain the maturity in terms of humic substances content was developed. For this, the stepwise method, within the regression procedure in SAS, was used (SAS Institute, 2008).

4.3. Evaluation of C rates in pots

4.3.1. Experimental design

One-year old nursery table grape (*Vitis vinifera)* plants of the Thompson seedless variety, cultivated on their own roots (no rootstock), were used, in a completely randomized experimental design. The evaluation period corresponded to one growing season (September to May) and one calendar year in total (from early May 2009 to May 2010). Plastic bags of 20 L of volume were used.

Treatments consisted on the application of two organic matter sources at three rates each: 1) mature compost from grape pomace and 2) liquid humus (humic substances extracted by alkaline treatment). Evaluated rates were the following: Compost, 0, 125, 250 y 500 g C pot^{-1}; liquid humus, 0, 25, 50 and 100 g C pot^{-1}; Compost and liquid humus at their maximum C rates were also evaluated in absence of chemical fertilization. Both products at all rates were evaluated in the presence or absence of a microbial inoculant; besides, compost and liquid humus (extracted according to methodology described in 4.3.1.1) at their maximum rates (2000 g pot^{-1} and 4000 ml ha^{-1}, respectively) were evaluated in absence of chemical fertilization. With these different combinations, eighteen treatments were arranged with 6 replications, using two controls: only chemical fertilization and an absolute control without any application. Equivalent C rates for each treatment are presented in Table 4.2.

Table 4.2. Evaluated treatments using different organic ammendments in Pot experiment.

Treatment	C source	Chemical fertilization[1]	Compost (g pot^{-1})[2]	Humic + Fulvic acids (L pot^{-1})[3]	Microbial Inoculant mL/pot $^{-1}$	C rate (g pot^{-1})
1	Compost	Yes	500			125
2	Compost	Yes	1000			250
3	Compost	Yes	2000			500
4	Compost	No	2000			500
5	Compost	Yes	500		400	500
6	Compost	Yes	1000		400	500
7	Compost	Yes	2000		400	500
8	Compost	No	2000		400	500
9	humic+fulvic acids	Yes		10		25
10	humic+fulvic acids	Yes		20		50
11	humic+fulvic acids	Yes		40		100
12	humic+fulvic acids	No		40		100
13	humic+fulvic acids	Yes		10	400	25
14	humic+fulvic acids	Yes		20	400	50
15	humic+fulvic acids	Yes		40	400	100
16	humic+fulvic acids	No		40	400	100
17	None	Yes				0
18	None	No				0

[1]Chemical fertilization: Entec 21 (stabilized ammonium with nitrification inhibitor DMPP): 20 g pot $^{(-1)}$, phosphoric acid 15 mL/pot and potassium sulfate 10 g/pot. [2]Application once at the beginning. [3] Four applications 1, 4-6 [4] two applications, one at 1st week and other at week 6th.

Fig. 4.3. Pot experiment, detail

Compost was obtained from an optimized co-composting process of grape pomace with goat manure (Martínez *et al.,* 2011); liquid humus was extracted from the same compost by the fractionating method (described below), and one microbial inoculant was obtained by isolating bacteria from the compost (method described below).

4.3.1.1. Extraction of humic substances

The best extraction ratio was used to prepare the extraction of humic substances at large scale 1000 L (Ortega, personal communication).Liquid humus was obtained following a fractioning method of soil humic substances described by Anderson and Schoenau (1993), using a ratio 1:10 compost: extractant (0,1M KOH) and 18 hours for extraction time. As a result of this fractioning process, humic (HA) and fulvic acids (FA) were obtained (Fig. 4.4).

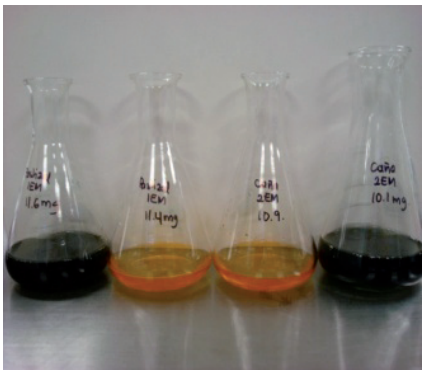


Fig. 4.4. Liquid humus obtained by fractionation method

4.3.1.2. Microbial Inoculant Production

The inoculant was a mixture of enzyme-producer microorganisms including *Pseudomonas sp., Ochrobactrum anthropi, Brevundimonas sp.* and *Sphingomonas paucimobilis*, isolated from compost made of grape pomace and goat manure. These microorganisms were grown to the late exponential phase in a sterilized medium prepared and standardized at the Microbiology Laboratory of the Advanced Center of Technology for Agriculture (CATA), of Federico Santa Maria University (Chile). The resulting cultures contained 6.2 x 10^{10} cfu ml^{-1} and were applied at the rate of 200 mL pot^{-1} in the corresponding treatments (Table 4.1).

The liquid humus and inoculant were applied at the surface of the pot, surrounding the vine plant. Compost was also surface applied once, at the beginning of the experiment. Liquid

humus was split applied ten times during the season, while the inoculant was applied twice. Fertilized treatments included the application of 25 g N $pot^{(-1)}$, 9 g P_2O_5 $pot^{(-1)}$ and 30 g K_2O $pot^{(-1)}$, applied as Novatec Solub 21 (21%N), phosphoric acid, and potassium sulphate (50 % K_2O), respectively; fertilizers were applied in solution in six equal splits during the season. The cultural practices, including irrigation and manual weed control, were the same for all treatments.

4.3.2. Measured variables

Initially, organic materials were evaluated by chemical, biochemical and microbiological analyses, according to the Chilean Standard NCh 2880/2004 (INN, 2004) as well as by the standard parameters proposed by the Test Methods for Examination of Compost and Composting (TMECC, 2002).

Soil samples from each pot were collected at 20 cm depth at the end of the experiment and analyzed in laboratory to determine soil quality parameters.

4.3.2.1. Soil variables

Chemical analysis were done according to the methods recommended by the Chilean Normalization and Accreditation Commission for soil analysis (CNA, 2006), and included: NO_3-N ($mg*kg^{-1}$), NH_4-N ($mg*kg^{-1}$) (Self y Rodriguez, 1998, modified by Ortega y Mardónez, 2005) Olsen-P (ppm), organic matter (OM%), total N (%), Total C (%), water soluble C (WSC), pH, and electrical conductivity (EC-dS* cm^{-1}); humic and fulvic acids content (%-HS) were determined by the method described by Anderson and Schoenau (1993) and E_4/E_6 ratio was determined according to Chen et al.(1977). Microbial populations were determined by the plate count method for isolating total bacteria (Bact) (nutrient agar), fungi (F) (PDA agar), yeasts (Y) (YPD agar), actinomycetes (Acty) (oat agar), and phosphate solubilizer microorganisms (Psol) (SMRS1 agar) (Martinez *et al.,* 2010); enzymatic activities were determined following the methods recommended by Dick *et al.* (1996), including β-glucosidase (β Glu: *p*-nitrophenol method), and acid and alkaline phosphatase (AcP and AlkP) activities (µg de para-nitrophenol $g^{-1}h$) (*p*-nitrophenol method); urease (U) activity (µg de NH_4 $g^{-1}h$) was determined by the method proposed by Kandeler *et al.* (1999) determining NH_4 by the indophenol blue technique.

4.3.2.2. Agronomic variables

Two agronomic parameters, trunk diameter (TD) (in cm) and plant height, were measured at times 0, 8, and 13 months from plantation. The diameter was measured with a caliper and the shoot cross sectional area (SCSA) was calculated as indicator of vigor [2].

$$SCSA = \frac{\pi * d^2}{4} \quad [2]$$

On the other hand, at the final evaluation time, roots of each treatment were collected to determine root dry matter (DM) (g) by oven desiccation at 70°C until constant weight; the root density (RD) ($g\ L^{-1}$) was determined by washing a 250 mL of soil sample throughout a sieve to collect the rootlets, which were desiccated in oven at 70°C until constant weight to determine their dry matter; results were expressed as density units dividing the mass into the volume of soil washed.

4.3.2.3. Statistical analysis

Data were analyzed using SAS statistical software (SAS, 2008). Statistical analysis included analysis of variance (ANOVA) with protected LSD test ($p<0.05$), and correlation analysis. Regression analysis was used to define the frequency response of measured on different matrices (soil, compost, humic extract (HE)).

4.4. Crop Production

4.4.1. General description

This study was carried out during two successive seasons (2009 -2010 and 2010-2011) on a year old Thompson seedless Table grape orchard, freedom rootstock, planted on sandy soil under drip irrigation system in a field located within Bauza Company. Plants were maintained with basic fertilization: Basacote (slow release fertilizer NPK:16-8-12+2Mg; effective durability, 3-4 months according to producer. COMPO®, 2012) 50g/vine, goat manure 1 kg year^{-1}, and nematicide. The vines were cane pruned with three wire trellis, supported by wooden poles and irrigated via drip irrigation system.

Three base line soils were used: the soil from the mountain xerophilic forest (BLM), the soil from the riparian vegetation around Rio Claro River (BLR) and finally, the uncultivated soil from the table grape agroecosystem (AGS) (Fig. 4.5). The experimental soils were alluvial inceptisols with low organic matter content (Table 4.3, Fig. 4.6), under routine chemical fertilization.

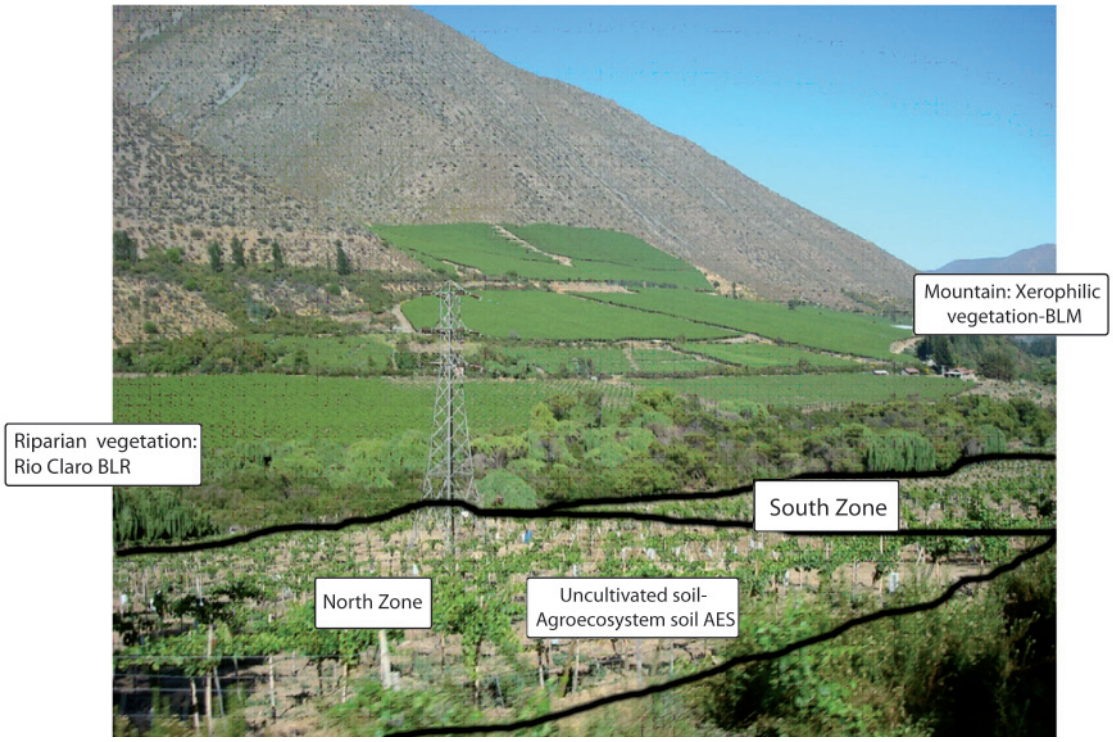


Fig. 4.5. Limits of Field experiment and Base Lines location.

Total area of table grape including North and South distributed experiments, and Mountain Xerofilic vegetation, Riparian vegetation around Rio Claro River and Agroecoystem Uncultivated asoil (AES) used as base line.

Each treatment was replicated five times with one vine per treatment and the randomized complete block design was arranged in two areas (North and South) as true replications (Table. 4.3). At the end, 10 vines/ treatment were used totalling 160 plants. The texture of the soil is sandy; the physical and chemical properties of the experimental soil are presented in table 4.4.

Fig. 4.6. Field experiment distribution 3b. Inceptisol soil detail

Table 4.3. Distribution of treatments in Field experiment

North

T5-6	T12-3	T4-10	T5-9	
T11-9	T12-7	T13-6	T9-8	T7-7
T9-8	T7-10	T5-8	T4-9	T2-3
T11-8	T12-9	T13-4	T15-3	T16-7
T9-1	T7-10	T6-7	T4-8	T2-1
T9-6	T7-8	T13-2	T3-4	T1-5
T14-1	T10-5	T7-9	T6-8	T16-10
T1-3	T4-8	T4-6	T14-3	T15-2
T3-1	T2-4	T13-1	T15-1	T16-9
T2-5	T15-4	T5-7	T13-5	T12-6
T9-4	T1-2	T8-2	T10-2	T6-5
T13-3	T3-2	T1-4	T16-8	T4-10
T12-8	T14-5	T8-1	T5-10	T8-9
T14-2	T11-7	T6-4	T9-5	T11-10
T16-6	T10-4	T14-4	T4-5	T6-3
T8-10	T3-3	T15-5	T2-2	T3-5
T11-6	T8-3	T10-6	T1-1	T12-10

South

T10-9	T9-9	T6-1		
T6-10	T13-7	T9-2	T5-4	T14-10
T12-2	T3-7	T15-10	T4-4	T7-2
T8-6	T1-6	T11-5	T2-6	T16-4
T1-8	T7-4	T8-5	T16-3	T6-6
T4-3	T13-10	T5-2	T7-6	T14-7
T15-6	T10-7	T9-3	T3-10	T2-9
T12-4	T16-1	T1-9	T8-8	T11-3
T10-3	T5-3	T2-10	T14-9	T3-9
T14-6	T12-5	T10-8	T5-1	T8-4
T11-4	T3-6	T15-7	T9-10	T4-7
T6-9	T1-10	T16-5	T7-3	T2-7
T15-8	T11-2	T4-2	T13-9	T14-8
T8-7	T12-1	T10-10	T7-5	T13-8
T11-1	T6-2	T15-9	T2-8	T5-5
T4-1	T16-2	T9-7	T3-8	T1-7

4.4.2. Fertilization

As for mineral fertilization complement, potassium sulphate and phosphoric acid were added per each vine and placed 10 cm under the soil surface on both sides of the vine (Table 4.4). The microbial inoculants were obtained as described in 4.3.1.2. The organic amendment (HS) were side dressed in a band of 50 cm wide on both sides of the vine rows and mixed with the soil surface. Vines treated with humic acids received a liter of HS (humic substances 12% humic acids) added on the soil surface. Humic substances were applied ten times during the season on a weekly basis. The other cultural practices were the same for all treatments.

Table 4.4. Fertilization program during the field experiment

			NOVATEC 21				KSO_4	Phosphoric ác.	Liquid Humus 6
			g plant-1				g $plant^{-1}$	mL $plant^{-1}$ (1%)	L/plant/week
Treatment	C rate (kg ha^{-1})	N rate (kg/ha)	W1	W 2-3	W 4	PH	W1-6	W1-4	w1 to w10
1	0	0	0	0	0	0	30	600	0
2	125	0	0	0	0	0	30	600	2,5
3	250	0	0	0	0	0	30	600	5
4	500	0	0	0	0	0	30	600	10
5	0	30	29	23	17	23	30	600	0
6	125	30	29	23	17	23	30	600	2,5
7	250	30	29	23	17	23	30	600	5
8	500	30	29	23	17	23	30	600	10
9	0	60	57	46	34	46	30	600	0
10	125	60	57	46	34	46	30	600	2,5
11	250	60	57	46	34	46	30	600	5
12	500	60	57	46	34	46	30	600	10
13	0	120	114	91	69	91	30	600	0
14	125	120	114	91	69	91	30	600	2,5
15	250	120	114	91	69	91	30	600	5
16	500	120	114	91	69	91	30	600	10

T: treatment; C: carbón rate; N: nitrogen rate; W: week; H:Harvest; PostH: Post Harvest

4.4.3. Measured tissue variables

To determine plant nutrient concentration, leaf samples were taken at veraison i.e. ripening onset (20 per experimental unit), in both seasons. Samples were dried at 65°C and were later grinded with a 40 mesh sieve. Nitrogen (N) (%) concentration was determined using a LECO (CNS-2000 Macro Elemental Analyzer; Leco Corp, St. Joseph, MI, USA). For Olsen P (ppm P), the ashes were analyzed forming a complex with molybdate-vanadate and then measured using a molecular absorption spectrophotometer. Micronutrients were determined by atomic absorption. Leaf mineral contents (total N (N), Nitrate reductase (NRed), Metabolized nitrogen (MetN), N-NH_4, N-NO_3, Total N (%N), Chlorophyll a (Chl a), b (Chl b) and total (TChl), and Total C (%C), were determined in blades from mature leaves (5-7th leaves from shoot top) opposite to basal clusters according to the methods described in Sadzawka et al., 2007. Total chlorophyll was determined according to Parra y Criedeman (1989), SPAD (Rozas y Echaverria,1998) and nitrate reductase defined as UNR :µg NO_2 g^{-1}*h, was determined according to Godoy (2004).

4.4.4. Measured soil variables

Soil analyses were done, for the top 30 cm, before the beginning of the experiment and at harvest, on both growing seasons. Samples were taken from each experimental unit and pH and electric conductivity (EC) were determined. At the same time chemical, microbiological and biochemical analysis (described in 4.3.2) were determined.

4.4.5. Measured Yield and Berry Quality Characteristics

At harvesting time (late February) the yield expressed in weight (kg) and harvest in T ha^{-1} of exportable fruit was determined. All bunches were collected from all plants (a total of 160 plants), and with the help of the harvesters team, the clusters were selected in the field. All clusters were classified according to their characteristics in “discarding” and “packing”. The weight of the discarded was obtained and this grape results in grape raisins.

The harvest boxes were transported from field to packing plant, and there took place the calssification of clusters according to size of the berry, color, weight, giving the properties for small (S: <700g), medium (M:700-800 g), large (L: 800-1000g) or extra large (XL: >1000g) size, all for English market (Fig. 4.7).

Fig. 4.7 Packing clasification in Varillar, Bauza Company.

A sample of 6 clusters per each treatment were randomly taken from each replicate to determine berries quality in terms of Brix, Chlorophyll a and b (µg g^{-1} fw), Total Chlorophyll and total acidity (TA) (expressed as g tartaric acid 100 g^{-1} of juice); properties were determined as outlined in A.O.A.C. Polyphenols (Poly) (*100g^{-1}) and Anthocyanins (Ant) were determined according to Longo and Vasapollo (2005) and, polyphenol oxidase content (one unit is defined as UPPO=µg TBQ/g*h) was determined according to Casado et al. (2005), modified method. The remaining bunches were stored at 0◦C for 30 days, and after that period, berry firmness was determined. Additionally, in each season, grape bunches were classified in exportable and national quality according to Bauza, International Market standards.

4.5. Selection of compost, soil and fruit variables for a quality

A proper index to measure quality should have certain characteristics:

1. Be simple to measure.
2. Be sensitive to changes in time and management.

The sensitivity to changes can be measured in at least two ways: 1) establishing if the property changes over time, as a result of the management imposed, which technically can be proved by regressing the property of interest against time, and 2) through the variance of each property.

In order to select the properties for compost, soil and fruit quality indices, the following steps were performed.

1. Each measured property was standardized either obtaining a Z score (with mean=zero and standard deviation=one) or dividing each observation by the maximum among all observations in order to obtain 0 to 1 values.

2. In the case of compost and soil for both, the pot and field experiments, each measured property for each treatment, and for all treatments together, was regressed against time (in months) from the baseline. The outcome for these regressions could be a positive slope, a negative one, or a zero slope, which will mean no change of that particular property with respect to the base line (Figure 4.8). Hence, the slope will corresponded to the weight of each property. Only those variables with significant (P<0,05) slopes were considered. Frequency response was estimated by dividing the number of significant (P<0,05) responses among the evaluated treatments over the total number of them. Corrected weights were estimated by multiplying the average response coefficient (slope) times its frequency response. Final weights were corrected to add to one.

3. In the case of fruit, since there were not measurements over time, only the variability among treatments was used as a criterion to select the properties that would be part of a quality index. In this case a composed index based on coefficient of variation of each property (Ortega and Santibáñez, 2007) was estimated. Only those properties varying more than the average CV + 0,5 (standard deviation) were considered. Thus, only properties at the 30% superior in terms of variability were considered.

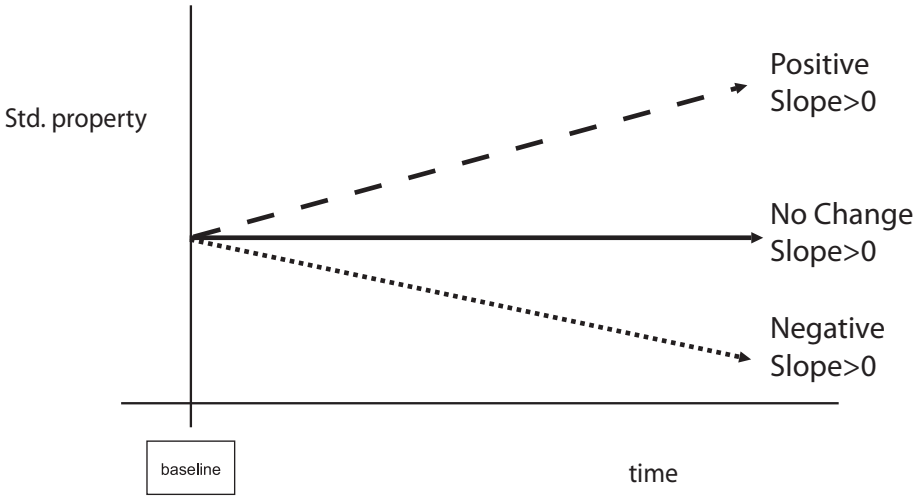


Fig. 4.8. Potential Outcomes respect to the base line.

CHAPTER V

RESULTS AND DISCUSSION

5.1. Compost process monitoring

5.1.1. Compost Chemical and physical characteristics

The composting process was conducted in the winter-spring months with temperatures averaging 12 °C. However, as shown in Figure 5.1, the overall temperatures in all treatments (piles) showed a mesophilic phase of two weeks and then a thermophilic phase that lasted between 3-8 weeks, with a maximum temperature of 62 °C (pile 1) with a mean of 50 °C. Subsequently it remained between 40 and 50 °C, with a tendency to increase; this temperature was maintained during 6 weeks and significantly decreased after the 20th week. Temperature is not only a consequence of the composting process (microbial metabolism) and turning, but also a control parameter.

According to Haug (1993) and Bernal *et al*. (2009) temperatures providing the maximum degradation velocity are in the range of 40-70ºC. The optimization of the composting process for grape pomace is possible, using goat manure, and bunch stems which appears to be an ideal bulking agent, providing C and improving physical properties such as porosity (given by its branch-type structure) and resistance to biodegradation of the hard-wood fraction (Tuomela *et al*., 2000); its chemical properties are also optimal; bunch stems' C/N ratio is high (around 40) and equilibrates the low C/N ratio of goat manure (around 5). While this behavior is typical of composting processes (Fig. 5.2) with C/N ratio appropiate close to 25:1, it could be improved, reducing the composting time to 10-12 weeks, controlling aspects associated with handling and turning.

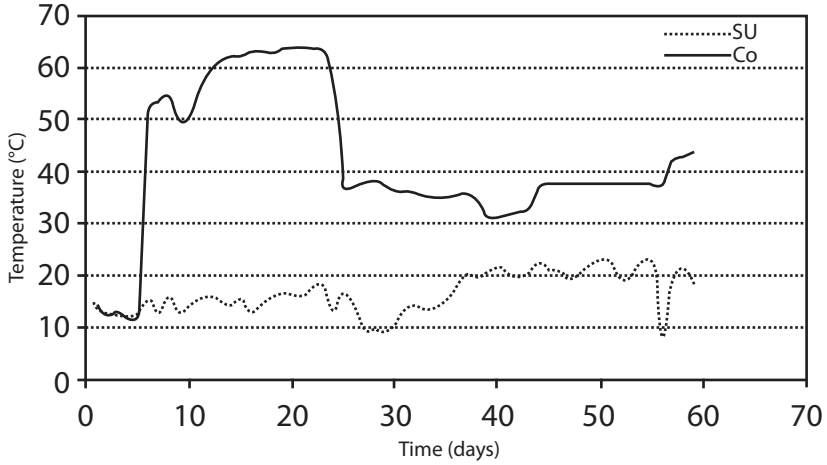


Figure 5.1. Temperature evolution during the mineralization process of composting
(Su: surface; Co: Compost pile).

The main results of the physical and chemical characteristics obtained in the different treatments are shown in table 5.1. In connection with the physical characteristics, the obtained compost materials presented loose texture corresponding to porosity near 70%, dark color, a pleasant scent throughout the process; this allowed the absence

of leachates and therefore flies and other vectors. Pisco industry generates large amounts of residues, in a short period of time, along the year, during the end of summer to fall seasons (March to May). Average grape yield, for Muscatel variety, are close to 45 to 50 ton ha^{-1}, 30% of which (12 to 15 ton* ha^{-1}) will be grape pomace and bunch raquises that could be mixed with other materials to decrease the C/N ratio, improve quality of the final product, and use it as organic matter and nutrient source, adding beneficial microorganisms to activate soil functions, and biological control (Rivera and Avallay, 2008). Besides Pisco residues, goat and horse manure, available in the area, are used as N source; but in co-composting with Pisco solid wastes, it was possible to obtain a solid stabilized organic fertilizer, suitable for application to vineyard crops.

Table 5.1. Chemical properties of materials after 220 days of composting.

Treatment	pH	EC	WSC	N	NH_4-N	NO_3-N	NH_4-N / NO_3-N	C/N
		dS cm^{-1}	g kg^{-1}	%	mg kg^{-1}	mg kg^{-1}		
1	7,13±0,0	1,24±0,0	3,69±0,0	1,79±0,0	137,54±0,0	46,41±0,1	2,98±0,1	15,01±0,1
2	7,25±0,0	1,79±0,0	4,67±0,8	2,17±0,0	207,02±0,0	86,56±0,0	2,39±0,0	15,94±0,0
3	7,12±0,0	1,52±0,0	3,97±1,1	2,16±0,2	33,08±0,1	55,01±0,0	0,61±0,2	17,57±0,0
4	8,03±0,0	4,02±0,0	8,72±1,5	2,25±0,1	117,42±0,0	393,97±0,0	0,30±0,0	10,05±0,0
5	8,54±0,0	5,10±0,0	12,86±3,6	2,63±0,0	263,06±0,0	132,62±0,0	1,99±0,0	10,41±0,0
6	8,25±0,0	3,18±0,0	6,54±2,4	2,26±0,1	370,92±0,0	78,17±0,0	4,75±0,0	13,37±0,0
7	8,29±0,0	2,93±0,0	5,61±4,3	2,15±0,0	395,01±0,0	19,46±0,1	20,35±0,0	13,00±0,0
8	8,01±0,0	3,76±0,0	13,11±1,1	1,95±0,0	186,78±0,0	437,45±0,0	0,43±0,0	11,77±0,1
9	8,46±0,0	3,75±0,0	11,72±0,0	2,13±0,0	349,38±0,0	225,36±0,0	1,55±0,0	11,60±0,2
Class A*	5,0-9,0	<3,0	Nd	>1	<500	Nd	<3	<25
Class B*	5,0-9,0	<8,0	Nd	>1	<500	Nd	<3	<30

EC: Electrical conductivity. WSC: Water soluble carbon. N: Total nitrogen.;; NH_4-N/NO_3-N :Ammonium-Nitrogen/ratio; C/N: Carbon:NitrogenCarbon: Nitrogen Ratio C: Total carbon. Nd: not defined.

* NTCh 2880/04. Bold numbers did not meet the standard. + Bold numbers are significant (p<0,05)

Figure 5.2. a)Compost piles and b) Measurement of the temperature of pile

The initial pH values in the piles were between 7,1 and 7,8, depending on the amount of manure included. At the end of composting, all treatments showed slightly alkaline pH, similar to those reported by other authors (Beltran, 2004; Bustamante, 2009). The levels of total organic carbon were similar to those observed in composting waste products of winery-distillery (Ranalli, 2001; Bustamante, 2009) and are above the reference value (15% C) of the Chilean Standard (NTCh 2880/04), although compared with the European standard values (30% C) are lower (European Commission, 2001). Abad *et al*. (2001) stated that a value of total organic matter above 80% should be adequate for potting media; all compost treatments had adequate organic matter levels (>50%) but no significant differences among them were observed.

Mineralization processes include degradation of polymeric substances, such cellulose, lignin, protein, using the catalytic activity of different enzymes, or groups of them, that show the evolution of the compost process in terms of the decomposition of organic matter, nitrogen and phosphorous transformation, and humic substances production. Therefore, final compost is a stabilized, deodorized, safe material for plants and humans, and rich in humic substances (Rallani *et al.,* 2001; Islam *et al.,* 2004).

5.1.2. Sanitary parameters and metal content

As complement of quality compost properties, the concentration of human pathogens, fecal indicators, and metal content was determined, thinking in the role of some bacteria in the foodborne diseases (Islam et al., 2004) and the effect of trace metal on human health. All the evaluated treatments met the specifications for *Salmonella* sp. and *E coli*, presenting values less than 100 MPN g^{-1}. They also complied with the maximum metal contents, which indicates that all compost treatments could be classified as Compost type A (Table 5.2), according to NTCh 2880 (2004), Biowaste EU Network (2011) and Compost Council Quality of California -CCQC (2001).

Table 5.2. Metal content, percent germination and sanitary parameters measured in compost.

Treatment	Cr	Cu	Ni	Pb	Cd	Zn	Germination	Fecal Coliforms	*E. coli*	*Salmonella* sp.
	----------mg kg^{-1}----------						%	MPN 100 g^{-1} DM		MPN $4g^{-1}$ DM
1	15,5±4,9	33,5±2,1	6,95±1,2	4,6±0,9	<0,01	35,5±2,1	95±0,1	31,09±0,1	0,23	<0.02
2	7,1±1,1	31±1,4	6,5±0,8	3,95±0,7	<0,01	40±2,8	98±0,8	245,28±0,1	0,26	<0.02
3	13,65±6,5	30±0,0	6,5±0,4	4,3±0,0	<0,01	35,5±0,7	94±0,8	6,08±0,1	0,24	<0.02
4	6,2±0,1	31±1,4	8,5±0,7	5,75±0,9	<0,01	55±0,7	95±0,8	0,20±0,1	0,20	<0.02
5	10,65±4,7	31±0,0	8,8±0,2	9,85±0,2	<0,01	56,5±3,5	100±0,0	0,21±0,1	0,21	<0.02
6	12,5±3,5	34±1,4	6,9±0,1	5,0±0,1	<0,01	44,5±0,7	90±0,7	641,67±0,1	0,21	<0.02
7	18,5±2,1	33±0,0	8,15±0,6	5,45±0,9	<0,01	43,5±0,7	98.1,2	480,70±0,1	0,22	<0.02
8	9,25±0,0	36±1,4	9,15±0,6	32,5±0,3	<0,01	49,5±0,3	92±0,0	570,89±0,1	0,23	<0.02
9	13±1,4	31,5±0,7	9,35±2,3	5,2±1,2	<0,01	48±0,7	93±0,1	613,16±0,1	0,24	<0.02
Class A*	120	100	20	100	2	200	>80	<1000	Nd	Absent
Class B*	600	1000	80	300	8	2000	>80	<1000	Nd	Absent

* NTCh 2880/04. Nd: Not determined

5.1.3. Microbial populations

Regarding microbiological properties, the behavior of heterotrophic bacteria and fungi populations was the expected in all evaluated compost (Figure 5.3). As reported by others authors (Dazeell *et al.*, 1991; Sánchez, 2009) the bacterial population was markedly dominant during the entire stabilization phase of composting, with all evaluated mixtures providing a good substrate for microorganisms of agricultural interest. This means that applications of compost as a soil amendment will provide a significant amount of microorganisms capable of exerting different effects at the level of nutrient mobilization (immobilized phosphorus) and activating functions of mineralization of organic matter (cellulolytic, proteolytic, and amylolytic microorganisms). Isolates showed interesting organisms that have potential in biological control and disease suppression of soils, which is a significant contribution to an integrated plant disease management.

During the active phase on the composting process, organic C decreases in the material due to decomposition of the organic matter by microorganisms. The degradation rate of the organic matter (OM) decreases gradually as composting progresses because the reductions of new complex and polymerized organic compounds (humification) that occur during the maturation phase (Bernal *et al.*, 2009). Important enzymes are involved in this biochemical

process associated to C substrates; among them, the cellulase complex, β-glucosidase which hydrolyses glucosides, proteases and ureases, associated to N mineralization, and phosphatases that remove phosphate groups from organic matter (Nannipieri 2002; Aira *et al.*, 2007). The process offers stabilized end products, which can be used as C storage and slow release fertilizers for agricultural purposes. The residual organic matter is transformed by microorganisms to form humic-like substances, which form complexes with extracellular enzymes stabilizing them, and preventing their degradation and denaturation (Chen *et al.*, 1997, Burns *et al.*, 1972). Additionally, the microbial community, with different physiological profiles, is used as indicator of compost maturity. The decreasing trend of microbial biomass throughout the composting process is normal, and it is associated to temperature and the consumption of C and N (Klamer and Baath, 1998); the fungal biomass decreases during the active phase and maturation stage compared to initial compost. Mondini *et al.* (2004), demonstrated the relationship between enzymatic activity and quality of organic matter, and indicated that the humic-enzyme complexes should be considered as a process directly related with compost stability. At the same time, during the maturity phase, the CO_2 evolution, fulvic acids, and N-NH_4 concentrations decrease, while nitrification process increases the concentration of N-NO_3, showing a nitrification index <0,16 (Hue and Liu, 1995; Bernal *et al.*, 2009); the concentration of humic acids also increases. At the end of the process, and previous to the application of compost, the phytotoxicity test, heavy metal contents, and evaluation of presence of human pathogens and fecal coliforms, could be a good complement to define a safe agronomical use.

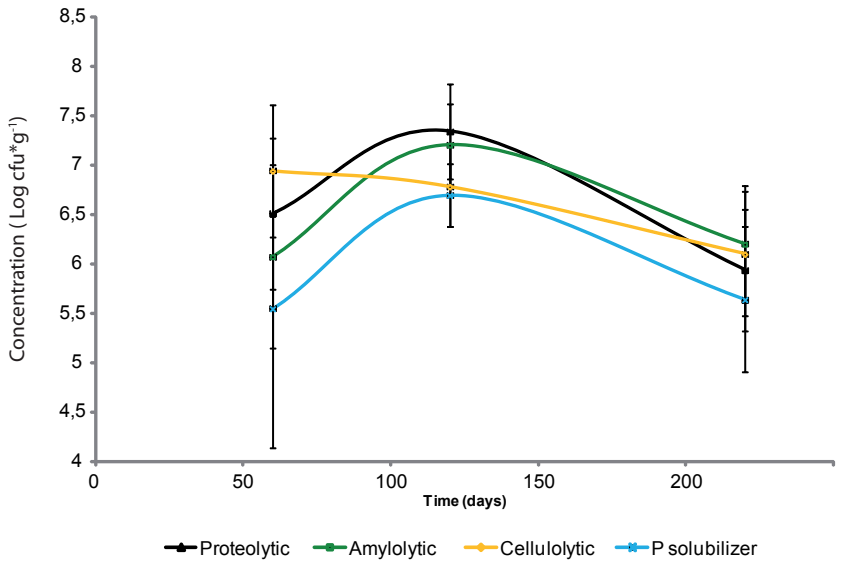


Figure 5.3. Microbial groups behavior along the composting process. Average of 9 treatments.

According to Choi and Park (1998), the presence of a high number of yeasts during the mesophilic phase is due to the presence of fruit residues, with low initial pH, and the ability of yeasts to grow at a lower pH than bacteria. In some occasions numbers of subpopulations of bacteria were higher than the total count. This probably was due to differences in media composition, incubation time and growth/degradation characteristics of the microorganisms counted. Thus did not attempt comparing absolute numbers of colonies grown on different media. Fungi and yeasts can survive the heat peak or are re-inoculated into the compost from the environment or from the edges of the pile.

Temperature is a determining factor on population diversity, because it defines the metabolism and the rate of enzymatic activity (Ishii *et al.*, 2000; 2003); the temperature range was around 55–60ºC and consequently, temperatures were not excessive. Temperatures of 60–75ºC, favor cellulose degradation, and the activity of cellulolytic and thermophilic organisms (Gray *et al.*, 1971). At the end of the composting process, the cellulose content is low due

to mineralization and humification process, and other C fractions could be inaccessible to enzymatic attack because of low water content or association with protective substances such as lignin (Stutzenberger *et al.*, 1970; Castaldi *et al.*, 2008).

This degradation process resulted in a decrease of the number of cellulolytic organisms, while heterotrophic bacteria showed high correlation ($r = 0,734$, $p<0,0001$) with β-glucosidase (β Glu) activity (Table 5.3). Amylolytic and proteolytic bacteria appeared mainly after the thermophilic phase, probably as result of the high amylose content, which was probably released after the biological degradation of protective substances such as cellulose, hemicellulose, and lignocellulose, and the large amount of proteins that are released when microorganisms die.

In this work most of the populations, during the maturing phase, had proteolytic, amylolytic, and cellulolytic capacities, corroborating the results from others authors (Diaz-Ravina *et al.*, 1989; Atkinson *et al.*, 1996). In agreement with this finding, Ishii *et al.* (2000) showed that Denaturation Gradient Gel Electrophoresis (DDGE) band patterns were more stable and more complex after cooling, indicating a higher microbial diversity. Because cellulose is an important constituent of grape pomace, the direct microbial biodegradation of cellulose will be restricted to a relatively narrow range of microorganisms, including cellulolytic bacteria, which appear frequently in this compost ecosystem (Hermann and Shann 1997).

Table 5.3. Correlation analysis among the compost evaluated variables.

	Date	AcP	AlkP	U	βglu	Bact	F&Y	Psol	Cellu	Proteo	Amyl	HA	FA	HS	HA/FA	pH	TotN	$N\text{-}NH_4$	$N\text{-}NO_3$	AvailN	$N\text{-}NH_4/N\text{-}NO_3$	TotC	C/N
Date	1,00																						
AcP	**-0,40**	1,00																					
AlkP	**-0,32**	**0,57**	1,00																				
U	**0,80**	**-0,33**	-0,19	1,00																			
βglu	**-0,32**	**0,24**	**0,23**	**-0,30**	1,00																		
Bact	**-0,61**	**0,48**	**0,24**	**-0,66**	**0,58**	1,00																	
F&Y	-0,08	**0,31**	**0,26**	-0,12	-0,13	0,18	1,00																
Psol	0,06	**0,21**	**0,24**	-0,03	**0,25**	**0,34**	**0,28**	1,00															
Cellu	**-0,46**	**0,40**	**0,39**	**-0,46**	**0,20**	**0,42**	0,15	0,19	1,00														
Proteo	**-0,36**	**0,28**	0,16	**-0,38**	**0,39**	**0,57**	**0,30**	**0,45**	**0,30**	1,00													
Amyl	0,02	**0,26**	**0,21**	-0,14	**0,21**	**0,44**	**0,33**	**0,53**	**0,47**	**0,42**	1,00												
HA	**0,20**	-0,18	**-0,21**	0,02	**0,39**	0,09	-0.15	0,12	-0,17	0,02	0,03	1,00											
FA	0,06	**-0,27**	-0,03	0,15	0,02	**-0,21**	**-0,41**	**-0,26**	-0,02	**-0,31**	**-0,21**	-0,03	1,00										
HS	0,16	**-0,33**	-0,13	0,14	**0,21**	-0,14	**-0,43**	-0,16	-0,10	**-0,26**	-0,17	**0,49**	**0,86**	1,00									
HA/FA	0,08	0,19	-0,17	-0,12	**0,28**	**0,32**	0,08	**0,26**	-0,08	**0,25**	**0,21**	**0,61**	**-0,63**	**-0,24**	1,00								
pH	-0,01	**-0,25**	**-0,34**	-0,02	**0,52**	0,11	**-0,50**	-0,11	**-0,33**	0,04	**-0,25**	**0,57**	**0,29**	**0,55**	**0,25**	1,00							
TotN	0,09	**-0,41**	**-0,23**	0,11	-0,17	**-0,23**	-0,12	-0,18	**-0,34**	-0,19	-0,14	0,15	0,19	**0,24**	-0,12	0,18	1,00						
$N\text{-}NH_4$	**0,41**	**-0,46**	**-0,31**	**0,70**	**-0,48**	**-0,71**	**-0,23**	**-0,27**	**-0,42**	**-0,44**	**-0,38**	-0,06	**0,22**	0,16	**-0,22**	0,01	**0,30**	1,00					
$N\text{-}NO_3$	-0,13	0,14	0,10	**-0,31**	**0,65**	**0,46**	**-0,30**	0,11	**0,20**	0,18	0,16	**0,38**	**0,36**	**0,51**	0,12	**0,59**	-0,16	**-0,51**	1,00				
AvailN	0,13	-0,15	-0,09	0,11	**0,43**	0,05	**-0,50**	-0,06	-0,05	-0,09	-0,07	**0,40**	**0,57**	**0,70**	-0,01	**0,68**	0,03	0,08	**0,81**	1,00			
$N\text{-}NH_4/N\text{-}NO_3$	**0,27**	**-0,29**	**-0,29**	**0,55**	**-0,35**	**-0,37**	0,11	-0,15	**-0,33**	**-0,31**	**-0,22**	0,04	-0,11	-0,08	0,01	-0,05	0,08	**0,64**	**-0,47**	-0,11	1,00		
TotC	-0,06	0,07	0,00	0,07	**-0,41**	-0,06	**0,32**	0,01	-0,16	0,06	-0,02	**-0,38**	**-0,25**	**-0,42**	-0,15	**-0,52**	**0,24**	0,15	**-0,59**	**-0,58**	0,17	1,00	
C/N	-0,12	**0,38**	0,18	-0,02	**-0,25**	0,10	**0,38**	0,14	0,11	0,19	0,09	**-0,43**	**-0,38**	**-0,55**	-0,02	**-0,61**	**-0,50**	-0,07	**-0,43**	**-0,55**	0,10	**0,72**	1,00

*Bold numbers are significant ($p<0.05$)

AcP: Acid Phosphatase; AlkP: Alkaline Phosphatase; Amyl: Amylolytic bacteria; Bact: Total Bacteria; β-Glu: β glucosidase; C/N: Carbon: Nitrogen Ratio; Cellul: Cellulolytic bacteria; FA; Fulvic acid; F&Y: Fungi and Yeast; HA: Humic acid; P sol: Phosphate Solubilizing Bacteria; HA/FA: Humic acid: Fulvic acid; Ratio; $N\text{-}NH_4/N/NO_3$:Ammonium: /Nitric Nitrogen ratio

5.1.4. Compost maturity indices

Germination tests are commonly used to assess the maturity and also the phytotoxicity of compost particularly associated with immature compost. A germination test is different to the growth test in which continuing changes in compost may affect the development of plants; it could represent damage at the beginning of composting, but a growth increase at the end. The germination test provides a fast answer about the presence of toxic substances in the material. Several organic substances, ammonia and heavy metals could be associated to phytotoxic response to compost (Ko *et al.*, 2008). In this work the heavy metal content was measured at the beginning of the maturing process, showing adequate concentrations according to the Chilean compost standards (Table 5.2). According to Zucconi *et al.* (1981), a germination evaluation below 50%, characterizes an immature compost; the present results showed germination over 80% in all treatments, indicating that the materials were free of substances that could reduce the seed germination or inhibit root development, such as ammonia, heavy metals and volatile organic acids (Ko *et al.*, 2008). With respect to heavy metal content, the Chilean national standard have set the maximum concentrations for Cd, Ni, As, Pb, Cr, Cu, and Zn, values of which are presented in Table 5.2. All compost treatments were well below to the limit levels proposed by the regulatory agency, and according to the maturity classification proposed by the Compost Council Quality of California CCQC (2001), the compost of treatment 5 (Table 5.3 and 5.5) showed to be very mature, with low levels of heavy metals and it could be used directly for soil and peat base container plant mixes and turf top-dressing.

Table 5.4. Comparison between the treatments evaluated in terms of the biochemical, chemical and microbiological parameters measured compost at final time, 120 days of composting.

T	βGlu	AcP	AlkP	U	Bact	F&Y	Psol	Cellul	Prot	Amyl	HA	FA	HA/FA	C/N	NH_4-N /NO_3-N
	----------µg p-nitrophenol $g^{-1}h$----------				---------- Log cfu g^{-1}----------						------mg g^{-1}------				
1	85,0	1720,8	2298,3	1051,7	5,9	7,2	5,8	6,4	5,9	6,5	1,3	8,8	0,1	15,0	3,0
2	76,2	420,1	1093,3	1109,5	6,4	5,2	5,8	6,8	5,9	6,7	3,4	9,9	0,4	15,9	2,4
3	62,4	1232,4	1809,1	949,8	5,3	8,1	6,2	5,6	6,5	6,2	0,9	8,1	0,1	17,6	0,6
4	133,7	328,7	1359,7	575,3	4,7	6,7	4,7	6,9	6,2	7,0	6,5	11,3	0,5	10,0	0,3
5	111,2	302,7	1240,1	1143,7	4,5	6,2	6,4	5,5	5,7	5,9	8,3	9,8	0,9	10,4	2,0
6	107,5	318,7	1453,4	1396,8	4,5	5,4	5,1	5,4	6,2	5,2	3,5	9,2	0,4	13,4	4,8
7	75,3	148,4	654,8	1749.,1	5,1	6,6	5,0	5,7	5,4	5,7	5,4	7,8	0,7	13,0	20,4
8	119,4	403,0	1633,8	1003.4	5,8	6,0	5,3	6,4	5,0	6,3	3,7	13,2	0,3	11,8	0,4
9	125,6	399,8	1354,7	1559,8	5,0	5,2	6,5	6,2	6,8	6,3	2,3	15,8	0,1	11,6	1,6
p*	0,0009	<0,0001	0,0124	<0,0001	<0,0001	0,0311	0,0404	0,0268	0,0383	0,0134	0,007	<0,0001	0,0005	0,006	<0,0001
LSD	28,8	325,8	651,9	160,7	0,3	1,5	1,1	0,9	0,9	0,7	3,6	2,3	0,3	3,2	0,9
RV**	180-200	4000-6200	200-270												

* ($p<0.05$)

β-Glu: β glucosidase; AcP: Acid Phosphatase; AlkP: Alkaline Phosphatase; U: Urease; Bact: Total Bacteria; F&Y: Fungi and Yeast; P sol: Phosphate Solubilizer Bacteria; Cellul: Cellulolytic bacteria; Prot: Proteolytic bacteria; Amylo: Amylolytic bacteria;HA: Humic acid-; Fulvic Acid; HA/FA: Humic acid: Fulvic acid Ratio; C/N: Carbon: Nitrogen Ratio; NH_4-N/NO_3-N :Ammonium Nitrogen/Nitric Nitrogen ratio.

**RV: Reference value for mature compost. *Castaldi et al., 2008, Bahacy and Kornillowicz, 2009.

Regarding nitrogen, total N content ranged between 1.8 and 2.6%, values similar to those reported by other authors for composted distillery residues (Ranalli, 2001); however the limits of NH_4-N in all treatments were below those suggested by Zucconi *et al.* (1981) for mature compost (400 mg g^{-1}). On the other hand, Bernal *et al*. (2009) indicated a NH_4-N/ NO_3-N ratio <0,16 for mature compost while a ratio <0.6 indicates stable compost (Bernal *et al.,* 1998). In this study the treatments 3,4 and 8 were closer to this proposed ratio; however, according to Brinton (2000) in a compilation of world compost standards, values of NH_4-N/ NO_3-N ratio <0.5 correspond to very mature compost, and ranges 0,5-3 to mature compost, in which case all treatments except treatment 7 were considered as mature compost. Throughout composting, occur nitrogen mineralization process. In the early stages, little or no nitrate-N is formed (depends on raw material) and in the thermophilic stage, the decomposition is faster, making the NH_4-N and NO_3-N appear from protein and other organic N sources.

Quantities over 50 ppm $N\text{-}NO_3$ can be an indicator of maturation of compost, as nitrification occurs until the levels of $N\text{-}NO_3$ exceed those of $N\text{-}NH_4$. Thus, with similar pH range, the $N\text{-}NH_4/N\text{-}NO_3$ ratio provides a useful parameter for defining compost maturity (Bernal *et al*., 1998). However, when the sum of ammonium and nitrate is less than 250 ppm in dry weight, this relationship does not provide a reliable measure of maturity, and may be associated, in contrast to excess nitrogen, and imbalance in the C/N in the original mixture (CCQC, 2001; Tiquia *et al*., 2002).

In general, the maturity of compost is related in part to the stability of the material and the presence of chemicals. Immature composts may contain high quantities of NH_3, organic acids and soluble organic substances that may limit seed germination and root development (Hue and Lui, 1995).

Stability refers to the degree of decomposition of organic matter determined by the complexity of the compounds; it is normally associated with the reduction of microbial activity, $C\text{-}CO_2$ and temperature (CEPA, 2002). The stability also is related to the potential effect of material on the availability of N and O_2 in the soil; in compost, nitrogen is more stable than on raw materials and therefore reduces the rate stabilization of organic N by soil microorganisms' mineralization, when applied as an amendment (Conti *et al*., 1997). At the same time mature compost reduce the risk of anaerobic conditions in soil and roots, because the microorganisms need less oxygen for the mineralization process (Mathur *et al*., 1993).

The C/N for the evaluated treatments was between 10,0 and 17,6, including the compost in type A or B according to Chilean standards, which are below the limit of 20, proposed by Golueke (1981). According to Rosen *et al*. (1993) a C/N ratio between 15 and 20 is ideal for ready-to-use compost, but depends on raw material. However the use of C/N ratio or total N content as indicators of compost maturity could not be the best, because these parameters are relatively stable along the maturity phase; however nitrification in terms of $N\text{-}NH_4/N\text{-}NO_3$ could be a better indicator. This ratio is associated to nitrification process; the $N\text{-}NO_{3^-}$ should be higher than $N\text{-}NH_4^+$ content, indicating the aerobic oxidation of NH_4^+.

5.1.5. Enzymatic activities and humic and fulvic acids as maturity indicators

Compost maturity can be assessed by its microbial stability, by determining microbial activity factors: biomass, count, metabolic activity, and concentration of easily biodegradable compounds. Aerobic respiration assesses the aerobic activity and stability because under these conditions the carbon derived from catabolism is attached to oxygen and produces CO_2, energy and heat; these products are high when the activity is high and when the maturity is not complete (Hue and Lui, 1995).

During the active phase on the composting process, organic C decreases in the material due to decomposition of the organic matter by microorganisms, but the degradation rate of the organic matter (OM) decreases gradually as composting progresses because the reductions of new complex and polymerized organic compounds (humification) that occur during the maturation phase (Bernal *et al.,* 2009). Important enzymes are involved in this biochemical process associated to C substrates; among them, the cellulase complex, β-glucosidase, which hydrolyses glucosides, proteases, and ureases, associated to N mineralization, and phosphatases that remove phosphate groups from organic matter (Nannipieri 2002; Aira *et al*., 2007). The process offers stabilized end-products which can be used as C storage and slow release fertilizers for agricultural purposes.

The enzymes are associated with biochemical indicators of decomposition of organic materials, confirming to be highly sensitive variables to changes in compost maturity. The presence of high content of degradable compounds in the initial mixture may have stimulated microbial

growth and enzyme synthesis that will be limited by the presence of substrate in biochemical reactions (Goyal *et al*., 2005). The mineralization process of organic matter can be studied by following the dynamics of enzymes over time and correlating it with other factors such as water soluble carbon, humic and fulvic acids concentration or the presence of microbial groups.

β-glucosidase is an extracellular enzyme associated to hydrolysis of terminations of β-D-glucose chains to yield β-glucose (Nannipieri *et al.*, 2002). This enzymatic activity decreases, during the composting process and, when the activity is high, it indicates the low stabilization process of the decomposing material (Castaldi *et al*., 2008); this decrease was evidenced in all the treatments ($p<0.0001$) evaluated (Figure 5.4), indicating the use of polysaccharides and the adequate stabilization phase.

Phosphomonoesterases catalyze the release of inorganic phosphorus (orthophosphate) from organic phosphomonesters (Alef et al., 1995) and are inhibited by substrate; for this, and due to the decrease of the organic induced by the phosphatases, in compost process, the phosphatases enzymes tended to decrease (López-Hernández *et al.*, 1989); this decrease was observed in almost all the treatments ($p<0.0001$) (Figure 5.4).

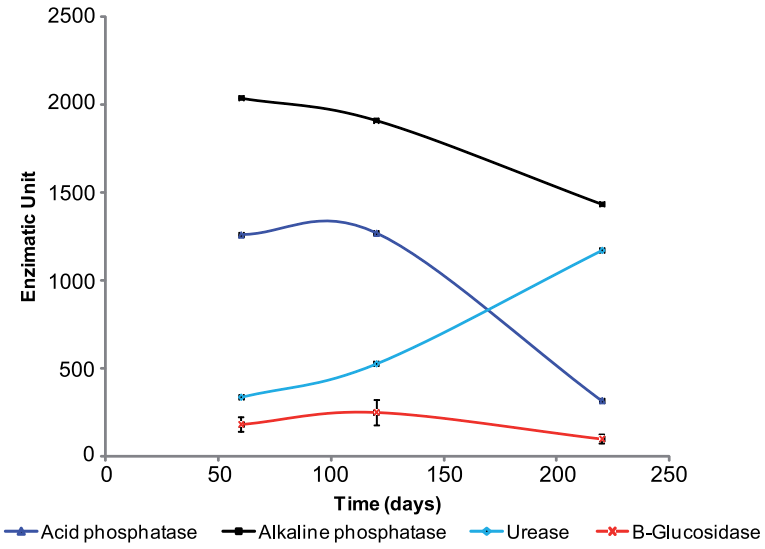


Figure 5.4. Enzymatic activities behavior along the composting process presented by all the different pile treatments evaluated in average.

*Enzymatic Unit: Acid phosphatase (UP: µg p-nitrophenol*g-1*h-1). Alkaline phosphatase (UPAlk: µg p-nitrophenol*g-1*h-1). β-glucosidase (UBG: µg p-nitrophenol*g-1*h-1). Urease (UU: µg NH_4*g-1*h-1).

The urease activity is related with the N metabolism because it hydrolyzes urea to release NH_4^+ (Makoi and Ndakidemi, 2008). This enzyme tends to increase at the beginning of the process because the initial high concentration of proteins induces the protease activity, releasing urea, and consequently induce the ureases enzymes. Typically, during the composting process urease activity presents fluctuations of decrease-increase due to the depletion of the substrate urea by ureases and the synthesis of urea as a result of the proteases; it is also reported a positive correlation between urease and NH_4^+ and negative correlation between urease and NO_3^- (Castaldi *et al.,* 2008). In this co-composting process, urease tended to increase along the stabilization phase ($p<0,0001$) (Figure 5.4).

The N was <2% in treatments 3, 5, 6 and 8, corresponding to grape pomace (91%); grape pomace: goat manure (50:50 and 33:73) and in addition with horse manure (33:20:42); in these cases, the addition of manure provided an initial C / N near 20-25:1, which made the process of proteolysis and subsequent mineralization of N resulting in a lower concentration of NH_4-N, compared to other treatments. This nitrification process favors the immobilization of N in the material, and is reflected in a NH_4-N / NO_3-N ratio <1. Also there is less free substrate for the enzyme urease, which is reflected in lower activity of this enzyme in these treatments (Castaldi *et al.,* 2008).

The residual organic matter is transformed by microorganisms to form humic-like substances, which form complexes with extracellular enzymes stabilizing them, and preventing their degradation and denaturation (Chen *et al.,* 1997, Burns *et al.*, 1972). Additionally, the microbial community, with different physiological profiles, is used as indicator of compost maturity. The decreasing trend of microbial biomass throughout the composting process is normal, and it is associated to temperature and C/N source consumption (Klamer and Baath, 1998); the fungal biomass decreases during the active phase and maturation stage compared to initial compost, but the bacteria continue with degradation activity, enzymatic production and the humification process. Mondini *et al.* (2004), demonstrated the relationship between enzymatic activity and quality of organic matter, and indicated that the humic-enzyme complexes should be considered as a process directly related with compost stability. At the same time during the maturity phase, the CO_2 evolution, fulvic acids, and NH_4 concentration decrease, while nitrification process increase the concentration of NO_3, showing a nitrification index <0,16 (Hue and Liu, 1995; Bernal *et al.,* 2009); the concentration of humic acid also increases. At the end of the process, and previous to the application of compost, the phytotoxicity test, heavy metal contents, and evaluation of presence of human pathogens and fecal coliforms, could be a good complement to define a safe agronomical use.

The concentration of humic acid (HA) increased gradually over the first 40 days of maturation process ($p<0,0001$), but also the fulvic acids concentration increased during the same period ($p<0,0001$) (Figure 5.5). In this study the behavior of humic substances was similar to that described by Bernal et al. (2009) using compost from animal manure; fresh and raw material contain lower levels of HA and higher contents of fulvic acids, compared to mature compost. However these contents could be variable depending on the source of raw material, therefore it would better to use humification indices rather than humic substances content as indicators of compost maturity. Jimenez and Garcia (1992), proposed the ratio HA/FA (humic acid content to fulvic acid content) as a parameter related to degree of compost maturity, and considered that a value higher than 1,6 indicates a mature compost. In this research, fresh compost made of grape pomace and goat manure, showed a ratio HA/FA< 0,5, which could be a suggested limit for immature compost, and the ratio increased at the end of the study (Table 5.4).

During composting HA contents of the raw material (grape pomace and goat manure) evolved and became over FA. HA/FA ratio in pile No.5 increased to over 70%, suggesting that the degree of humification in this pile was higher than others.

This results are coincident with the results obtained by Tiquia (2005), who also demonstrated that besides the mineralization of organic matter, in compost, this can be transformed into humic substances (HS), in correlation with respiration rate and oxidizing activity. In the present study the increase in humic acids concentration observed from 100 days during maturity, coincides with that of stabilization of β-glucosidase and acid phosphatases (Figure 5.4 and 5.5). However, during the later composting process (maturity) fulvic acid increased and humic acid decrease this could mean that the fraction of humic acid is used by microorganisms, probably fungi, through enzymatic mechanisms different to hydrolytic pathways.

Grinhut *et al.* (2007), proposed a model for humic substances degradation, in which lacases and lignin-peroxidase (LiP) attack directly the HS side chains and Mn and Cu chelates produced by basidiomycetes, could be mineralized humic substances. *Trametes* sp., a basidiomycete, isolated from biosolids compost showed the ability to degrade HS from leonardite, considered very stable OM. In compost as well as in soil, during humification processes a large heterogeneity of substances are formed, in terms of their source, physical, chemical characteristics and variability in time and space (Guggenberger, 2005)

Different characteristics are defined to define the compost quality, are used, especially chemical and microbial properties are include to define the classification and the use in yield of this material. However, it is possible use different organic material, but for horticulture systems the human pathogen and fecal indicators, could define the use. For this reasons, the compost

obtained from treatment No. 5 was selected to continue the pots experiment and the extraction of humic substances, because showed high concentration of humic acid (6,35 % HA, Table 5.2) with HA/FA ratio of 0,27 (Table 5.4) and MPN $100g^{-1}$ of fecal coliforms and 100% of germination.

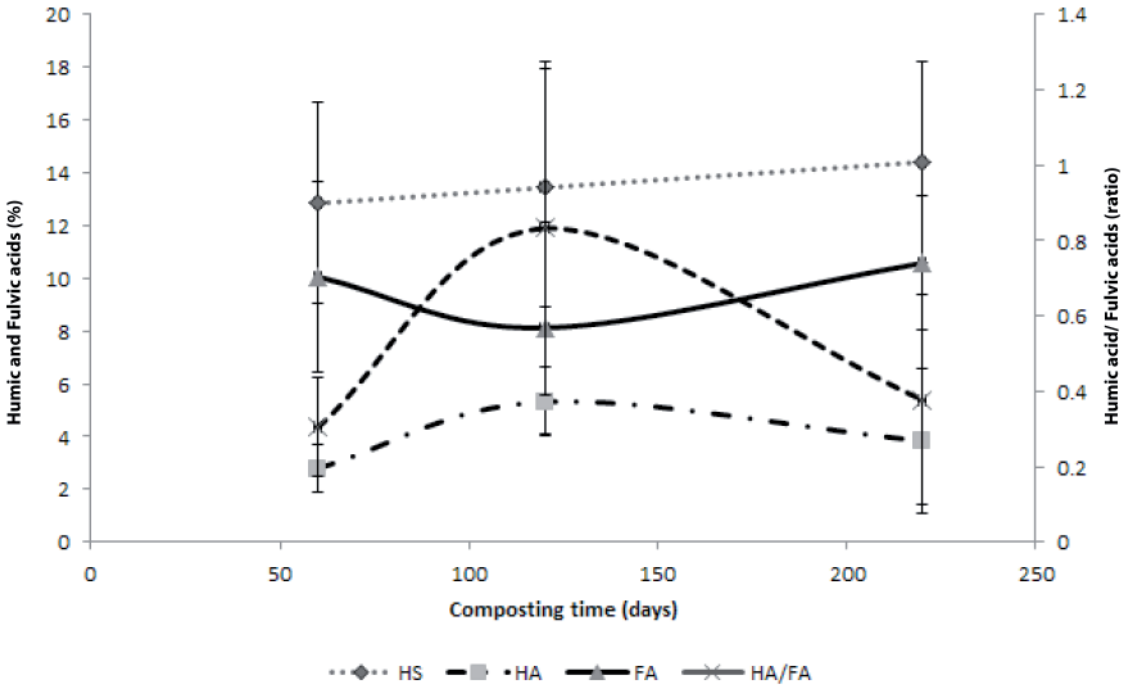


Figure 5.5. Humic acid (HA), Fulvic acid (FA), humic substances (HS) and HA/FA ratio evolution during the composting process (average of 9 treatments)

On the other hand, elemental analysis of HS using the optical densities of HS solutions, measuring the ratio E4/ E6, showed results in the same range as those obtained by other authors (Stevenson, 1994). Treatment 5 and 9 had the lowest E4/E6 ratio indicating the presence of low proportion of aliphatic compounds, and high degree of aromatic C network content (Fig. 5.6).

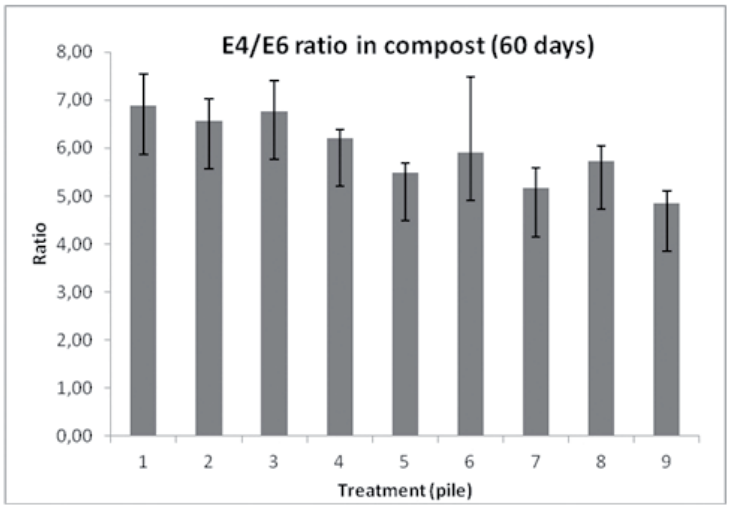


Fig. 5.6. E4/E6 ratio in compost after 60 days

The magnitude of this ratio is related to the degree of condensation of the aromatic C network; with a low ratio indicating a relatively high degree of condensation of aromatic humic components. Conversely, E_4/E_6, high, reflects a low degree of condensation and suggests presence of aliphatic compounds (Velasco *et al.*, 2004).

Compost used to obtain humus liquid, was the product of treatment 5, for presenting a greater degree of polymerization, absence of human pathogens and fecal indicators, and 100% germination in radish seed test, indicating the absence of phytotoxic substances.

5.1.6. Sensitivity of of each property for representing changes during maturation process

Normally, chemical parameters related to nutrient content, are applied as quality indicators in compost used in agriculture, however it is important to define these indicators, according to easy of interpretation, analysis, reference, and consistency during the processes (Gómez-Brandón *et al*., 2008); to the former group of desired characteristics I wanted to include the sensitivity of each indicator. Biochemical indicators, as enzymatic activities, and concentration of humic substances play an important and sensible role in monitoring the process of mineralization and maturity in compost, but it is important to adjust and standardize the methodologies in order to make the results comparable.

The content of humic substances (HS) can be a good indicator not only of the maturity of the compost but also of the potential of it as an organic amendment. Compost containing more humic substances would be a better stabilized amendment to apply to the soil.

Changes of each measured compost soil property on each treatment, with respect to the mineralization process (day 60), were determined. The objectives of this analysis were to the end of the mineralization describe the effect of treatments on several soil properties and to determine their weights which could be used in a maturity index.

Each of the 9 treatments was considered a population, to determine the proportion of them presenting changes in a given property over time (as compared to the base line). On the other hand, the weight (slope) of each property over time was estimated, in order to rank all the properties in terms of its sensitivity. Standardized variables were used and regressed on 4 times: 0, 120, and 180 days. When the slope was not significantly different from zero (P>0,05) it meant that there were not changes in time over the base line and the property was not sensitive in a given treatment. On the other hand the larger the coefficient (slope), the higher the weight of the property (Table 5.5).

The regression of each standardized variable on time in each treatment determined that the compost properties changing most frequently and with larger weights were urease (U), acid phosphatases (AcP), cellulolytic microorganisms (Cellul), acid phosphatases (AcP), NH_4^+ and $N\text{-}NH_4/N\text{-}NO_3$ ratio. Urease changed over the first sampling time (mineralization process) in 89% of the 9 populations (treatments) evaluated (Table 5.6), with 0,12 as corrected weight (Fig. 5.7). Several properties changing less frequently and with lower weights which mean that they would not be good indicators of changes in compost maturity.

Table 5.5. Ranking of all standardized compost variables measured during maturation process

Pile	AcP	AlkP	U	β-Glu	Bact	F&Y	Psol	Cellul	Prot	Amyl	HS	pH	N	NH_4	NO_3	NH_4/NO_3	C	C/N
1	0	0	1	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0
2	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0
3	0	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0	1	0
4	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1
5	1	0	1	1	0	0	0	1	0	0	1	0	1	1	0	1	1	1
6	1	0	1	0	1	0	0	1	0	0	0	0	0	1	1	1	0	0
7	1	1	1	1	0	0	0	0	1	0	0	0	0	1	0	1	0	0
8	0	0	1	0	0	0	0	1	0	0	0	0	0	1	0	1	0	0
9	0	0	1	0	1	0	1	0	1	0	0	0	1	0	0	0	0	0
Sum	5	4	8	3	3	2	2	5	2	1	2	0	2	5	1	4	3	2
%	56	44	89	33	33	22	22	56	22	11	22	0	22	56	11	44	33	22

1: property changes along the time 0: no change. Use four times (0, 6,12,18 months)

β-Glu: β glucosidase; AcP: Acid Phosphatases; AlkP: Alkaline Phosphatases; U: Urease; Bact: Total Bacteria; F&Y: Fungi and Yeast; P sol: Phosphate Solubilizer Bacteria; Cellul: Cellulolytic bacteria; Prot: Proteolytic bacteria; Amyl: Amylolytic bacteria; HA: Humic acid-; Fulvic Acid; HA/FA: Humic acid: Fulvic acid Ratio; C/N: Carbon: Nitrogen Ratio; NH_4: Ammonium Nitrogen; NO_3: Nitric Nitrogen; NH_4-N/NO_3-N :Ammonium Nitrogen/ Nitric Nitrogen ratio

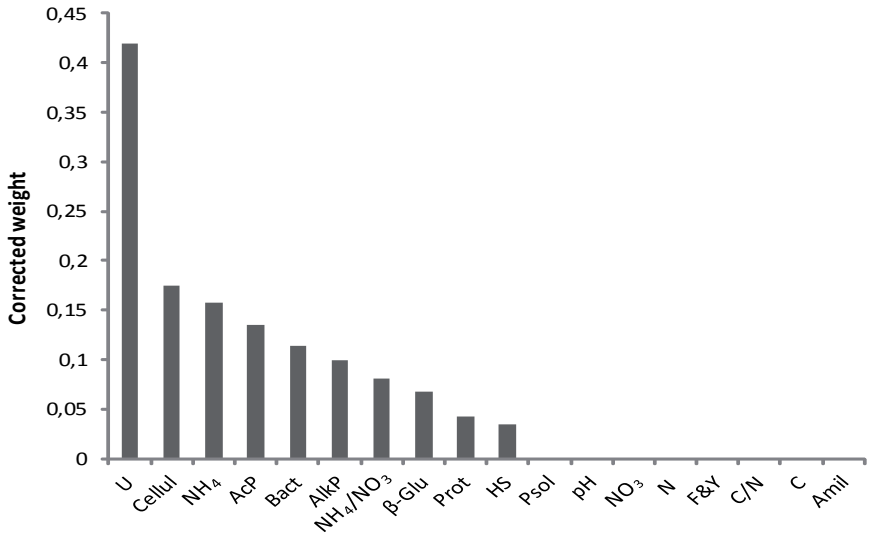


Figure. 5.7 Corrected weights of compost properties obtained by regression and frequency response analysis.

U: urease activity, Cellul: cellulolytic microorganisms N-NH_4: Ammonium nitrogen, AcP: acid phosphatases activity, Bact:total bacteria; AlkP: alkaline phosphatase activity , NH_4/NO_3: ratio, βGlu: β-glucosidase activity, prot: proteolytic microorganisms; HS: humic + fulvic acids, F&Y: Fungi and yeasts, Psol: Phosphate solubilizer bacteria; Amil: amilolytic microorganisms, N: total nitrogen; N-NO_3: Nitric nitrogen C: Organic Carbon; C/N ratio.

Several models were proposed as a Minumum Data Set (MDS) to explain the content of humic substances in compost using microbiological, chemical and biochemical properties. Selected models explained between 34 to 61% of the variation of humic substances and its ratio. The same models were run using standardized variables which allowed determining the weights of each variable included in the model. Overall the most important ones were available N, and pH, among the chemical properties, cellulolytic and P-solubilizer bacteria, among the microbiological characteristics, and acid phosphatase and β- Glucosidase, among the biochemical properties (Table 5.6).

Table 5.6. Selected models[1], for humic substances based on biochemical properties.

	HA	FA	HS
Intercept	0,00	0,00	0,00
AcP			-0,18
Bact		-0,37	
Avail. N		0,61	0,59
$N\text{-}NH_4/N\text{-}NO_3$		-0,22	
Amilo	0,21		
pH	0,65		
C/N			-0,19
F	25,3	24,2	39,9
R^2	0,39	0,49	0,61

*all coefficients but the intercept are significant at $p<0,05$.[1] using step wise procedure
HA: humic acids; FA: fulvic acids; HS: humic substances

Humic substances content can be considered as an overall indicator of the composting process. It can explain mineralization of organic material, as well as maturation and humification processes. In this case it was also considered as a quality parameter since I was looking for the treatment that yielded the most humic substances content in order to extract them to produce liquid C as humic extract.

5.2. Evaluation of C rates in pots

5.2.1. Quality of the organic materials used

Organic fertilization in Chile is based on the application of products or waste of productive activities, which can be classified by origin on: byproduct of animal waste, sewage treatment and industrial liquid byproducts of the industry or productive activities (Hirzel and Salazar, 2011).

The applications of organic products are in the form of semi-composted manure of poultry, hog and cattle, and sewage sludge, although the latter is not allowed for horticultural use (Hirzel and Salazar, 2011). The amount of amendments applied to the table grape plants is, usually, between 10 and 15 Ton ha^{-1} of manure, which contribute with extra nutrient supply, particularly nitrogen, phosphorous, and potassium, which are normally not considered in the nutritional balance. For a production of 30 Ton ha^{-1} of table grape, which corresponds to approximately 2000 exporting boxes/ha, the table grape plants require about 90 kg N ha^{-1}, 30 kg P_2O_5 ha^{-1}, and 120 kg K_2O_5 ha^{-1} (Ortega 2012, personal communication).

For this experiment the compost produced from co-composting grape marc and goat and horse manure, showed physical, chemical, biochemical, and toxicological characteristics that allowed its classification as compost type A, according to the Chilean standard for compost NCh 2880/04 (INN, 2004). On the other hand, as compared to commercial humic acid derived from leonardite, liquid humus extracted from compost, presented lower C content, but similar pH and EC values; the latter are common to extractions with strong bases; in order to avoid physicochemical instability (precipitation of water mixtures) and to intend preventing EC damage to plant, liquid humus was diluted 10 times before application.

The soil used had low organic matter content and medium-low fertility, typical of inceptisols from semi-arid regions (Table 5.7).

Table 5.7. Chemical and biochemical characteristics of soil, compost and liquid humus used for the pot experiment.

Parameter	Unit	Soil	Compost	Liquid Humus
Acid Phosphatase (AcP)	µg p-nitrophenol $g^{-1}h^{-1}$	203	849	3
Alkaline Phosphatase (AlkP)	µg p-nitrophenol $g^{-1}h^{-1}$	185	1338	33
B-glucosidase (βGlu)	µg p-nitrophenol $g^{-1}h^{-1}$	78	238	ND
Urease (U)	µg NH_4 $g^{-1}h^{-1}$	84	221	14
Humic substances (HS:HA+FA)	%	0,54	5	2,34
pH		8,1	8,6	12,2
EC	dS m^{-1}	0,7	0,88	29,6
$N\text{-}NH_4$	mg kg^{-1}	8,0	72	49
$N\text{-}NO_3$	mg kg^{-1}	5,0	309	43
Olsen P	mg kg^{-1}	7,0	55,6	-
Total P	mg kg^{-1}	13	-	3,93
K sol	mg ml^{-1}	-	-	9,7
Organic Matter (OM)	%	1,4	44,5	0,80
Organic C (OC)	%	1,5	24,7	0,25
Total N (TN)	%	0,1	1,8	0,04
C/N ratio		11,2	13,4	6,25

Olsen-P: Phosphorus Olsen;N; $N\text{-}NH_4$: Ammonium nitrogen $N\text{-}NO_3$: Nitric Nitrogen; Avail N: Available N; HS: Humic substances (HA+FA) :): OM: organic matter; WSC: Water Soluble Carbon; EC: electrical conductivity

5.2.2. Effect of organic matter application on soil properties

Regarding chemical parameters (Table 5.8), soil pH and electrical conductivity (EC) presented significant differences ($p<0,05$) among treatments; as expected, treatments with liquid humus had higher pH and EC values compared to those with compost; however, at the end of the experiment the maximum EC values were slightly lower than for the control with no organic matter. The increase in EC values, observed at the beginning of the experiment in the liquid humus, caused a significant reduction on plant shoot and root growth, as well as microbial activity, which could not be recovered during the season, even though soil was heavily washed to reduce EC. For this reason the effect of liquid humus will be evaluated only over soil chemical properties and not on biochemical or plant evaluations.

Soil content of humic and fulvic acids (HS) showed to be higher ($p<0,05$) in all treatments in comparison with the control, particularly when the maximum level of C was added. $N\text{-}NO_3$ was also significant ($p<0,05$), showing higher values in compost treatments.

Table 5.8. Effect of the applied treatments on soil chemical properties[1] - pot experiment

Treatment	Olsen-P	$N\text{-}NH_4$	$N\text{-}NO_3$	Avail. N	HS	OM	WSC	pH	EC
	----------mg kg^{-1}----------				----------%----------		mg kg^{-1}		dS m^{-1}
1	24,99±4,9	4,33±4,0	2,43±0,3	6,76±4,1	0,28±0,1	0,69±0,0	15,9±1,2	7,02±0,7	0,16±0,1
2	33,02±13,2	2,47±1,3	3,06±1,0	5,54±2,3	0,65±0,2	1,6±0,3	21,5±6,6	7,28±0,0	0,13±0,0
3	22,59±2,4	5,17±0,8	2,2±1,6	7,37±0,8	1,31±0,4	2,61±0,7	25,0±6,2	7,23±0,4	0,12±0,0
4	25,66±4,7	1,69±0,8	2,16±1,2	3,86±0,9	1,65±0,3	3,04±1,2	22,2±4,3	7,86±0,8	0,17±0,0
5	24,16±9,6	9,08±9,5	3,16±2,2	12,25±10,9	0,43±0,1	0,93±0,2	15,9±1,2	7,20±0,3	0,22±0,1
6	28,19±11,4	8,88±10,7	3,33±1,2	12,21±10,9	0,99±0,2	1,47±0,7	18,7±2,0	6,95±0,1	0,21±0,1
7	26,54±10,6	2,9±0,7	3,1±0,8	6,01±1,6	1,61±0,3	2,49±0,5	27,08±4,1	7,02±0,2	0,14±0.0
8	18,79±8,3	6,71±5,6	3,06±1,6	9,77±7,0	2,29±0,9	3,20±0,5	28,5±5,2	7,21±0,3	0,16±0.0
9	15,36±5,8	1,09±1,0	1,06±0,4	2,16±0,9	0,33±0,0	0,52±0,0	12,5±2,0	8,18±0,1	0,18±0.0
10	16,95±7,4	2,68±1,6	1,56±0,4	4,24±2,0	0,42±0,0	0,60±0,1	14,5±5,5	8,63±0,4	0,22±0.1
11	24,3±22,6	0,63±0,5	1,9±1,1	2,56±1,6	0,40±0,1	0,60±0,0	16,67±2,0	8,81±0,4	0,31±0.1
12	12,0±7,0	1,16±1,4	1,83±0,6	2,99±1,5	0,44±0,1	0,61±0,0	15,97±5,2	8,9±0,08	0,23±0.0
13	14,6±7,0	2,8±2,9	1,13±0,5	3,93±3,0	0,36±0,1	0,66±0,1	12,5±2,0	8,09±0,01	0,22±0.0
14	24,2±19,6	1,12±0,7	0,93±0,3	2,05±0,9	0,35±0,0	0,64±0,1	11,1±5,2	8,4±0,1	0,18±0.0
15	26,11±2,2	2,71±1,5	1,46±0,4	4,19±1,6	0,41±0,1	0,5±0,04	18,06±4,3	8,94±0,5	0,34±0,2
16	11,83±5,7	1,55±1,2	0,6±0,3	2,15±0,9	0,43±0,1	0,66±0,17	17,36±3,1	9,01±0,4	0,52±0,0
17	11,12±2,6	3,23±1,5	0,66±0,3	3,89±1,8	0,23±0,1	0,67±0,1	13,19±3,4	7,45±0,5	0,17±0,0
18	15,7±2,7	1,72±1,4	1,23±0,8	2,95±2,2	0,35±0,1	0,56±0,12	12,5±2,8	7,58±0,5	0,17±0,0
LSD2_TR	Ns	Ns	1,73	Ns	0,50	0,74	6,67	0,91	0,19
LSD3_OM	5,80	2,76	0,59	2,58	0,31	0,44	2,95	0,32	0,076
LSD4_IN	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns	Ns

[1]Variation is expressed as standard deviation. Olsen-P: Phosphorus Olsen; $N\text{-}NH_4$: Ammonium nitrogen $N\text{-}NO_3$: Nitric Nitrogen; Avail N: Available N; HS: Humic substances (HA+FA): OM: organic matter; WSC: Water Soluble Carbon; EC: electrical conductivity.
[2] LSD: Least significant difference; 3Compares compost vs. liquid humus; 4 compares inoculated vs. non inoculated
Ns: not significant (p>0,05)

Meanwhile, soil organic matter (SOM) showed an increase with C rate only on those treatments with compost (Figure 5.8a), while for those with liquid humus (humic extracted) it remained about constant (Figure 5.8b); however, in terms of water soluble carbon (WSC), both evaluated materials increased its content in soil with C rate (Figure 5.9). Interestingly, the slope of the curve of WSC increase with C rate was larger for liquid humus (0,048, Figure 5.9b) than for compost (0,0257, Figure 5.9a), meaning that humic and carbon substances extracted would be more efficient than compost to provide C for soil microorganisms, especially soluble C. This would be a logical finding since the compost extraction with a strong base will allow obtaining a significant amount of WSC as compared to compost applied as it is.

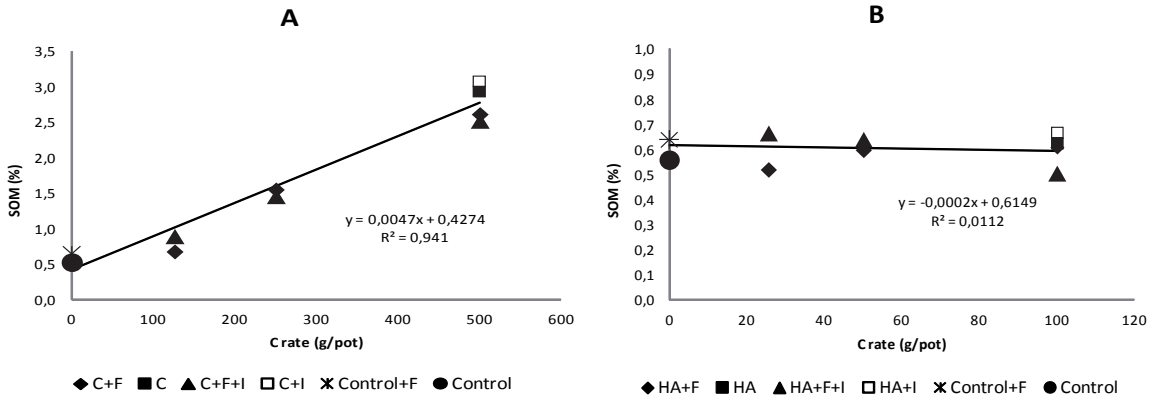


Figure 5.8. Variation of Soil Organic Matter (SOM) on pot experiment, as function of C rate applied: A) Compost and B) Liquid humus.

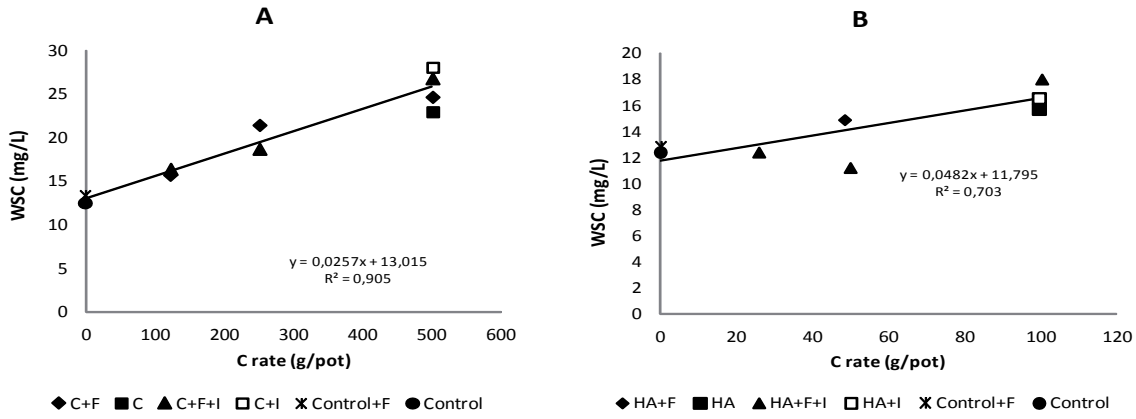


Figure 5.9. Variation of Water Soluble Carbon (WSC) in soil, as function of C rate applied: A) Compost and B) Liquid humus.

C+F: compost and chemical fertilization; C+F+I: compost, chemical fertilization and microbial inoculant; C+I: compost and microbial inoculant; HS: humic substances; HS+F+I: humic substances, chemical fertilization and microbial inoculant, HS+I: humic substances plus microbial inoculant, C+F: control plus chemical fertilization; C:control.

5.2.2.1. Effect of Compost application on Biochemical Properties

Biochemical analysis revealed that all compost treatments increased the activity of β-glucosidase, and acid phosphatases ($p<0,05$), compared with control (Table 5.10). On the other hand, urease activity showed a different pattern been decreased at the highest C rates from compost and liquid humus ($p<0,05$). The presence of inoculant increased activity of β-glucosidase and decreased that of urease (Table 5.10).

Table 5.9. Effect of compost applications on soil enzymatic activities[1]

Treatment	β-Glucosidase (β Glu)	Acid phosphatase (AcP)	Alkaline phosphatase (AlkP)	Urease (U)
	----------µg p-nitrophenol $g^{-1}h^{-1}$----------			µg NH_4 $g^{-1}h^{-1}$
1	45,8±22,1	117,9±34,9	184,3±95,3	119.3±40,0
2	49,3±11,1	164,1±31,5	264,5±70,1	222.7±45,8
3	69,8±42,4	269,5±44,0	372,6±151,7	230.7±103,0
4	49,6±38,9	329,0±167,1	634,2±526,5	201.5±33,1
5	50,2±16,6	117,1±8,4	304,0±163,4	146.5±57,1
6	49,6±2,7	150,8±73,4	206,7±93,2	168.8±44,4
7	65,2±5,9	251,4±47,3	366,0±75,5	151.1±8,0
8	69,6±19,7	369,0±149,4	415,7±208,1	165.7±56,3
17	26,74±8,0	138,7±47,6	219,2±51,1	258.3±38,6
18	47,5±9,0	158,7±26,1	205,2±17,0	229.6±13,5
LSD2_TR	36,69	185,29	Ns	Ns
LSD3_OM	18,91	112,9	Ns	Ns
LSD4_IN	20,54	ns	Ns	34,5

[1] Variation is expressed as standard deviation

[2]LSD: Least significant difference; [3]Compares compost vs. control; [4] compares inoculated vs. non inoculated

Compost application stimulates biological activity by mineralizing it, increasing the levels of soil available N and P and of some micro elements, depending on the source. In response to biological activity, it is reported that compost generates an increment of soil microbial biomass, soil respiration, and enzyme activities such as phospho-, mono- and di estereasesesterase, dehydrogenases, β-glucosidases, arylsuphatases, deaminases, ureases and proteases; however, some cases a decrease on protease, urease and deaminase activities is observed due to a toxic effect caused by the presence of trace elements (Heargreaves *et al.*, 2008).

In the present study, a significant increase on β-glucosidase, acid and alkaline phospatasephosphatases activities was showed in compost treatments. Sources of C as cellulose, lignin, starch, N as proteins, organic P and other nutrients, present in compost stimulate biological activity and continues mineralization process; in the case of humic extract the main C sources are polyphenolic compounds with low availability and mineralization rate (Anderson, 1979), although WSC can also be an important C source for soil microorganisms. Probably, the application of liquid humus at the proper dilution would have caused the desired effects, including stimulating microbiological populations and improving enzymatic activity. It is worth noting that soil enzymes can be protected by humic substances and its activity potential depends on pH (Burns, 1978); under the conditions of this experiment a strong negative correlation between pH and all enzymatic activities evaluated was observed.

Urease activity has to be analyzed carefully because this activity can be very variable because it is affected by the presence of trace elements, oxygen concentration, and N availability. Soils with permanent availability of organic or inorganic sources of NH_4, reduce significantly their urease activity; the use of urea as fertilizer in agricultural soils can cause interference on the urease laboratory determination (García *et al.,* 2003). The results of this experiment indicated a decrease on urease activity due to an inhibition by product, because compost and chemical fertilizers maintained N levels in soil causing a depression in urease activity.

5.2.2.2. Effect of compost application on Microbial Population

Microbiological analysis revealed no significant effect of treatments on populations evaluated, except on yeasts ($p<0,0001$), where compost at minimum C level, had the highest yeasts concentration. On the other hand, the presence of inoculant tended to increase fungi populations (Table 5.10).

Table 5.10. Effect of compost applications on microbial populations.

Treatment	Psol	Actinomycetes (Acty)	Fungi (F)	Yeasts(Y)
	----------Log10 UFC g^{-1}----------			
1	6,4±0,3	6,0±0,2	6,3±0,3	6,5±0,3
2	6,2±0,5	6,0±0,2	6,2±0,3	6,5±0,4
3	6,3±0,2	6,2±0,2	6,2±0,3	6,2±0,5
4	5,2±1,4	5,7±0,6	5,8±0,8	7,4±0,3
5	6,4±0,3	5,8±0,4	6,7±0,6	6,4±0,6
6	6,2±0,3	6,6±0,3	6,3±0,2	6,7±0,4
7	6,7±0,2	6,2±0,3	6,6±0,1	6,1±0,3
8	6,4±0,2	6,6±0,3	6,5±0,4	5,8±0,2
17	6,3±0,0	6,1±0,1	6,3±0,3	6,0±0,3
18	6,4±0,4	5,9±0,4	6,7±0,2	6,5±0,4
LSD2_TR	Ns	0.53	Ns	0,65
LSD3_OM	Ns	Ns	Ns	0,54
LSD4_IN	Ns	Ns	0,31	Ns

[1] Psol: Phosphate solubilizer bacteria. Variation is expressed as standard deviation.
[2]Least significant difference; [3]Compares compost vs. control; [4] compares inoculated vs. non inoculated

Addition of organic matter into the soil enhances microbial diversity as well as it biomass; numerous authors had demonstrated the increase in functional groups as mycorrhizal fungi and beneficial rhizosphere bacteria (Heargreaves *et al.,* 2008). Visser (1985) found that the presence of humic acids at concentrations of up to 30 mg L^{-1} normally resulted in increased numbers of soil active microbes. Observed increases could be as much as 2000-fold. Microbes in a humus-rich organic soil were more stimulated by humic substances than organisms from a sandy soil.

Organic matter content is an important factor influencing microbial population, particularly the labile and organic sources of C, P, and N; from them, soil microbes construct aggregates and can proliferate within the soil ecosystem (Magdoff and Wiel, 2004). The benefit associated to microbial diversity is the plant growth promotion, due to several factors such as direct phytohormone production, or indirectly by mineralization of organic matter or improvement of soil conditions; there is another important indirect way which contributes to promoting the plant growth: the suppression of plant pathogens and the systemic induced resistance that protect the plants of potential pathologies and pests (Magdoff and Wiel, 2004).

Particularly in vineyards, yeasts populations are very important because they strongly influence the grape quality, due to the fact that compose major part of the natural *terroir*, which is correlated to fruit quality and also with nutrient and organic matter content, among other climate and geographical factors (Probst and Schüler, 2008). The results obtained suggest that treatments had a significant effect on yeast population (Table 5.11), indicating that high C rates depressed its population, probably by competence for substrate and space between native *terroir* yeasts and microbes added with compost; in the case of liquid humus, it is possible that the high pH and EC, particularly at high C rates could have suppressed yeasts populations.

Regarding the reduction of microbial population in those treatments receiving liquid humus in

comparison to the control treatment, it could be due to the EC effects previously described; however it has been reported that humus-like substances extracted from compost seem to exert higher stimulated effects on microbial rhizosphere and vegetative biomass production (Valdrigh *et al.,* 1995, Avis *et al.,* 2008).

5.2.2.3. Effect of compost application on agronomic variables: Effect on root development

Results indicate that the treatment including compost, microbial inoculant and chemical fertilizers (Treatment 5), generated the best root production; Root density showed a clear tendency ($p<0,12$) to increase with compost rate (Fig 5.10).

Fig. 5.10. General aspect and detail of the pot experiment.

a) Organization of the pots in the field. b) Root density (roots 2a.) c) root mass after washing.

The application of inoculant improved this effect as treatments including it showed larger root density (Figure 5.11). Similar results were observed for the increment on cross sectional shoot area, ISCSA (data not shown).

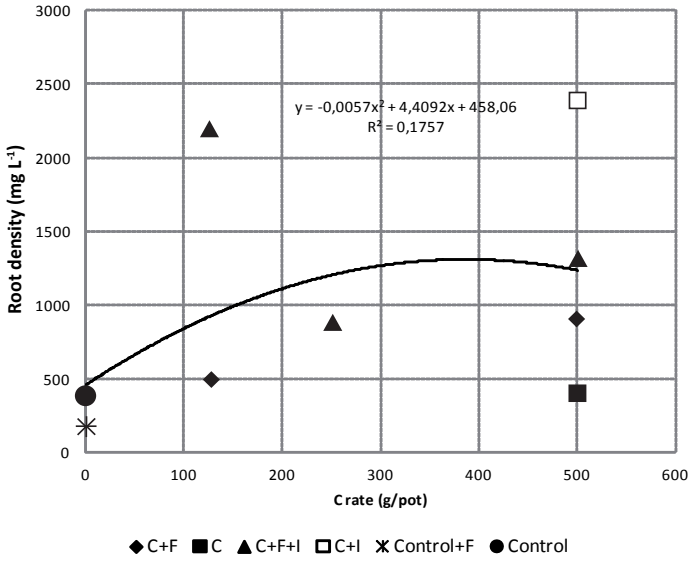


Figure 5.11. Root density as function of C rate from compost (C) in presence or absence of chemical fertilization (F) and inoculant (I)

Results obtained in correlation analysis are summarized in Table 5.11. The root dry mass, enzymatic activity and microbial population were negatively correlated with pH and EC; on the other hand, it was found a direct relationship between root density and: available N, acid phosphatase, and β-glucosidase. It was evidenced, that humic and fulvic acids (HS), % organic matter, and WSC content where positively correlated with phosphatases and β-glucosidase activity, and with yeast populations.

Nitrogen is acknowledged to be one of the most important and most likely limiting nutrients in grapevines, being more critical in the spring after the plant has exhausted its reserves to produce its initial growth. Usually, the soil N is minimal very early in the season and fertilizers should be applied when vines can best absorb and assimilate N to build or supplement reserves, while minimizing losses thorough leaching and denitrification (Conradie, 2005; Peacock *et al*, 1989). Nitrogen absorption is most rapid between bloom and veraison, with the developing clusters being the largest sink for N during this time (Conradie, 2005; Peacock *et al*., 1989). Therefore, N fertilization is best applied late in the spring, after the risk of frost, when uptake and demand is optimal (Christensen, 2008).

The fertilizer applied as N source, Novatec Solub 21™, contains ammonium stabilized by the addition of the nitrification inhibitor DMPP, which improves the absorption N efficiency by avoiding lixiviation losses in form of NO_3-N (Molina and Ortega, 2006); for this reason, the control with chemical fertilization had less NO_3-N forms, but also had less total available N than compost treatments (Table 5.8), indicating that the amendment can contribute to avoid N leaching losses by improving soil structure and water retention capacity; additionally, the larger available N content is related with the fact that organic N from compost is mineralized gradually, offering constant availability of this element, because organic fractions are broken down by microorganisms, constituting an important N source for plant nutrition (Magdoff and Wiel, 2004). In general terms, NO_3-N contents were correlated with root dry matter content and had a positive effect on soil enzymatic activities phosphatases and β-glucosidases (Table 5.11), because compost improves the porosity, aeration and water retention capacity of the soil, which favors these enzymatic activities (Magdoff and Wiel, 2004).

Table 5.11. Pearson correlation analysis among measured agronomic and soil parameters in pot experiment.

	RDM	RD	ISCSA	EC	pH	O M	HS	WSC	AvailN	$N\text{-}NO_3$	$N\text{-}NH_4$	OIs-P	F	Y	Psol	Acty	βGlu	AcP	AlkP	U
RDM	1,00																			
RD	**0,71**	1,00																		
ISCSA	0,28	0,20	1,00																	
EC	**-0,61**	-0,40	0,10	1,00																
pH	**-0,91**	**-0,66**	-0,22	**0,70**	1,00															
OM	0,38	**0,55**	0,21	-0,43	**-0,53**	1,00														
HS	0,29	**0,57**	0,20	-0,34	-0,44	**0,96**	1,00													
WSC	0,29	**0,53**	0,26	-0,22	-0,43	**0,89**	**0,90**	1,00												
Avail N	**0,68**	**0,73**	0,18	-0,30	**-0,71**	0,43	0,43	0,46	1,00											
$N\text{-}NO_3$	**0,57**	**0,67**	0,16	-0,42	**-0,66**	**0,60**	**0,58**	**0,68**	**0,81**	1,00										
$N\text{-}NH_4$	**0,59**	**0,67**	0,05	-0,22	**-0,61**	0,28	0,30	0,28	**0,96**	**0,66**	1,00									
OIsP	0,36	0,23	0,45	-0,30	-0,40	0,37	0,29	0,43	0,43	**0,70**	0,23	1,00								
F	0,37	0,31	-0,12	0,17	-0,26	0,10	0,13	0,19	0,30	0,19	0,28	0,14	1,00							
Y	0,30	0,45	0,24	-0,30	-0,34	**0,53**	**0,48**	**0,51**	**0,49**	**0,69**	0,36	**0,53**	-0,03	1,00						
Psol	0,17	0,30	-0,20	-0,11	-0,24	-0,10	-0,04	0,14	0,28	0,32	0,30	0,11	0,39	-0,05	1,00					
Acty	-0,17	-0,17	-0,11	0,15	0,06	-0,09	0,01	-0,05	-0,03	-0,13	0,02	0,08	0,41	**-0,55**	0,30	1,00				
βGlu	**0,68**	**0,73**	0,29	**-0,52**	**-0,79**	**0,85**	**0,78**	**0,83**	**0,73**	**0,84**	**0,58**	**0,57**	0,27	**0,69**	0,19	-0,18	1,00			
AcP	**0,51**	**0,70**	0,50	**-0,51**	**-0,59**	**0,92**	**0,89**	**0,83**	**0,55**	**0,67**	0,39	0,43	0,01	**0,67**	-0,16	-0,30	**0,88**	1,00		
AlkP	0,42	0,44	0,25	-0,46	**-0,54**	**0,93**	**0,85**	**0,76**	0,33	**0,52**	0,18	0,34	0,00	**0,53**	-0,32	-0,23	**0,77**	**0,88**	1,00	
U	**0,68**	0,30	-0,18	**-0,77**	**-0,76**	0,44	0,28	0,20	0,29	0,28	0,22	0,12	0,05	0,24	-0,13	-0,25	**0,52**	0,46	**0,54**	1,00

RDM: root dry matter. RD: root density. ISCSA: increase of shoot cross sectional area. ; EC: electrical conductivity. OM: Organic Matter. HS: humic + fulvic acids WSC: Water Soluble Carbon. $N\text{-}NH_4$: Ammonium nitrogen, $N\text{-}NO_3$: Nitric nitrogen; OIsP: Phosphorous-Olsen. U: urease activity; AlkP: alkaline phosphatase activity. AcP: acid phosphatases activity. βGlu: β-glucosidase activity. Psol: Phosphate solubilizing bacteria; Acty: Actinomyces; F: Fungi; Y: yeasts Significant correlation coefficients are bolded, p-value <0.05.

The amount of N remobilized from permanent structures between bud break and fruit set accounts for up to 40% of that needed by shoots, leaves and clusters (Conradie, 1980). In addition to the needs of N as a nutrient for plant growth, fruit production and quality, supply and immobilization of available N present in the compost or organic amendments should be considered in fertilizer programs. The total nitrogen presents in compost does not become completely available to the vines. About 30% of the total nitrogen becomes available during the season, and this value varies based on compost composition, application method, soil conditions, microbial activity in soil, and environmental conditions after the application; recommendation is to discount the amount of N supplied by compost from chemical fertilizers, to reduce the risk of excess of N (Amlinger *et al.,* 2003; Travis *et al.,* 2003). Under the conditions of this experiment, an equivalent of 36 kg N ha^{-1} was applied with the maximum compost rate, out of which about 10 kg N ha^{-1} * season, was mineralized; this amount of N probably did not influence plant response since enough N was added to all treatments. It is worth noting that during establishment N needs are about 30 kg N ha^{-1}. Flavel *et al*. (2005), showed that grape marc compost, obtained with 2,7% total N, applied in the 0-10 cm depth in sandy soils of Australia, produced high mineralization rates during the first days application (2,4 to 7,4 mg N kg^{-1} day^{-1}), due to the decomposition of the soluble fraction of compost. Over time the mineralization of N decreased, but the net balance, in 148 days, was equivalent to 18 kg N $ha^{-1}day^{-1}$ at 20 cm depth.

This experiment resulted in an increase of HS content with the C rate, especially when compost is used as organic amendment (Table 5.8), being significantly higher in the treatment with high rate of compost + inoculant and no fertilizers (Treatment 8, Table 5.8); these results are due to the fact that the inoculant microbes increase enzymatic activity in soil, using compost as substrate, with mineralization of organic matter and HS production. Additionally, a positive correlation between HS and both alkaline and acid phosphatases as well as with actinomycetes population was found (Table 5.11), indicating that possibly, hydrolytic bacteria are responsible for most of hydrolysis of organic carbon sources. However, a negative correlation between HS and dry root biomass (Table 5.11) was observed, because the use of liquid humus, which had high pH and EC generating suppression on root synthesis, masking the beneficial effect that humic substances cause on the root system.

Previous studies have shown increases in soil carbon at higher amendment loading rates. Albaladejo *et al*., (2008) observed doubling of soil carbon concentrations using 260 ton ha^{-1} of uncomposted organic municipal solid waste, and Morlat and Chausson (2008), observed after cumulative loading rate of 256-320 ton ha^{-1} of compost in vineyards over 16 year period, doubled carbon concentration on surface respect to soil control.

On the other hand, the labile fraction of organic matter is composed by soluble forms of C, which are easily degradable and therefore the most susceptible to mineralization (Cook and Allen, 1992), acting as an immediate energy source for microorganisms. In this experiment concentration of WSC in soil increased with C rate using both OM sources, however the rate of increase was larger for liquid humus as compared to compost, probably because the higher content of WSC in liquid humus.

Composting is a widely-used treatment whose objective is to transform organic wastes in organic amendments for agronomic use; it produces several benefits for plant growth as it improves chemical, physical and biological soil characteristics, generating an effect of plant growth promotion by different ways (Heargreaves *et al.,* 2008). Thus, grape marc (GM) composting to obtain an organic amendment could be an economically and ecologically acceptable way to use it.

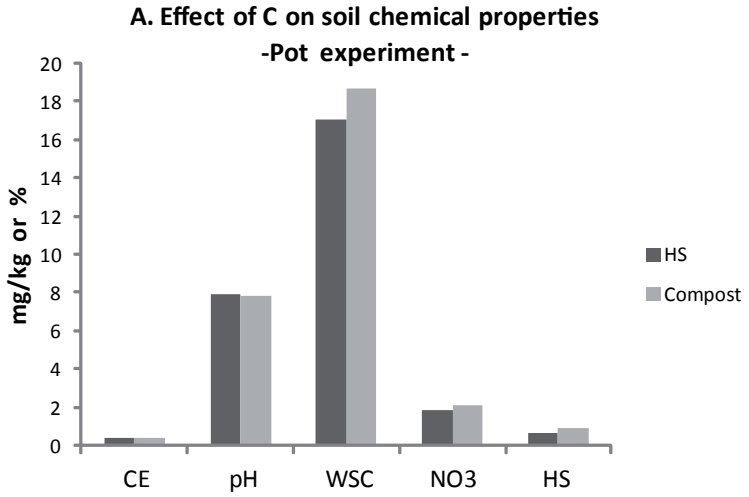
Grape marc is characterized by low electrical conductivity (EC) values, high organic matter (OM) and significant P and K concentrations, as well as low heavy metal contents, being all of them important factors in agricultural soils. However, this residue is also notably acidic and contains significant polyphenolic compounds, having these potential phytotoxic and

antimicrobial effects (Bustamante *et al*., 2008; Martinez *et al*., 2011); therefore composting of GM is required, in order to produce a more stable and manageable agricultural end product. This composted material can constitute a feasible option to increase soil OM content improving physical, chemical and biological properties to this ecosystem, because soil organic matter, nutrients and biological activity contribute to ecosystem level process and are important for productivity, community (Anderson, 2003; Avis *et al.,* 2008), structure and fertility (Gil *et al*, 2008); however the influence of organic matter on soil properties depends on amount, type and size of added organic materials (Barzegar *et al*., 2002).

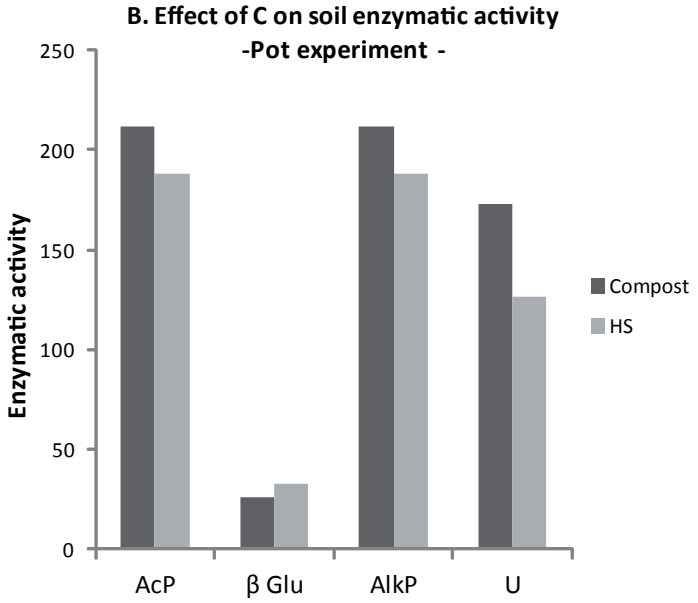
Ferrer *et al*. (2001) reported positive effect using 1-4 ton ha^{-1} of grape marc compost as a soil conditioner for corn seed germination in greenhouses. The chemical analysis of compost used in this experiment, revealed levels of free potassium, in the range of 2-3% w/w, plant macronutrients such as Ca, S, Mg were present at low levels (<1% w/w), while phosphorus (0,1-0,3% w/w) and nitrogen (1-2% w/w) levels were not very high and present in organic form. All the grape marc composts analyzed provided some benefit in returning nutrients into the vineyard and all were significant potassium sources (Issa *et al*., 2009). However, Flavel *et al*. (2005) observed negative effects on soil when grape marc compost was used as an organic fertilizer, which included an initial net immobilization of nitrogen. In the present study, it was observed that compost generated an improvement on root synthesis, and no negative effects were observed, suggesting that the nutrient and salt content were appropriate as amendment to promote rhizogenesis process especially in nursery vines.

At the end of the composting process, compost contains a large amount and diversity of microorganisms, so application of this amendment, improve the nutritional content of soil, the diversity and abundance of beneficial organisms, acting at the same time as a source of C and energy for native soil organisms (Heargreaves *et al.,* 2008). Perucci (1990) observed a significant increase in microbial biomass carbon, nitrogen and phosphorous in soil after 12 months from the application of compost from municipal solid waste at a rate of 2,5% (w/w). In the present research, the results indicated strong root development in plants with compost application (Figure 5.12a), probably due to the fact that compost favors factors such as texture, aeration, temperature, water and nutrient availability and organic matter content, factors that affect root distribution of grapevines (Morlat & Jaquet, 1993; Richards, 1983).

Use of compost from GM mixed with chemical fertilizers and microbial inoculant resulted in the best root production ($p<0,05$) , due to the fact that chemical nutrition provides much part of the elements required by the plant (Fig. 5.12c), compost generates an adequate environment in terms of physical and chemical conditions for availability of these nutrients, and also, together with the inoculant, provide beneficial microorganisms that mineralize organic matter by enzymes (Fig. 5.12b). The β-glucosidase showed less activity in treatments with compost compare with HS, expected result if the highest concentration was observed with respect to WSC in the treatments with compost. This succession of events, results in solubilization of nutrients and and synthes of humic substances, while promoting the plant growth by other mechanisms like stimulation of root elongation through the production of phytohormones like indol acetic acid (IAA) (Kloepper *et al.,* 1998, Avis *et al.,* 2008). Some microorganisms are described associated with grapevine, according to a study by Compant *et al*., (2011), they are *Pseudomonas* sp, *Pseudomonas fluorescens*, *Pseudomonas cannabina*, *Bacillus sp*, *Bacillus pumilus, Paenibacillus lautus*, *Arthrobacter sp*, *Variovora paradoxus*, *Rhodococcus sp* among others.



HS: humic + fulvic N-NO_3: Nitric nitrogen; WSC: Water Soluble Carbon; CE: electrical conductivity.



AlkP: alkaline phosphatase activity. βGlu: β-glucosidase activity;
AcP: acid phosphatases activity; U: urease activity.

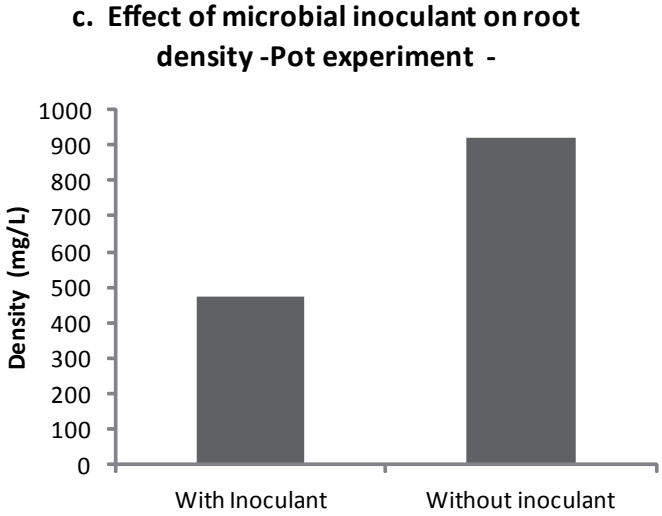


Fig. 5.12. Effect of Compost and Inoculant on (a), Soil chemical properties, (b) enzymatic activity and (c) effect of microbial inoculants root density by orthogonal contrast analysis

Chizhevsky and Dikusar, since 1995, suggested that beneficial effects of humic substances on plant growth may be mediated via microbial breakdown products, or by stimulation or inhibition of enzymatic activity which could contribute to growth promotion. Cecco and DÁngolla (1984), showed a stimulating effect on pea growth of liquid humic and fulvic substances at concentrations below 5 mg L^{-1}, and an inhibitory effect above this concentration; this stimulatory effect was attributed to the presence of free phenolic compounds in humic extracts, exhibiting a similar stimulatory action of indoleacetic acid, or by increasing the concentration of hormones in plants due to the inhibitory effect of humic substances on the enzyme IAA oxidase. In this regard, Ortega and Fernandez (2007) reported an increase in above ground and root biomass with C rate applied as humic substances extracted from vermicompost and leonardite. In the present study, the pH and EC of humic extract caused negative effects in root development, despite the dilution performed before its application; this negative effect was due to the fact that the salts concentration causes physiological stress by osmotic unbalance (Bassoi *et al*., 2003).

5.2.3. Sensitivity of each property for representing changes in management

Changes of each measured soil property on each treatment, with respect to the soil used as base line, were determined. The objectives of this analysis were to describe the effect of treatments on several soil properties and to determine the weights of each property in order to propose a possible model.

Each of the 18 treatments (including compost and humic extracts), was considered a population, with the idea of determining the proportion of them presenting changes in a given property over time (as compared to the base line). On the other hand the weight (slope) of each property over time was estimated and used to rank all the properties in terms of its sensitivity. Standardized variables were used and regressed over 3 times, 0, 4, 8 months. When the slope was not significantly different from zero (P>0,05) it meant that there were not changes in time over the base line and the property was not sensitive enough in a given treatment. On the other hand the larger the coefficient (slope), the higher the weight of the property (Table 5.12).

The regression of each standardized variable on time in each treatment determined that the soil properties changing most frequently and with larger weights were Olsen-P (Ols-P), $N\text{-}NH_4$ content, electrical conductivity %C and β-Glucosidase (β-Glu). Phosphate solubilizing bacteria changed over the base line in 94% of the 18 populations (treatments) evaluated (Table 5.12).

Table 5.12. Frequency analysis for all soil properties in pot experiment*

Treatment	AcP	AlkP	U	β-Glu	F&Y	Psol	HS	pH	NH_4	NO_3	C	EC	OlsP
1	0,00	0,00	0,26	0,00	0,00	0,35	-0,09	0,00	-0,27	0,00	-0,31	-0,42	0,27
2	0,00	0,00	0,32	0,00	0,00	0,32	0,00	0,12	-0,32	0,00	0,00	-0,43	0,41
3	0,00	0,00	0,00	0,00	0,00	0,33	0,00	0,00	0,25	0,00	0,00	-0,44	0,23
4	0,00	0,00	0,26	0,00	0,25	0,00	0,41	0,00	-0,35	0,00	0,00	-0,41	0,28
5	0,00	0,00	0,00	0,00	0,00	0,34	0,00	0,00	0,00	0,00	-0,29	-0,37	0,00
6	0,00	0,00	0,00	0,00	0,00	0,32	0,00	0,00	0,00	0,00	0,00	-0,37	0,00
7	0,00	0,00	0,00	0,00	0,00	0,38	0,39	0,00	-0,31	0,00	0,00	-0,43	0,00
8	0,00	0,00	0,00	0,00	0,00	0,34	0,64	0,00	0,00	0,00	0,00	-0,42	0,00
9	0,00	0,00	0,00	-0,32	0,00	0,29	-0,07	0,32	-0,35	-0,30	-0,33	-0,40	0,00
10	0,00	0,00	0,00	-0,34	0,00	0,28	-0,04	0,42	-0,32	0,00	-0,32	-0,37	0,00
11	0,00	0,00	-0,19	-0,35	0,00	0,35	0,00	0,46	-0,36	0,00	-0,32	-0,18	0,00
12	0,00	0,00	0,00	-0,35	0,00	0,34	0,00	0,48	-0,31	0,00	-0,32	0,00	0,00
13	0,00	0,00	0,00	-0,35	0,00	0,33	0,00	0,30	-0,28	-0,30	-0,31	0,00	0,00
14	-0,31	0,00	0,00	-0,34	0,00	0,34	-0,07	0,37	-0,35	-0,32	-0,32	0,00	0,00
15	-	0,00	-0,17	-0,35	0,00	0,39	0,00	0,49	-0,32	0,00	-0,33	-0,08	0,29
16	-0,31	0,00	0,00	-0,35	0,00	0,30	0,00	0,50	-0,35	-0,35	-0,31	0,00	0,00
17	-0,27	0,00	0,31	-0,35	-0,43	0,30	-0,11	0,00	-0,30	-0,35	-0,31	0,00	0,00
18	-0,25	0,00	0,27	0,35	-0,32	0,29	0,00	0,00	-0,35	-0,29	-0,32	0,00	0,00
All	-0,08	0,00	0,18	-0,23	0,00	0,31	0,14	0,19	-0,29	-0,20	-0,23	-0,32	0,19
Freq**	22,22	0,00	38,89	55,56	16,67	94,44	44,44	50,00	83,33	33,33	66,67	66,67	27,78

AlkP: alkaline phosphatase activity. AcP: acid phosphatases activity. U: urease activity; βGlu: β-glucosidase activity; F&Y: Fungi and yeasts, Psol: Phosphate solubilizing bacteria; HS: humic + fulvic acids, N-NH_4: Ammonium nitrogen, N-NO_3: Nitric nitrogen; C: Organic Carbon. EC: electrical conductivity. OlsP: Phosphorous-Olsen.

Frequency calculated as the number of responsive treatments divided by the total number of treatments.

On the other hand their average corrected weight was 0,12 (Figure 5.3). On the other hand there were several properties changing less frequently and with lower weights which mean that they would not be good indicators of changes in management under the evaluated conditions.

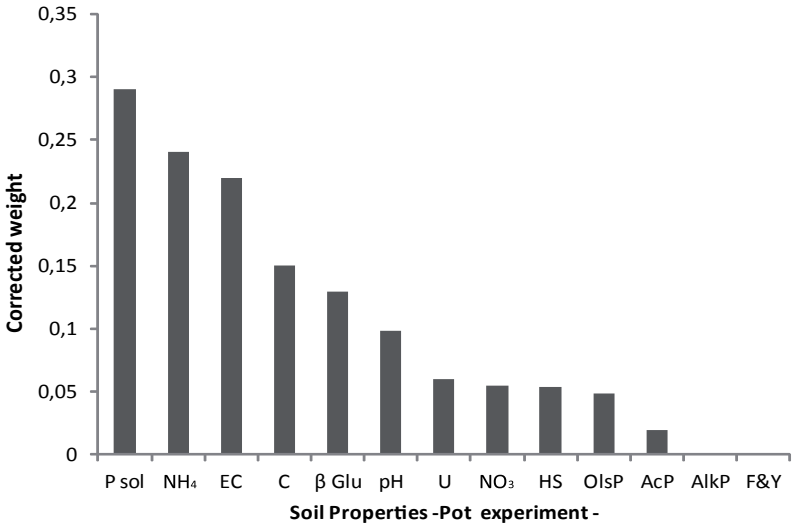


Figure 5.13. Corrected weights of soil properties obtained by regression and frequency response analysis (Pot experiment).

Psol: Phosphate solubilizing bacteria, N-NH_4: Ammonium nitrogen. EC: electrical conductivity, C: Organic Carbon, βGlu: β-glucosidase activity, U: urease activity; N-NO_3: Nitric nitrogen, HS: humic + fulvic acids, OlsP: Phosphorous-Olsen AcP: acid phosphatases activity. AlkP: alkaline phosphatase activity. F&Y: Fungi and yeasts.

Finally, the properties defined to explain changes in soil as minima data set under semi controlled conditions (pot experiment), were: phosphate solubilizer bacteria, ammonium nitrogen concentration, electrical conductivity, organic carbon, β glucosidase and pH.

5.3. Evaluation of C and N rates in commercial Table Grape yield

Simultaneously with the pot experiment, a field study was conducted in an established, 1-year old, table grape orchard, Thompson Seedless variety grafted on *"Freedom"* rootstock variety, under drip irrigation (Fig. 5.14). The effects of different C and N rates on soil, plant and fruit were assessed. Besides, quality indices for soil and fruit were developed.

Figure. 5.14. b. Field experiment under drip irrigation system. Uncultivated soil used as baseline – Agroecosystem baseline (AES)

5.3.1. Changes of soil properties over the base line.

Limary Valley is one of the most important areas in Chile in terms of grape production (table grape and pisco grapes). The area is characterized by mountainous landscape, subtropical, semiarid climate (Fig. 5.14), with winter temperatures ranging from -6°C to 15°C, and 16 to 23°C in summer; the area has an average annual temperature of approximately 18°C. The average rainfall is slightly over 125 mm/year and the area has excellent radiation levels. Soils in this semiarid region are mainly inceptisols, aridisols and entisols; they are relatively rich in sand (>50%), have low levels of organic matter (<1-2%), and tend to have alkaline pH. These factors represent extreme conditions for the soil mineralization process and, normally, under these conditions the rate of humus synthesis in crop land is low (Martínez *et al*., 2003). Studies by Etienne *et al.* (1993), in Ovalle et al. (1993), indicated that this region presents a high degree of degradation, situation that was confirmed when sampling the base line.

Figure 5.15 Valle del Limarí, Chile

Xerophitic soil cover shows predominantly “espinales” like *Acacia aven* (Aronson *et al.*, 1993) associated with woodlands “Lilén” (*Azara celastrinal*), “molle” (*Schinus latifolius*), “litre” (*Lithraea caustic*), “Guayacan” (*Porlieria chilensis*) and some grazing areas (Fig. 5.15 b and c). Riparian vegetation is dominated by Sauce (*Salix spp.*), and Guayacan, and uncultivated agroecosystem soil shows the presence of some weeds like Quingüilla (*Chenopodium spp.*) and Chamico (*Datura stramonium*) (Fig. 5.16a).

Figure 5.16. (a) Riparian vegetation –River Base line (BLMR), (b) Mountain Base line (BLM), Xerophilic forest, (c) detail of “Espinales” in Limarí Valley

5.3.1.1. Comparison among base lines at the beginning of the experiment

Three baselines were considered: 1) xerophytic forest at the mountain (Mountain baseline, BLM), 2) riparian vegetation by Rio Claro (River baseline, BLR), and 3) uncultivated soil (AES). Predominant soil chemical characteristics are: low organic matter and N contents, slightly acidic pH, low levels of P and medium levels of exchangeable bases (Table 5.13).

Table 5.13. Comparison among base line soils in terms of chemical, biochemical and microbiological characteristics

	Units	Xerofilic Forest Mountain (BLM)	Agroecosystem Soil (AES)	Riparian Vegetation (BLR)
AcP	µg de para-nitrofenol/g*h	201,4±59,5	98,9±20,3	280,6±0,0
AlkP	µg de para-nitrofenol/g*h	176,36±77,7	128,5±30,8	315,7±0,0
U	µg de NH4/g*h	105,7±7,28	32,87±13,0	95,7±0,0
β Glu	µg de para-nitrofenol/g*h	95,6±31,25	19,8±4,24	45,00±0,0
Bact	Log UFC/g	4,7±0,3	6,22±1,85	6,7±0,0
F	Log UFC/g	5,09±1,2	5,63±0,46	6,0±0,0
Y	Log UFC/g	3,85±0,15	5,01±0,95	1,03±0,0
Cellul	Log UFC/g	4,56±0,26	5,06±0,31	5,03±0,0
Prot	Log UFC/g	5,77±0,4	5,12±1,0	5,48±0,0
pH		6,76±0,0	7,6±0,14	7,7±0,0
HS	%	0,58±0,0	0,49±0,1	0,69±0,0
N	%	0,18±0,0	0,08±0,0	1,7±0,0
$N\text{-}NH_4$	$mg*kg^{-1}$	13,6±1,1	15,75±6,2	0,16±0,0
$N\text{-}NO_3$	$mg*kg^{-1}$	12,3±2,5	14,7±5,2	18±0,0
Ols-P	$mg*kg^{-1}$	9,7±4,1	19,6±4,2	36±0,0
OM	%	3,83±0,7	1,52±0,44	5,84±0,0
C	%	2,23±0,7	0,87±0,2	3,4±0,0
C/N		13,03±2,5	10,54±2,1	2±0,0

βAcP: Acid Phosphatase; AlkP: Alkaline Phosphatase; βGlu: β glucosidase . U: urease. Bact: Total Bacteria.; ; F: Fungi, Y: Yeast ; Cellul: Cellulolytic bacteria, Prot: Proteolitic bacteria. HS: Humic Substances (HA+FA).N: Nitrogen. NH_4-N/NO_3-N :Amoniacal Nitrogen/Nitric Nitrogen ratio. Ols-P: Olsen Phosphorous. OM: Organic Matter. C/N: Carbon:Nitrogen Ratio;

Overall, the riparian vegetation (BLR) had better quality soils than, BLM and this in turns than AE (Table 5.13, Figure 5.17a and 5.17b).

The analysis of variance made to compare the three baselines determined that AcP, AlkP, U, β-Glu, concentration of amilolytic bacteria (Amil), and some chemical characteristics like pH, N, $N\text{-}NH_4$, $N\text{-}NO_3$, Ols-P, %OM, %C, C/N showed significant differences among base lines (Fig 5.17a and 5.17b). All these properties showed larger values in BLR and BLM than in agroecosystem base line (AES); BLR has permanent organic matter supply in different degradation grade. Also, the larger water availability and above ground biomass in riparian base means higher organic matter content in soil. However, apparently the xerophytic mountain base line (BLM) has organic matter rich in cellulolytic compounds and probably fresh nitrogen, according to higher urease β Glucosidase and Urease activity. In addition, BLR ecosystem receives inputs of organic matter by dragging the river in summer. The NH_4 and pH was higher in BLR and agroecosystem AES, probably by the effect of run off.

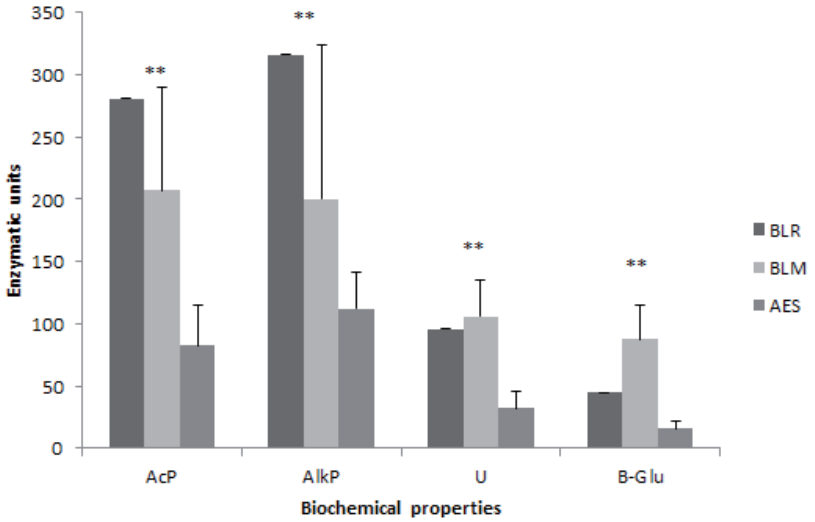


Fig. 5.17a Comparison among base line soils in terms of biochemical properties.
** high significant difference
BLR: Base line River-Riparian vegetation; BLM: Base line Mountain-xerophitic forest; AES: Base line_agroecosystem. AcP: acid phosphatase; AlkP: alkaline Phosphatase; U: Urease; β Glu: β Glucosidase.

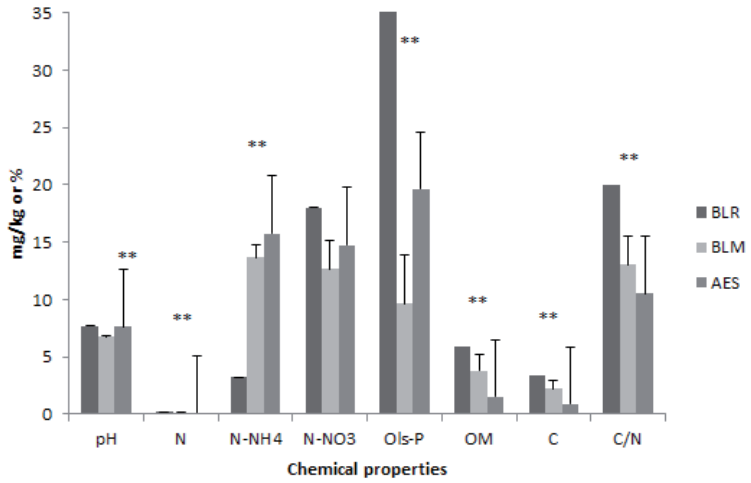


Fig. 5.17b Comparison among base line soils in terms of chemical properties.
** high significant difference
BLR: Base line River-Riparian vegetation; BLM: Base line Mountain-xerophitic forest; AES: Base line_agroecosystem. N: total N; $N\text{-}NH_4$: ammonium nitrogen; $N\text{-}NO_3$: nitric nitrogen; Ols- P: Olsen Phosporous; OM: organic matter; C: organic carbon; C/N:_ ratio

Uncultivated soil (AES) showed the lowest organic matter (OM) concentration (1,52±0,44 %) followed by mountain baseline (BLM) with 3,83±0,7 %, and riparian vegetation (BLR) (5,84±0.0), being consistent with the results of enzymatic activity which was also the lowest. According to Dick *et al.* (1996), enzyme activity is a property of soil rapidly changing by cultural practices, fertilizer management and crop type. The results obtained in this study, showed significant differences between cultivated soil, with only one year under table grape management, including basal fertilization (nitrogen, phosphorous and potasium), and the uncultivated agroecosystem (table grape) soil.

5.3.1.2. Changes in time and by management over the agroecosystem base line.

Considering the agroecosystem base line (AES), on the average, there were positive changes in enzymatic activities (AcP,AlkP, U, and βGlu), microbial groups (Fungi, Yeast, Cellulolitic, and Proteolitic bacteria) and some chemical properties such as HS,OM, WSC, NH_4, NO_3, C, pH and Olsen P. Negative changes was observed in terms of total, amilolytic and phosphate solubilizing bacteria, electrical conductivity, total nitrogen and C/N ratio. In terms of frequency of change, the properties changing most often, in the sixteen agroecosystems (AE) (treatments) evaluated, where acid phosphatase activity (AcP), humic substances content (HS), and β-glucosidase activity (βglu), with 100, 100, and 94 % of the AE changing, respectively (Fig. 5.14). Regarding AE comparison, the ones receiving C and/or N showed similar responses as compared to the check treatment in terms of rate (slope) of change in key soil properties.

Table 5.14. Change frequency, with respect to the base line, for the measured properties in the 16 evaluated AES

Treatment	AcP	AlkP	U	β-Glu	Bact	F&Y	Yeast	Psol	Cellul	Prot	Amil	HS	OM	WSC	pH	CE	N	NH_4	NO_3	NH_4/NO_3	C	C/N	Ols-P
1	1	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1
2	1	1	0	1	0	0	0	0	1	1	0	1	0	0	1	0	0	0	1	0	0	0	1
3	1	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1
4	1	0	1	1	0	0	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0
5	1	0	0	1	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0
6	1	1	0	1	0	0	0	0	1	0	0	1	1	0	1	0	0	0	0	0	1	1	1
7	1	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	1	1	1
8	1	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
9	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1
10	1	1	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
11	1	0	1	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
12	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0
13	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1
14	1	0	0	1	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	0	1
15	1	1	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0
16	1	0	0	1	0	1	0	0	1	1	0	1	0	0	1	0	0	0	0	0	0	0	1
Sum	16	6	3	15	0	1	2	0	4	5	0	16	3	3	13	0	0	1	2	0	3	3	12
%	100	37,5	18,75	93,75	0	6,25	12,5	0	25	31,25	0	100	18,75	18,75	81,25	0	0	6,25	12,5	0	18,75	18,75	75

AcP: acid phosphatases activity, ; HS: humic + fulvic acids, βGlu: β-glucosidase activity, OlsP: Phosphorous-Olsen, AlkP: alkaline phosphatase activity.Port: proteolytic microorganisms, WSC: water soluble carbon; Yeast; Cellul; cellulolytic microorganisms; F&Y: Fungi and yeasts; C: Organic Carbon, U: urease activity; , N-NO_3: Nitric nitrogen; Bact: total bacteria; Psol: Phosphate solubilizing bacteria, Amil: amilolytic bacteria;; EC: electrical conductivity. N: Organic Nitrogen; N-NH_4: Ammonium nitrogen; C/N ratio

When estimating the weigth of each property, considering its average change (singless), and the frecuency of change, the most sensitive properties were acid phosphatase (AcP), humic substances (HS) and β glucosidase (β Glu) (Fig. 5.18).

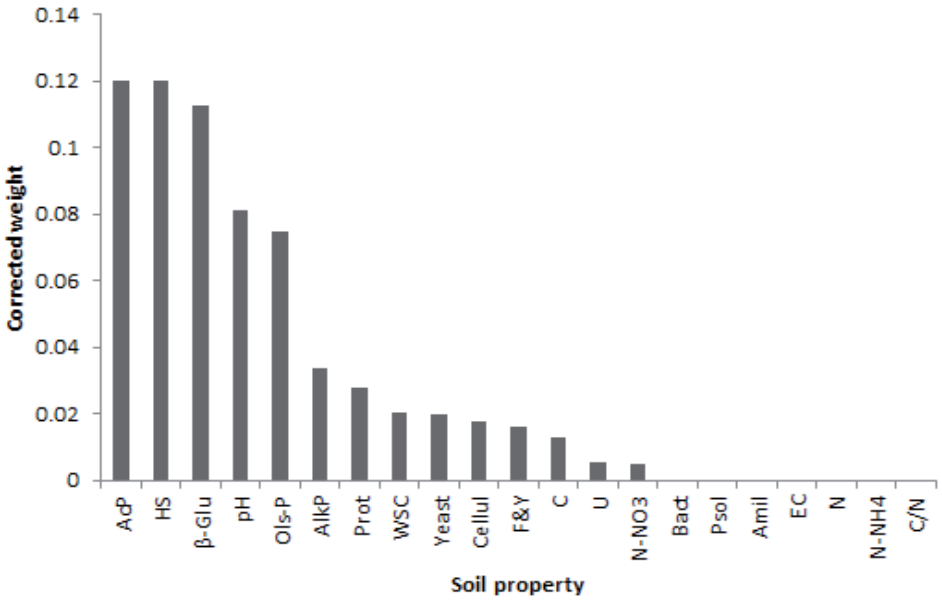


Fig. 5.18 Corrected weights of soil properties obtained by regression and frequency response analysis (Field experiment).

AcP: acid phosphatases activity; HS: humic + fulvic acids, βGlu: β-glucosidase activity, OlsP: Phosphorous-Olse, AlkP: alkaline phosphatase activity.Port: proteolytic microorganisms, WSC: water soluble carbon; Yeast; Cellul; cellulolytic microorganisms; F&Y: Fungi and yeasts,; C: Organic Carbon, U: urease activity; $N\text{-}NO_3$: Nitric nitrogen; Bact: total bacteria; Psol: Phosphate solubilizing bacteria, Amil: amilolytic bacteria; EC: electrical conductivity. N: Organic Nitrogen; $N\text{-}NH_4$: Ammonium nitrogen; C/N ratio.

5.3.2. Effect of C and N rates on soil properties

The quality of the organic matter applied, defined in terms of its reactive components (nutrient supply and storage), and physical characteristics (water retention capacity, structure, and others), is difficult to define. In general SOM components can be divided into humic acids (HA) fulvic acid (FA), and humin, which are quantified based on their solubility in alkaline and acid solutions (Stevenson, 1994). Soil humic substances (HS), composed by HA and FA, are stable polycyclic fractions, synthesized from simple organic matter by biochemical and microbial activity (Ortega and Fernandez, 2007). These C fractions show different absorbance at 465 (E_4) and 665 nm (E_6). The quality of SOM can be evaluated by C-HA/C-FA and the E_4/E_6 ratio and or/ through the classification of HS. The C-HA/C-FA ratio defines humification process, while E_4/E_6 ratio is used to characterize HA and FA; this ratio indicates the molecular size or chemical complexity degree of HS. These characteristics depend on the OM source; in this case, on the organic amendment. Depending on the source, and extraction process, they exhibit different properties in soil.

Analysis of variance revealed a strong interaction treatment by date, meaning that the effect of C and N rates on soil properties depended on the sampling date. Thus, after 6 months from establishment, C rate increased ($p<0,1$) HS, WSC, and pH, while N rate significantly affected organic matter concentration and C/N ratio. At 12 months, C affected $N\text{-}NO_3$, while N did with EC, and pH. Few interactions were observed between C and N rates.

At the end of the experiment, after 18 months from establishment, a significant effect of C applied as humic substances in soil on the absorbance of an alkaline soil extraction, at both 465 (E_4) and 665 (E_6) nm was observed; this finding corroborates the accumulation of humic substances in soil by the application of humic extract that included C in several forms, including water soluble C (WSC), and that in fulvic and humic acids (Figure 5.19). A the same

time, ANOVA defined, C and N rate affected (p<0,1) HS, AlkP, pH, and Olsen P, and N rate significantly affected the urease activity (Fig. 5.22). Most probably, humic extract applied is contributing to the accumulation or in situ formation of aliphatic groups in the soil (Velasco *et al.*, 2004). On the other hand, C rate produced an increase in soil organic C (SOC) only at the lowest N rate used, 30 kg N ha^{-1} (Figure 5.20d).

Besides, the C rate, the type and quality of the organic matter applied define the efficiency of the application. Especially for intensive horticulture systems, like table grape under drip irrigation, the use of some organic matter amendments, like manure or compost, results difficult to apply, are expensive, and their components (particularly in fresh manure) could result in damage for the plant because of phytotoxic components (NH_4, organic acids, phenolic components content). On the other hand, liquid humus or humic substances, are easy to apply through the irrigation system and along with supplying WSC, result in a stable source of C, and due to complex chemical structure, could be considered the storage of C in soil.

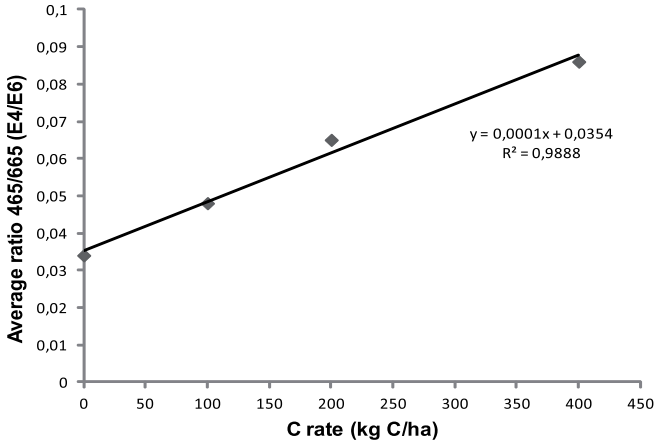


Fig 5.19. Average E4/E6 ratio of humic substances (HS) fraction with different Carbon (C) rate

Carbon is used as storage of energy and substrate for soil microorganisms, and contributes to plant metabolism in different forms. While C reserves obtained from photosynthesis contribute to vegetative growth and reproduction, also maintains root respiration and production of exudates that contribute to increasing and maintaining the beneficial micro flora in the rhizosphere. At the same time, the application of organic amendments contributes to maintain or increase SOC concentration in soil (Conradie, 1980).

Humic substances (HS) application as C source results significantly (p<0,05) increased soil pH, which was expected since the HS had alkaline pH (Figure 5.19). This would be a positive effect for acidic soils, however under the alkaline conditions of the experiment it would not since it might affect the availability of some nutrients, particularly P and micronutrients. However, under drip irrigation, fertigation is made controlling the pH of the water close to 6, therefore, in practical way, this would not be a problem.

In terms of biochemical indicators alkaline phosphatase and β-Glucosidase activities decreased with the increase in C rate (Fig. 5.20a and b). On the other hand the population o cellulolytic bacteria also decreased with C rate (Fig. 5.20d). Probably, observed effects are related to changes in pH and also to the increment of humified C sources such a humic acids, after the most available C sources have been exhausted (WSC and fulvic acids) (Fig. 5.20c). On the other hand, one year before the beginning of the experiment, each plant was planted with 1 kg of semicomposted goat manure, a traditional cultural practice in table grape. This organic amendment contains organic carbon in different decomposition stages, as cellulose, hemicelluloses and protein, a complex organic substrate for cellulolytic microorganisms, which decompose the material through enzymatic activity. When this organic source completes the degradation process, the enzymatic activity stops and some functional groups as cellulolytic and proteolytic bacteria of microorganisms reduce in concentration. Humic acids can be

extracted from different fresh, composted, soil or marine sources and contain entirely aliphatic copolymers of several principal monomeric units originating from polysaccharides with enormous variability in composition (because there are so many possible monomeric units in the molecule) (Susic, 2008). However during the extraction process from compost, the fraction of cellulose is degraded increasing the aliphatic, aromatic and monomeric components in the extract, with low enzymatic activity (Table 5.7). For this reason, in soil the enzymatic activity is higher than base line (Table 5.14) and humic extract (Table 5.7) but over the time, decreased with humic substances application, data confirmed in pots experiment (Table 5.9).

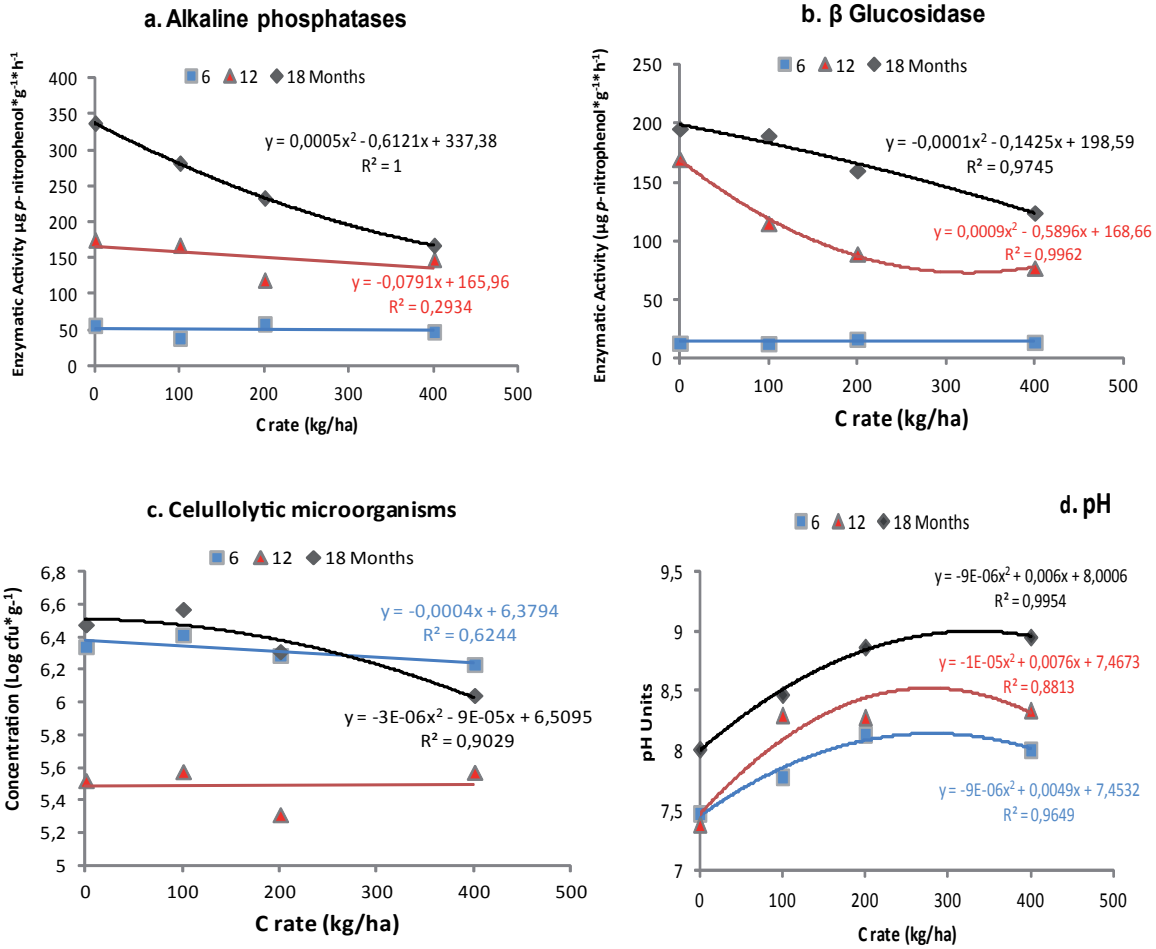


Figure 5.20 Effect of C application as humic extract on selected soil properties at three sampling dates (average of 4N rates). a). Alkaline phosphatase b) β Glucosidase c) Cellulolytic microorganisms d)pH

Nitrogen is one of the most critical nutrients in vine crops, especially in spring, during the period of rapid shoot growth, flowering and berry formation (Winkler et al., 1974). In midsummer this need decreases, as the berries begin their process of maturity, so the availability and distribution of nitrogen previously stored in roots, trunk and canes defines the period of rapid growth (Alleweldt, 1984; Araujo and Williams, 1988). Therefore, it can be inferred that nitrogen should be applied when the vine can better absorb it and incorporate it as part of the reserve, while the losses of nitrogen in the soil by leaching or denitrification, are reduced. Usually, there are two fertilization moments, during late spring up to fruit set and at postharvest. Nitrogen can be used by the vine plant, both in the form of ammonium (NH_4^+) and nitrate (NO_3^-); when the former is available, it is rapidly metabolized in roots and translocated to the shoots, leaves and clusters, while the NO_3^- is present throughout the plant and throughout the year. Then, the plant starts forming amino acids, which may occur in the root tips, leaves, and even berries, mediated by the nitrate reductase enzyme.

The movement of N through the xylem occurs where it is possible to find both inorganic and organic forms, limited to a few amino acids such as aspartic acid, glutamic acid and its amide,

arginine. In petioles (used as a diagnostic tissue to analyze plant nutritional status), a NO_3^+ fraction larger than 2.3% of dry weight can be found, depending on solar intensity and variety (Wermelinger, 1991).

Bell and Robson (1999), working with different nitrogen fertilizer rates (0, 50, 00, 200 and 400 g N/plant-vine) applied by irrigation in 12-year- old Cabernet Sauvignon vines, defined that use moderate rate of N (100 g N/ plant), stimulated vine growth and vigor (shoot extension), increased canopy density, reaching maximum petiole nitrate concentration at flowering, and leaf area. An excess of nitrogen fertilization increases the concentration of mineral nitrogen, with accumulation of NO_2^- and NO_3^- on berries and leaves of vine (Motasser et al., 2003). In sandy soils, with low organic matter concentration, it is important to minimize the input of mineral nitrogen, because there is more risk of absorption and tissue accumulation or leaching losses. In this experiment the product Novatec Solub 21, was used as N source; it s, contains the nitrification inhibitor DMPP, which acts blocking the ammonium monooxigenase (AMO) enzyme, produced by nitrificant bacteria in soil. This N fertilizer combined with stabilized organic matter with good C / N, can reduce nitrogen losses and increase nitrogen use efficiency (NUE), also activating the rhizosphere microbial flora.

In the present study, after 18 months from the establishment of the experiment nitrogen application significantly affected alkaline phosphatase and urease activities (Fig. 5.21a and b), which increased and decreased their activity with the N rate, respectively. On the other hand, soil pH decreased with the maximum N rate (120 kg N/ha), while SOC was lower with the minimum N rate (30 kg N/ha), (Fig. 5.21c).

The increase in C rate resulted on an increment of pH in soil, but increasing the N rate resulted in a slight acidification process. Under the conditions of this experiment, these were independent processes caused by the application of an alkaline solution in case of C and a ammonium fertilizer in case of N. This finding results specially interesting for horticulture systems in acid soils, as was mentioned before.

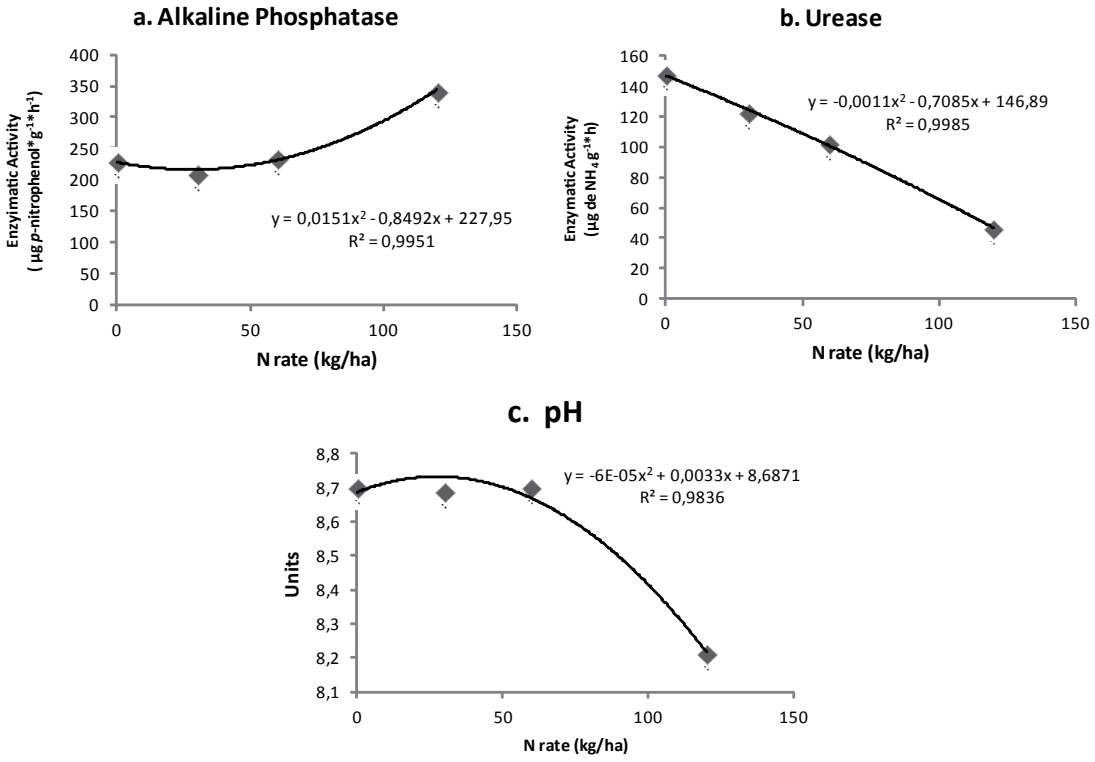


Fig. 5.21 Effect of C and N on soil properties field experiment
a) alkaline phosphatase, b) Urease, c) pH

On the other hand, the application of high rates of C (400 kg C/ha) in combination with 60 or

120 kg N/ha resulted positive in relation to the increase in soil organic carbon compared to the Agroecosystem Baseline (AES). This increment can be explained by the application of humic extracts which includes soluble carbon, besides aromatic and aliphatic compounds which are slowly degradable and stable in soil. The humic substances (HS) have been related, by several authors, with improving agronomic parameters like stimulating root development (Vaughan and MacDonald, 1976) and nutrient uptake (Vaughan *et al.*, 1985; Ortega and Fernandez, 2007). Low molecular weight fractions (including in WSC fraction) induced morphological changes in plants, similar to those caused by indole-3-acetic acid (IAA) (Muscolo *et al.*, 1993). Studies by Lui *et al.* (1998) showed a positive effect of humic substances on chlorophyll leaf content, root and shoot dry weight and number of flowers and buds in grass. Also, the extracted humic substances contained biologically active substances not from the original parent compounds, but as products of microbial metabolism; these promotion effects are not completely studied, but HS contribute to plant nutrition improving N and K availability, soil structure, water-air retention capacity, increasing soil microbial population, and acts as a buffer solution in cation exchange capacity and pH (Anderson, 1979; Margdof and Weil, 2004). In addition, humic substances can serve as carrier of micronutrients or growth factors; a theory even is proposed on which humic substances can act as a direct stimulator of plant growth by entering into the plant tissue, resulting in various biochemical effects at the cell wall, membrane, or in the cytoplasm (Magdoff and Weil, 2004).

5.3.2.1. Correlation among measured soil variables

Since there was interaction between sampling time and treatment, the correlation analysis was done by sampling date.

At six months from establishment a positive correlation was observed between the soil C content and hydrolytic enzyme activity of β-glucosidase, and acid and alkaline phosphatases. At the same time OM, fungi and yeast and N-NO_3, showed many correlations with biochemical and microbiological properties (Table 5.15). This relationship held up to 12 months from establishment, especially with the β-glucosidase. B- glucosidase, wich also showed a positive correlation with soluble carbon and pH (Table 5.16). These results confirm the usefulness of this enzyme as an indicator of changes in soil; its increased activity is probably related to an increase in WSC, and not necessarily the SOC, caused by the applications of C as humic acid extracts.

The enzyme β-glucosidase is hydrolyzes glycosides in the β-D-glucose dimer as cellobiose, intermediate in the degradation of cellulose. Numerous studies report this enzyme as an indicator of changes in the soil as a result of application of organic matter which is confirmed in the present study. Similar results were obtained in the first and second sampling date, coinciding with 6 and 12 months after application. In the third sampling date (18 months from establishment), however, this correlation is not observed, but there is negative correlation between this enzyme and the pH, which suggests that the increase in soil pH, resulting from the application of C as humic extracts, could have affected the activity (Table 5.17).

Regarding alkaline phosphatases (AlkP), correlation analysis indicated similar results as compared to other studies (Frankenberger and Dick 1983) with high correlation coefficients with organic C, pH and EC in first and third sampling time (Table 5.15 and 5.17); this enzyme exhibits better activity in alkaline pH, and depends on availability of organic phosphates in the system. It is produced by plant and microorganisms and its activity could be increased if the plant rizospheric exhudates exhibit higher phosphate concentration. The humic extracts applied could be a possible inductor of different P and N metabolic pathways in plant being beneficiated by this specific activity.

On the other hand a positive relationship between alkaline and acid phosphatases and β- glucosidase was observed.

In general, C application during 18 months, resulted in an increased activity of AlkP and β-Glu,

with respect to the AE base line; these biochemical properties resulted better indicators than selected microbial groups or other chemical characteristics (Fig. 5.22). These increased activities can be explained mainly by the augmented C fractions in soil, particularly HS and WSC as these properties increased with time and were modified by management (Fig. 5.22 and 5.23). Natural increased was probably due to the goat manure added to each plant as explained before. After 18 months of management many treatments surpassed the BLM and BLR bases lines, meaning that table grape production can be as sustainable as any natural ecosystem (Fig. 5.22). Nonetheless, bacteria and yeasts exhibited different relationships with organic matter depending on the sampling date. These results suggest that soil bacteria were positively affected by the increment in SOM in soil; with other changes occurring in soil, especially in pH, and NH_4 and NO_3 availability, the yeast increased their concentration.

Table 5.15. Pearson correlation Soil properties, first sampling -6 months(n=32)

	AcP	AlkP	U	β Glu	Bact	F&Y	Psol	Cellul	Prot	Amil	HS	OM	WSC	pH	EC	N	$N\text{-}NH_4$	$N\text{-}NO_3$	C	CN	OlsP
AcP	1																				
AlkP	0,35	1																			
U	-0,001	-0,001	1																		
β Glu	-0,05	**0,66**	0,08	1																	
Bact	0,34	**0,53**	-0,06	0,29	1																
F&Y	-0,33	-0,28	0,04	-0,04	-0,27	1															
Psol	-0,03	-0,001	-0,07	0,12	-0,09	0,39	1														
Cellul	0,07	0,27	0,22	0,17	0,08	0,32	0,09	1													
Prot	0,015	0,14	0,07	0,06	0,007	0,03	0,03	**0,44**	1												
Amil	-0,09	0,21	-0,01	-0,05	-0,01	**0,75**	**0,5**	0,26	-0,09	1											
HS	0,25	0,06	-0,15	0,05	0,23	**-0,38**	-0,26	-0,19	-0,02	-0,17	1										
OM	**0,44**	**0,59**	-0,11	**0,35**	**0,51**	**-0,66**	**-0,46**	0,09	0,08	**-0,43**	0,31	1									
WSC	0,19	-0,13	-0,14	-0,02	-0,05	-0,03	0,07	0,24	-0,01	0,23	0,39	**-0,03**	1								
pH	0,16	-0,28	-0,07	-0,22	0,13	0,32	**0,31**	0,08	-0,17	**0,47**	0,19	-0,13	0,25	1							
EC	-0,06	0,19	0,02	0,22	-0,23	0,01	0,39	-0,12	-0,06	0,06	-0,03	-0,14	0,17	**-0,41**	1						
N	0,27	**0,39**	-0,24	**0,3**	0,01	**-0,44**	-0,03	-0,2	-0,17	-0,13	0,16	**0,5**	**0,37**	-0,3	**0,41**	1					
$N\text{-}NH_4$	-0,19	0,11	**0,44**	0,25	-0,22	-0,01	0,05	0,17	-0,01	-0,13	-0,04	-0,14	-0,05	**-0,41**	**0,56**	0,16	1				
$N\text{-}NO_3$	0,06	**0,51**	-0,08	**0,36**	0,27	**-0,42**	-0,18	**0,34**	0,31	**-0,4**	0,1	**0,52**	-0,14	-0,37	0,02	0,19	0,1	1			
C	0,44	0,59	-0,11	0,35	0,51	-0,66	-0,46	0,09	0,08	-0,43	0,3	0,5	-0,03	-0,24	-0,14	-0,5	-0,14	**0,5**	1		
CN	0,29	**0,41**	0,02	0,19	**0,58**	**-0,4**	**-0,48**	**0,26**	0,24	**-0,37**	0,25	**0,75**	-0,26	-0,07	**-0,38**	-0,17	-0,24	**0,46**	**0,75**	1	
OlsP	0,12	0,08	0,37	0,04	0,08	**-0,41**	**0,37**	-0,15	-0,007	**-0,48**	0,24	0,14	0,05	-0,25	**0,1**	0,22	**0,39**	0,14	0,14	0,002	1

AcP: acid phosphatases activity, HS: humic + fulvic acids, βGlu: β-glucosidase activity, OlsP: Phosphorous-Olse, AlkP: alkaline phosphatase activity.Port: proteolytic microorganisms, WSC: water soluble carbon; Yeast; Cellul; cellulolytic microorganisms; F&Y: Fungi and yeasts; C: Organic Carbon, U: urease activity; , $N\text{-}NO_3$: Nitric nitrogen; Bact: total bacteria; Psol: Phosphate solubilizing bacteria, Amil: amilolytic bacteria;; EC: electrical conductivity. N: Organic Nitrogen; $N\text{-}NH_4$: Ammonium nitrogen; C/N ratio

Table 5.16. Pearson correlation Soil properties, second sampling -12 months(n=32)

	AcP	AlkP	U	β Glu	Bact	F&Y	Psol	Cellul	Prot	Amil	HS	OM	WSC	pH	EC	$N\text{-}NH_4$	$N\text{-}NO_3$	C	OlsP
AcP	1																		
AlkP	-0,17	1																	
U	-0,16	**0,41**	1																
β Glu	0,4	0,3	-0,07	1															
Bact	-0,13	**0,35**	**0,46**	-0,04	1														
F&Y	0,05	0,02	-0,21	0,04	**0,34**	1													
Psol	0,26	0,33	-0,05	0,21	0,29	0,2	1												
Cellul	-0,06	0,28	0,34	-0,2	0,18	0,01	0,2	1											
Prot	0,2	0,28	-0,08	-0,2	-0,03	-0,02	0,01	0,16	1										
Amil	-0,02	0,09	0,12	-0,05	**0,41**	0,06	-0,02	0,27	-0,08	1									
HS	0,26	0,23	-0,11	0,31	0,03	-0,08	0,06	0,001	-0,26	0,09	1								
OM	0,12	-0,11	-0,11	**0,68**	-0	-0,01	-0,08	-0,19	-0,22	0,26	**0,54**	1							
WSC	0	0,17	-0,12	**-0,38**	-0,25	-0,13	-0,01	0,01	-0,01	0,02	0,04	-0,13	1						
pH	0,3	-0,33	0,26	**0,68**	0,22	-0,05	-0,13	-0,08	0,13	0,001	-0,14	**-0,54**	0,32	1					
EC	0,01	-0,22	-0,05	0,11	-0,27	0,28	-0,05	-0,16	-0,02	-0,01	0,16	0,22	0,03	-0,2	1				
N-NH4	0,006	0,11	-0	0,014	0,03	-0,02	-0,28	-0,18	0,11	-0,27	0,26	-0,14	-0,26	-0,06	0,005	1			
N-NO3	0,02	0,07	-0,07	**0,46**	-0,16	-0,11	-0	0,16	-0,14	0,01	**0,41**	**0,41**	-0,08	**-0,54**	**0,66**	-0,16	1		
C	0,12	0,17	-0,1	**0,68**	-0	-0,03	-0,11	0,15	-0,23	0,26	**0,54**	**0,99**	-0,13	**-0,55**	0,22	-0,14	0,44	1	
OlsP	0,03	0,03	-0,13	0,29	-0,21	-0,17	-0,17	0,17	0,07	0,27	0,18	**0,51**	-0	**-0,47**	0,05	0,01	0,04	**0,52**	1

AcP: acid phosphatases activity, ; HS: humic + fulvic acids, βGlu: β-glucosidase activity, OlsP: Phosphorous-Olse, AlkP: alkaline phosphatase activity.Port: proteolytic microorganisms, WSC: water soluble carbon; Yeast; Cellul; cellulolytic microorganisms; F&Y: Fungi and yeasts, ;: C: Organic Carbon, U: urease activity; , $N\text{-}NO_3$: Nitric nitrogen; Bact: total bacteria; Psol: Phosphate solubilizing bacteria, Amil: amilolytic bacteria;; EC: electrical conductivity. N: Organic Nitrogen; $N\text{-}NH_4$: Ammonium nitrogen; C/N ratio

Table 5.17. Pearson correlation Soil properties, third sampling -18 months(n=32)

	AcP	AlkP	U	β Glu	Y	F	Cellul	Prot	Amil	HS	OM	WSC	pH	EC	N	$N\text{-}NH_4$	$N\text{-}NO_3$	C	C/N	OlsP
AcP	1																			
AlkP	**0,36**	1																		
U	0,13	-0,2	1																	
β Glu	**0,35**	**0,7**	-0,13	1																
Y	-0,13	0,17	-0,03	**0,37**	1															
F	0,27	0,13	0,03	0,18	0,17	1														
Cellul	0,3	**0,46**	0	**0,34**	0,18	0,17	1													
Prot	-0,05	-0,1	-0,18	0,11	**0,43**	0,1	0,14	1												
Amil	-0,07	0,1	-0,06	0,06	-0,03	0,15	-0,09	-0,1	1											
HS	0,25	0,2	0,12	0,26	**0,4**	0,24	0,18	-0,11	0,06	1										
OM	0,13	**0,38**	-0,17	0,27	-0,17	0,19	0,2	0,14	0,11	0,23	1									
WSC	-0,01	-0,13	0,16	-0,31	-0,16	-0,06	-0,07	-0,1	-0,22	-0,26	-0,14	1								
pH	-0,11	**-0,68**	0,22	**-0,45**	**0,35**	0,02	**-0,48**	-0,05	-0,23	-0,27	-0,15	0,31	1							
EC	0,09	0,33	-0,17	0,16	-0,03	-0,25	0,33	-19	-0,01	0,12	0,24	-0,27	**-0,68**	1						
N	0,06	0,25	0,23	0,16	-0,08	0	0,12	0,08	0,27	0,22	0,3	-0,2	-0,13	0,11	1					
$N\text{-}NH_4$	-0,15	-0,05	-0,48	-0,16	**0,37**	-0,3	0,15	0,22	0,02	-0,14	-0,23	0,04	0,3	0,21	-0,36	1				
$N\text{-}NO_3$	0,2	0,3	-0,15	**0,41**	**0,4**	0,009	0,24	0,1	0,03	0,18	0,18	-0,16	**-0,51**	**0,45**	-0,12	0,3	1			
C	0,13	**0,38**	-0,17	0,27	0,32	0,19	0	0,15	0,11	**0,22**	**0,99**	-0,14	-0,15	0,24	0,3	-0,23	0,18	1		
C/N	0,18	0,12	-0,1	0,16	0,21	0,33	0	0,04	-0,33	-0,06	**0,48**	0,08	-0,09	0,011	**-0,51**	-0,12	0,11	**0,43**	1	
OlsP	-0,07	0,04	-0,05	0,18	-0,17	0,14	0,28	0,27	-0,14	0,18	0,13	0,01	0,11	-0,3	0,22	-0,1	0,08	0,14	-0,1	1

AcP: acid phosphatases activity, ; HS: humic + fulvic acids, βGlu: β-glucosidase activity, OlsP: Phosphorous-Olse, AlkP: alkaline phosphatase activity.Port: proteolytic microorganisms, WSC: water soluble carbon; Yeast; Cellul; cellulolytic microorganisms; F&Y: Fungi and yeasts, ;: C: Organic Carbon, U: urease activity; , $N\text{-}NO_3$: Nitric nitrogen; Bact: total bacteria; Psol: Phosphate solubilizing bacteria, Amil: amilolytic bacteria;; EC: electrical conductivity. N: Organic Nitrogen; $N\text{-}NH_4$: Ammonium nitrogen; C/N ratio

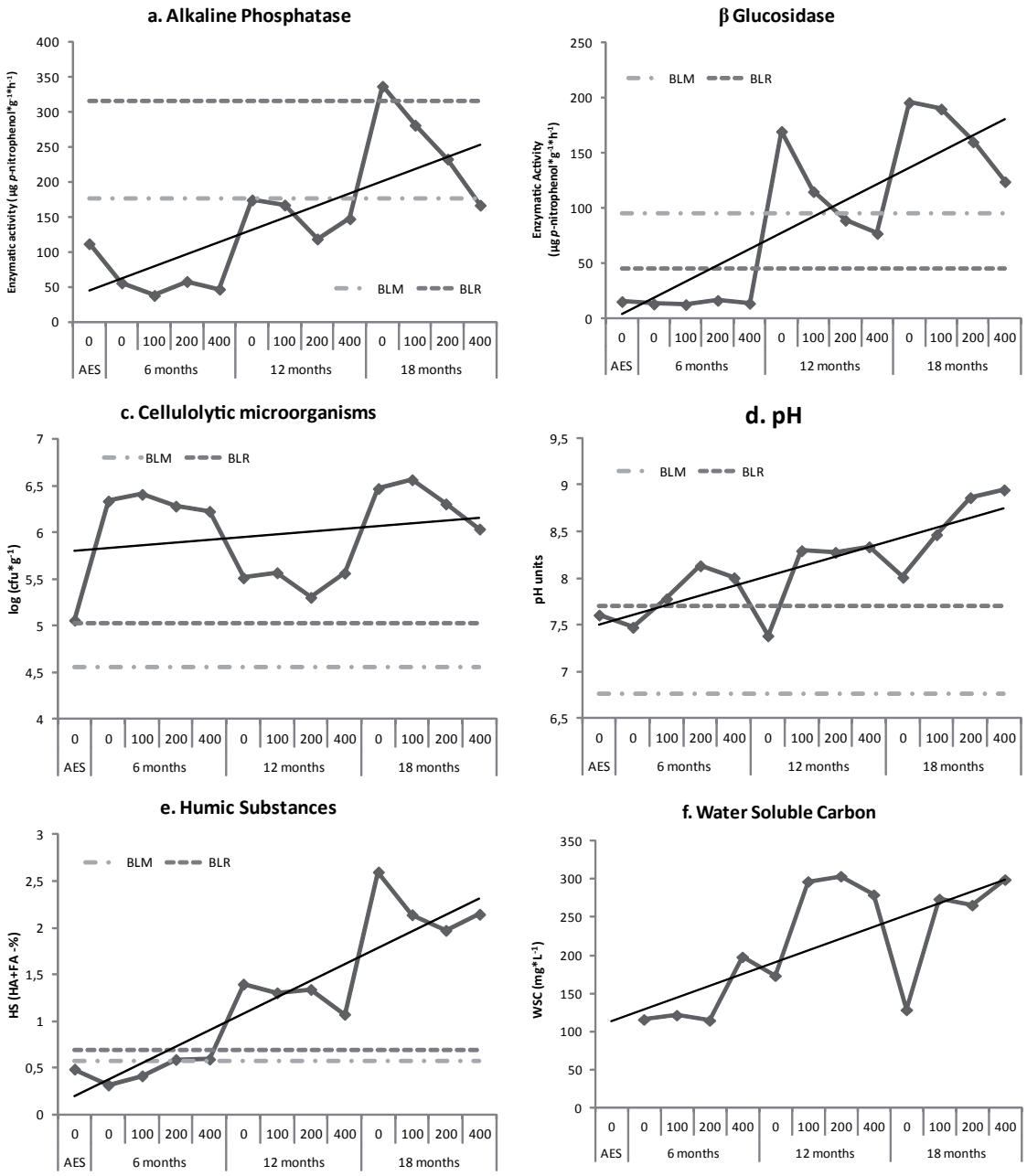


Fig. 5.22. Changes in chemical, biochemical and biological activities in soil – Field experiment- . a) alkaline phosphatase, b) β Glucosidase c) cellulolytic microorganisms, d) pH, e) humic substances and f) water soluble carbon

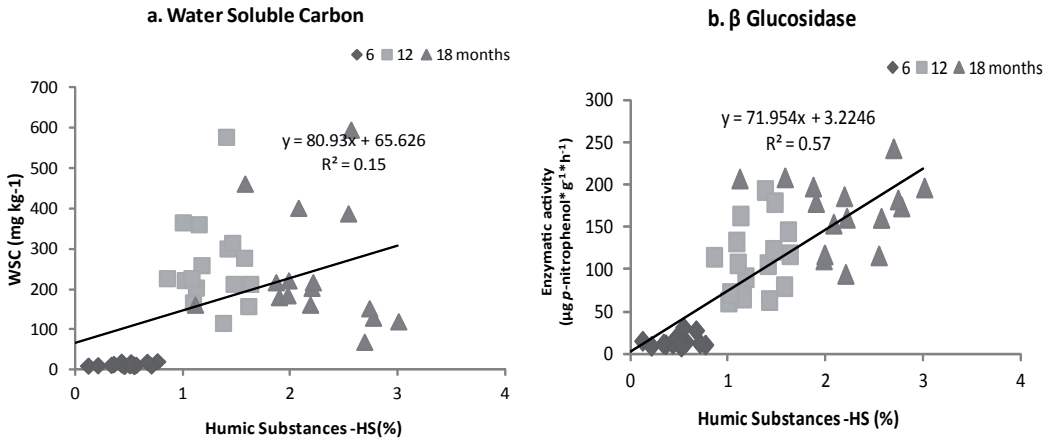


Fig. 5.23. Relation between HS in soil and a)WSC concentration and b) β Glucosidase in table grape along 18 months

In sandy soils, enzymes are associated more to soil organic matter and their activity depends on the changes in electrical charge related to pH, or presence of soluble organic matter. In this case, the applications of humic extracts leave soluble carbon available in soil solution (increase the WSC concentration in soil) and this carbon is used by microorganisms producing active enzymes and changes in cationic exchange capacity in soil. Changes in CEC and pH affect the availability of different elements, and particularly P, which is involved in root growth stimulation.

The humic extract applied to soil, had 0,8% (on wet basis) of organic matter, and 3,93 mg kg^{-1} of total P, which was not detected by Olsen technique, since it measures only inorganic, P when done by UV-VIS spectroscopy (section 5.2.1); at the same time, HS showed low enzymatic activity for acid phosphatase, presenting detectable activity only for alkaline phosphatase and non detectable for β- glucosidase. Organic P added with the humic extract, plus the organic phosphorus present in soil, as result of goat manure application at planting, increased Organic P concentration and this could be used as consequence for microbial activity.

5.3.3. Effect of C and N rates on nutritional status of table grape plants

Analysis of variance performed on chemical and biochemical properties of leaf tissue, indicated no significant differences among treatments. Similarly, it was not possible to demonstrate any effect of different C or N rates on chlorophyll content. The leaf N status often correlates with higher chlorophyll content and SPAD values; authors such as Ferrara *et al.*(2007), have shown that application of humic acids significantly increased SPAD, total chlorophyll and chlorophyll a (Cha) in leaves of different grape types, as in grapevine rootstocks 41B and 110 Richter (Zachariakis *et al.* 2001).

Nutrient values found in all treatments were inside the normal ranges for table grapes and plants did not show any deficiency or toxicity symptom (Table 5.18).

Table 5.18 Macro and micronutrient foliar content (2d. season)

Treatment	N	C	K	P	Ca	Mg	Na	Cu	Mn	Fe	Zn	B
			%						mg*kg^{-1}			
1	2,5±0,2	45,0±0,7	1,7±0,0	0,2±0,0	2,6±0,6	0,3±0,0	433±101,1	10,3±0,6	45,8±1,4	149,5±16,1	65,2±22,2	53,7±20,5
2	2,5±0,1	44,9±0,1	1,8±0,2	0,2±0,0	2,1±0,3	0,2±0,0	368,5±21,9	8,9±0,4	52,6±2,1	151,9±2,0	79,7±14,2	56,2±19,7
3	2,4±0,0	45,7±1,4	1,5±0,4	0,2±0,0	2,6±0,1	0,3±0,0	408,5±41,7	11,0±0,0	64,3±3,7	158,4±6,6	74,8±16,5	46,2±21,6
4	2,6±0,3	45,7±0,48	1,5±0,2	0,2±0,0	2,5±0,21	0,3±0,0	439,0±60,8	11,9±1,07**	74,0±40,3	176,7±23,2	91,8±19,9	55,7±25,35
5	2,3±0,07	45,5±0,16	1,3±0,13	0,2±0,11	2,6±2,5	0,3±0,0	372±40	8,6±0,2*	59,8±5,8	139,7±11,2	75,4±7,7	52,4±15,9
6	2,4±0,24	45,6±0,76	1,70,15	0,2±0,04	2,1±0,66	0,2±0,06	384±91,9	9,5±0,86	53,6±3,9	159,4±1,85	84,4±2,46	48,7±2,79
7	2,6±0,02	46,3±1,9	1,5±0,33	0,2±0,0	2,5±0,85	0,3±0,07	470±28,3	10,5±0,13	56,3±4,2	155,8±10,2	90,8±5,4	47,6±4,63
8	2,5±0,0	45,6±0,8	1,8±0,41	0,2±0,04	2,4±0,3	0,3±0,04	418,0±42,4	8,6±0,09*	69,3±27,7	142,4±3,6	77,3±14,9	60,1±32,0
9	2,4±0,2	46,6±0,12	1,6±0,08	0,3±0,09	2,3±0,15	0,2±0,01	343,0±1,41	11,4±0,57	67,9±10,44	188,8±18,1	54,8±10,5	39,2±6,9
10	2,6±0,03	45,4±0,26	1,7±0,14	0,3±0,07	2,4±0,15	0,3±0,03	397,0±41,0	9,9±1,48	71,9±1,6	169,3±26,7	65,5±6,6	45,0±11,7
11	2,7±0,02**	46,0±1,4	1,5±0,06	0,3±0,0	2,6±0,0	0m2±0,0	361,2±14,2	10,8±0,7	79,2±15,6	179,3±38,7	76,8±1,3	51,9±10,2
12	2,5±0,13	44,9±0,5	1,6±0,03	0,3±0,0	2,5±0,16	0,3±0,08	371,5±20,5	8,9±0,34	74,4±27,2	150,0±21,4	56,7±10,1	41,6±4,07
13	2,7±0,12**	45,5±0,2	1,5±0,03	0,3±0,02	3,0±0,89	0,3±0,02	435,5±80,9	10,1±1,2	103,2±19,2	161,3±23,0	77,5±9,1	47,7±6,7
14	2,5±0,16	45,5±1,37	1,7±0,37	0,2±0,01	2,3±0,4	0,2±0,01	342,0±25,1	8,9±0,8	72,0±6,2	139,4±8,9	60,1±16,0	41,0±4,6
15	2,4±0,0	44,6±0,2	1,7±0,3	0,2±0,04	2,3±0,0	0,2±0,0	457,0±1,4	9,8±0,76	74,4±0,3	161,3±5,7	101,4±36,2	56,5±12,1
16	2,3±0,06*	44,9±0,1	1,7±0,1	0,2±0,0	2,4±0,6	0,3±0,08	379,0±56,1	9,9±0,8	77,2±18,2	176,4±9,8	106,8±2,3	61,1±2,7

The N fertilizer, together with the humic extract, was applied during spring up to fruit set, to correspond with rapid uptake and demand by developing clusters, and to a lesser extent by shoots and leaves. This N was metabolized during the plant, growth but during the first season, rates over 60 kg N ha^{-1} resulted in the decrease of internal metabolic process (Fig. 5.24a). This was expected since there was more N available than the one needed. Nitrate reductase showed strong negative correlation with metabolized N (Table 5.19). Increasing N rates up to 60 kg N ha^{-1} increased NR activity and decreased metabolized N (Annex 1).

Nitrate reductase (NRed) is an enzyme inducible by substrate, that catalyzes the reduction of nitrate (NO_3^-) ion absorbed through the roots, to nitrite (NO_2^-) and has been reported as an indicator of nitrogen metabolism in Thompson seedless, under different nitrogen fertilization regimes. In this research the NR activity was inversely related to chlorophyll content and yield in the first year, and showed no association with nitrate or ammonium nitrogen concentrations. During the second year, NR activity was not correlated with C or N rates, but it showed correlations with some nutrients such as B and Zn as well as with SPAD (Table 5.20). Nitrogen application was related with more plant growth which in turn means less chlorophyll concentration (Fig.5.24 b) and more metabolized nitrogen.

Table 5.19. Pearson correlation analysis among measured foliar parameters in field experiment (n=32). first season

	CR	NR	Cha	Chb	Chl T	NRed	N	$N\text{-}NH_4$	$N\text{-}NO_3$	MetN	C	C/N
CR	1											
NR	,000	1										
Cha	-,287	**-,364**	1									
Chb	-,296	-,225	**,931**	1								
TChl	-,292	-,342	**,998**	**,954**	1							
NRed	,231	,174	**-,501**	**-,501**	**-,507**	1						
N	,000	,040	,119	,110	,119	,296	1					
$N\text{-}NH_4$	,122	,083	,117	,057	,107	,197	**,525**	1				
$N\text{-}NO_3$	-,214	,203	,077	,127	,087	,031	,032	,054	1			
Met N	,010	-,039	,322	**,361**	,333	**-,826**	**-,440**	-,175	-,130	1		
C	-,042	**-,448**	,116	,057	,106	-,226	-,022	-,206	-,213	,212	1	
C/N	-,006	-,137	-,181	-,189	-,184	-,192	**-,966**	**-,565**	-,065	,313	,205	1

CR: Carbon Rate. NR: Nitrogen Rate. Ch a: Chlorophyll a. Ch b: Chlorophyll b. T Chl: Total Chlorophyll. NRed: Nitrate reductase. N: Organic Nitrogen. $N\text{-}NH_4$: ammoniacal nitrogen. $N\text{-}NO_3$: Nitric nitrogen MetN: metabolized nitrogen. C:Organic Carbon. C/N: carbon:nitrogen ratio.

From bunch closure to veraison, when shoot growth slows, available N will also be allocated and incorporated into permanent vine structures for storage. The timing of N fertilizers, like other nutrients, should occur when demand is high and uptake is rapid. Nitrogen is needed most during the period of rapid vegetative growth, which occurs during the spring, from budbreak to early berry development. It is during this period that new growth may accumulate up to 50% of its annual N requirement (Conradie, 2005). Because active root growth and mineral uptake is generally minimal during the budbreak period, N demand is met primarily from reserves stored in the roots and other permanent woody structures (trunk, cordons and canes).

Table 5.20. Pearson correlation analysis among measured foliar parameters in Field experiment second season (n=32)

	C	N	OM	pH	N	NH_4	NO_3	B	Zn	Fe	Mn	Ca	Na	Mg	P	K	C	N	SPAD	Chl a	Chl b	TChl	Nred
C	1																						
N	,000	1																					
OM	-,029	,011	1																				
pH	**,692**	**-,384**	-,153	1																			
EC	-,118	,341	,252	**-,684**																			
N	-,204	**-,399**	,279	-,138	1																		
NH_4	-,263	,200	-,237	-,303	**-,357**	1																	
NO_3	-,161	**,382**	,189	**-,518**	-,125	,310	1																
B	,191	-,059	-,009	,133	**-,390**	,176	,152	1															
Zn	,315	,114	-,096	,115	-,293	,158	,177	**,703**	1														
Fe	,068	,099	,269	-,096	-,042	-,326	,011	,110	,207	1													
Mn	,127	**,449**	,172	-,062	-,346	**,468**	,342	-,111	-,041	-,161	1												
Ca	-,042	,034	,259	-,122	,189	-,065	-,022	**-,460**	-,280	-,101	**,436**	1											
Na	,104	-,084	,090	-,017	-,033	,115	**,383**	**,439**	**,510**	-,130	-,074	-,214	1										
Mg	,087	-,096	,002	-,014	,103	,077	,002	-,276	-,125	**-,476**	**,370**	**,734**	,074	1									
P	-,069	,195	,085	-,178	-,327	,343	,305	,195	-,202	,034	**,396**	,000	-,190	-,191	1								
K	,107	,039	,009	,147	-,145	-,046	-,206	**,578**	**,350**	,050	-,315	**-,674**	,152	**-,555**	,121	1							
C	-,136	-,136	-,025	-,097	,239	-,028	,131	-,269	-,184	**,378**	-,088	,026	-,063	-,205	-,003	-,332	1						
N	-,037	,044	,249	-,108	,168	-,293	-,025	-,335	-,230	,242	,028	**,441**	-,057	,038	-,045	-,214	,315	1					
SPAD	-,157	-,068	,003	-,112	-,031	,309	,303	-,183	**-,432**	-,198	**,471**	,243	-,232	,113	**,557**	**-,405**	,186	,052	1				
Chl a	-,134	-,182	**,427**	-,148	,215	**-,423**	-,004	,013	-,115	**,429**	-,168	,235	-,219	-,118	,203	,166	,120	,347	,055	1			
Chl b	-,203	-,302	,024	-,154	,022	**,395**	,087	,087	-,036	-,021	,106	,142	-,126	,013	**,486**	,060	,087	,026	**,442**	**,442**	1		
Chl total	-,161	-,224	**,387**	-,163	,197	-,298	,014	,029	-,110	**,380**	-,129	,238	-,221	-,103	,280	,160	,125	,315	,139	**,983**	**,597**	1	
Nred	,328	-,025	-,152	,112	,025	-,257	-,047	**,425**	**,600**	,349	-,321	-,292	,269	-,216	-,178	**,402**	-,015	-,253	**-,396**	,126	,004	,113	1

C: carbon rate; N: nitrogen rate; OM: organic matter; EC: electrical conductivity; N: organic nitrogen; Nitrogen. N-NH_4: ammoniacal nitrogen. N-NO_3: Nitric nitrogen, C: Foliar Total carbon; N: Foliar Total Nitrogen; SPAD: Chlorophyll by Spad; Ch a: Chlorophyll a. Ch b: Chlorophyll b. T Chl: Total Chlorophyll. NRed: Nitrate reductase.

Several authors report increases in N content due to humic acid applications, both in leaf and soil, in crops such as olive (Fernandez- Escobar *et al.*, 1996), tomato (David *et al.*, 1994), asparagus (Tejada and González, 2003), using different origin and concentration of HS. However, in this study no effect of C rate on the nutritional status of table grape plants could be demonstrated.

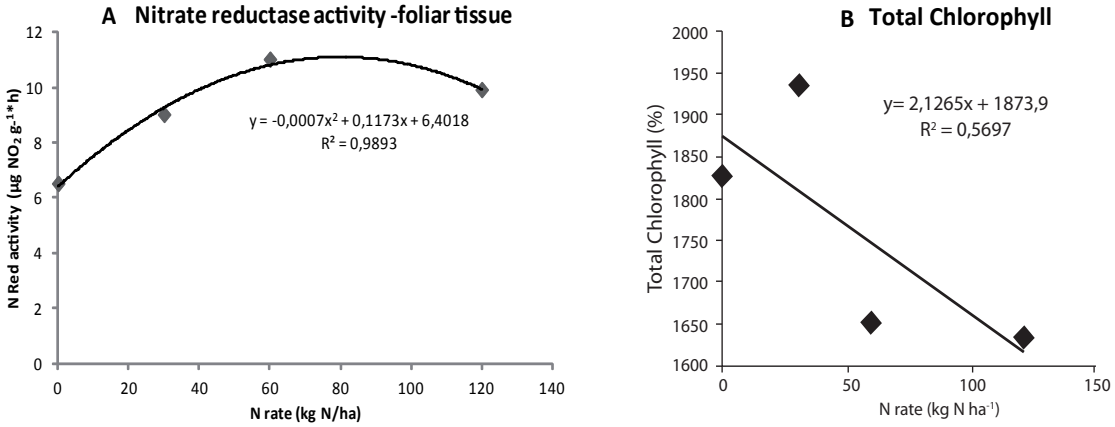


Fig. 5.24. Effect of C rate on a) Nitrate reductase activity (Nred) an b) Total Chlorophyll

5.3.4. Effect of C and N rates on fruit quality

The table grape is a non-climateric fruit with relatively low rate of physiological activity; it is subject of water loss following harvest. Consumers like a fruit with exportation quality characteristics, like berry firmness, without defects such as decay, cracked berries, stem browning, shriveling, sunburned, dried berries or insect damage. In this experiment, two harvest were performer; at the end of February 2010 and 2011, respectively. Properties used to confirm the harvest were sugar content (SS) 13-14,5%, yellowish-green color and no split or crush berries (Bauzá, Quality Control, Harvest. 2010, INN, 1991).

In terms of quality, table grapes harvest index reefers to minimum amount of sugar, expressed as soluble solids (SS). A value of 16,5% Brix is usually accepted as a valid harvest index for Thompson Seedless exporting procedures (USDA 1971, INN, 1991), and the composition of sugars, organic acids and amino acids in grape berries are crucial to define market destination. In this study, brix, pH and tritable acidity in all treatments were above the harvest index and no differences were observed among treatments (Table 5.21) differences ($p>0,05$) were analysis of variance performed on both evaluated seasons revealed that there were not significant effects ($P>0,05$) of either C or N rates on total yield or fruit quality parameters (Table 5.21). The results are similar to those obtained by Muñoz-Robledo (2011), for Thompson seedless in Chile, and confirm the proper maturity, in terms of sugar content, at harvest, and the importance of this parameter as a sugar content indicator and harvesting index. The natural pH of Thompson seedless grape is around 3,6 for fresh fruit (Zheng *et al.,* 2012). However, Pearson correlation for second season (Table 5.22), showed a strong relationship between pH an tritable acidity as was expected, and between pH, brix and tritable acidity and Polyphenoloxidase. At the same time this enzyme presented negative correlation with acid and alkaline phosphatases, and βGlucosidase activity in soil (data no shown, Annex 2).

Polyphenoloxidase is the enzyme associated to enzymatic browning process in fruits, and has been proved to be one of the main factors contributing to colour change of most processed fruits, including grape. The negative correlations with sugar content was also described by Zheng et al. (2012), who characterized the enzyme from Thompson seedless. In grapes, glucose and fructose account for at least 95% of the carbohydrates, and sucrose, the rest (Muñoz-Robledo, 2011) and this sugar concentration, specially, sucrose causes inhibition of PPO as well as low pHs (2,5-5,0).

Table 5.21 Fruit quality parameters for second harvest (2011).

Treatment	Total Chlorophyll µg/g	Total Polyphenols DO/100 g	Anthocyanins DO/100 g	Brix	Total Acidity g L^{-1} Ac tart	pH	Polyphenoloxidase PPO
1	1,4±1,3	57,6±10,7	0,4±0,0	19,8±0.6	3,7±0,1	3,66±0,1	613,4±442.5
2	1,6±1,5	48,7±6,3	0,3±0,0	17,8±3.0	3,6±0,1	3,64±0,1	523±315.7
3	1,8±1,6	50,4±5,3	0,4±0,0	16,8±0.9	3,5±0,2	3,53±0,1	737±696
4	2,1±2,1	48,5±0,5	0,3±0,0	18,0±1.2	3,6±0,1	3,61±0,1	780±704
5	2,4±2,1	48±3,3	0,4±0,0	16,4±3.7	3,7±0,2	3,56±0,1	760±686
6	3,1±0,4	51,9±7,0	0,3±0,1	17,8±2.3	3,6±0,0	3,66±0,1	545±264
7	1,6±1,3	55,3±0,6	0,4±0,0	19,4±0.6	3,7±0,0	3,62±0,1	773±519
8	3,9±0,0	44,5±3,4	0,4±0,1	18,6±2.5	3,7±0,0	3,68±0,0	423±241
9	2,4±1,6	42,6±14,9	0,4±0,0	18,5±2.3	3,6±0,2	3,66±0,0	851±784
10	3,4±3,4	54,5±0,3	0,4±0,0	18,4±0.4	3,6±0,1	3,58±0,1	383±143
11	2,4±1,4	39,8±12,8	0,4±0,0	19,2±0.1	3,6±0,0	3,62±0,1	477±204
12	2,5±1,3	50,9±7,1	0,4±0,1	19,7±0.7	3,6±0,0	3,61±0,0	937±813
13	3,4±0,5	48,2±8,1	0,4±0,0	18,3±0.4	3,7±0,2	3,66±0,1	238±81
14	4,6±0,8	42,2±0,2	0,4±0,1	18,2±2.3	3,6±0,1	3,56±0,1	492±339
15	4,2±1,0	46,4±3,2	0,4±0,1	19,4±0.8	3,7±0,1	3,67±0,0	268±13
16	3,8±1,2	36,37,0	0,3±0,0	20,3±0.1	3,8±0,2	3,79±0,1	407±53
LSD	NS	NS	NS	NS	NS	NS	NS

TChl: Chlorophyill content, Poli: Poliphenol content; Anto: Anthocyanins; TA : Total Acidity ;
PPO: Poliphenoloxidase activity

Table 5.21a Pearson correlation for fruit properties (2d. season)

	ExpH	TH	%EXP	Chlo	Poly	Antho	Brix	pH	TA	PPO
ExpH	1									
TH	**,917**	1								
%EXP	**,457**	**,87**	1							
Chlo	,134	,166	,001	1						
Poly	,078	,072	-,016	-,278	1					
Antho	,050	,016	-,045	,077	,123	1				
Brix	,154	,213	-,070	,314	-,108	,238	1			
pH	,046	-,007	,082	-,291	-,034	**-,370**	,061	1		
TA	-,033	,004	,001	,047	,133	,023	,000	**-,502**	1	
PPO	,007	-,051	,190	,265	-,066	,223	**,338**	**-,382**	**,374**	1

EH: Exportable harvest., TH: Total harvest, %EXP: % exportable; Chlo: Chlorophyill content, Poly: Polyphenol content; Anto: Anthocyanins; TA : Total Acidity ; PPO: Polyphenoloxidase activity.

Fig. 5.25 Harvest detail. Second season

During the first season exporting yields varied between 3,4 and 11,6 Ton ha^{-1}. Total and exportable yields during the second season (2010/2011) varied from 14,6 to 28,4 Ton ha^{-1} and 8,9 to 12,5 Ton ha^{-1}, respectively, with an exporting proportion varying from 59 to 77%.These values are within the range of the expected yield for a third-leaf orchard.

In both seasons there was a significant (P<0,1) effect of C rate on exportable yield. This increased linearly with C rate (Fig. 5.26 a and b).It was found that, averaging across all N rates, the application of 1 kg of C ha^{-1}, increase exporting yield by approximately 4 to 8 kg of grape/ ha (Fig. 5.27b). This means that with the maximum C rate (400 kg C ha^{-1}), the increase in yield will be 1600 to 3200 kg of fruit ha^{-1} or 250 to 500 boxes/ha (Figure 5.27c).

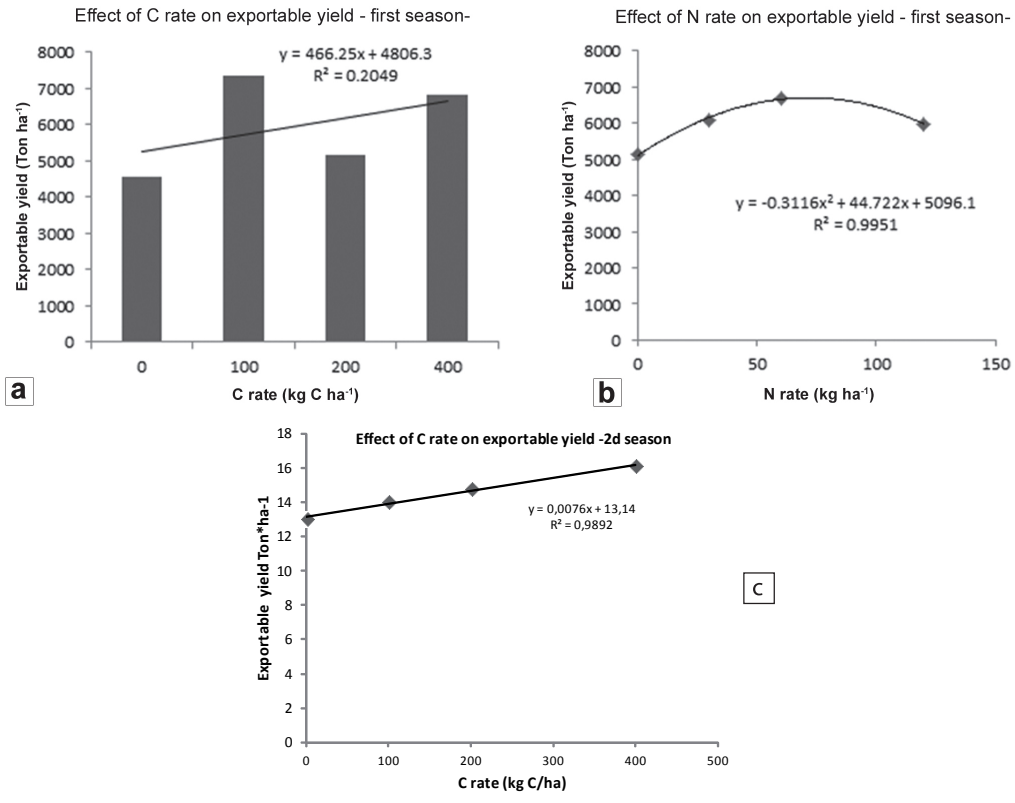


Figure 5.26. Effect of C and N rate on exporting yield. a) carbon rate – first season, b) N rate first season, c) C rate second season (mean of 4 N rates).

Nitrogen rate affected exportable yield only during the first season. Maximun exportable yield was achieved with the application of 60 kg N/ha, as Novatec Solub 21 (Fig. 5.26b). The null response to N during the second season was probably due that the plants of all treatments had sufficient reserves to reach good yields.

5.3.5. Compost, soil and fruit quality Minimum Data Set

The concept of integrated indicators for soil quality proposed by Larson and Pierce (1991), applies terms of soil ecology to assess the sustainability of the soil and ecosystem management, combining a variety of information, using multi-objective analysis (Andrews and Carroll, 2001).

The Minimum Data Set is the third steps in the definition of a Soil Quality Index (SQI). Four main steps are usually followed: (i) to define the management goal(s); (ii) to select a minimum data set (MDS) of indicators that best represent efficiently and effectively soil function, as determined by the specific management goals; (iii) to score the MDS indicators based on their performance of soil function; and (iv) to integrate the indicator score into an index of soil quality (Karlen *et al.*, 2003, Mandal *et al.*, 2008). Although the steps to obtain a SQI seem clear, there is little information about the practical way of obtaining the weights of each property and integrating them into an index. Ortega and Santibañez (2007) proposed a soil index based on the variance of each soil fertility property, giving more weight to those properties varying more and viceversa. In their work, the weights were estimated by Principal Component Analysis (PCA) or by the coefficient of variation (CV) of each property.

Crop yield can be an important indicator of soil quality because it serves as a plant bioassay of the interacting soil characteristics. However, productivity alone may not always be the main criteria by which sustainability of an agricultural system should be judged.

A proper index to measure compost, soil and fruit quality should have certain characteristics:

1. be simple to measure.

2. be sensitive to changes in time and management.

The sensitivity to changes can be measured in several ways: 1) establishing if the property changes over time, as a result of the management imposed, which technically can be proven by regressing the property of interest against time, 2) establishing how frequently the property changes over time under different agro ecosystem managements, and 3) through the variance of each property. In general, those properties showing more variability among treatments are supposed to be more sensitive to changes in management. Since the variance is dependent upon the measurement unit of the property, coefficient of variation is used instead.

When several properties are measured on compost, fruit, and soil, the first step is to see if the data set can be reduced to two or three properties that can explain most of the variance in it. In this work, principal component analysis (PCA) was performed on compost, soil and fruit variables. Results demonstrated that the three first components explained 62, 47 and 67% of the total variance for compost, soil and fruit, respectively. This means that, in the three matrices, but particularly in the case of soil, most variables should be included to explain the total variance of the data set. If an index was to be built using PCA, this should be done including all the measured variables, which is impractical.

In this study, the corresponding compost, fruit and soil minimum data sete were estimated according to sensitivity of each property.

In the case of the field experiment, changes of each measured soil property on each treatment, with respect to the agroecosystem base line, were determined. Each of the 16 treatments (including C and N rates), was considered a population, so the idea was to determine the proportion of the 16 populations presenting changes in a given property over time (as compared to the base line). The weight (slope) of each property over time was determined, in order to

rank all the properties in terms of its sensitivity. When the slope was not significantly different from zero (P>0,05) meant that there were not changes in time over the base line and the property was not sensitive to changes in time in a given treatment. On the other hand the larger the coefficient (slope), the higher the weight of the property and vice versa.

The same procedure was done in the case of compost and the soil of the pot experiment.

The regression of each standardized variable on time in each treatment determined that the soil properties changing most frequently and with larger weights (>0,1) were acid phosphatase (AcP), humic substances (HS), and β-Glucosidase (β-Glu).

In the case of compost, the variable changing most frequently and with weights > 0.1 were AcP, N-NH_4, U, Cellul, and Bact. On the other hand, in the case of soil from the pot experiment, the variables changing the most and more frequently, over the baseline, were β-Glu, Psol, N-NH_4,EC, C, and pH. Table 5.23 shows the absolute weights of each property, originally greater than 0,1, corrected for its sum to be equal to one. It can be seen that each matrix (soil or compost), and experiment, had its own set of properties as part of a potential compost or soil quality index.

The signs of each property should be assigned by expert criteria. Thus, probably, in the case of soil of the field experiment one would like to give negative sign to the β-Glu if the idea were to reduce OM cycling.

Table 5.23 Absolute weights for biochemical properties selected for estimating matrix quality.

	Compost	Soil		Fruit
		Pots	Field	
AcP	0,14		0,34	
HS			0,34	
β Glu		0,11	0,32	
Psol		0,26		
N-NH_4	0,16	0,22		
EC		0,19		
C		0,14		
pH		0,08		
U	0,42			
Cellul	0,17			
Bact	0,11			
UPPO				0,56
T Chl				0,44
Total	1	1	1	1

AcP: Acid Phosphatase; HS: humic substances; B (beta) Glu: B(simbolo beta) glucosidase; Psol: phosphate solubilizing bacteria; N-NH4 (4 subindice): ammonium nitrogen; EC: electrical conductivity; C:Organic Carbon; U: ureases; Cellul: cellulolytic microorganisms; Bact: total bacteria; UPPO: polyphenoloxidase, T Chl: total chorophyll.

In case of a fruit quality Minimum Data Set (MDS), since normally quality variables are measured at maturity, it does not make sense to study its evolution over time to determine the weight of each variable, but trying to estimate their weights based on their variation within the data set. Thus, variables having larger Coefficient of Variation (CV) will have more weight and viceversa (Ortega and Santibáñez, 2007). Only those properties that weighted more than the average weight plus 0,5 standard deviation were selected. Thus, only those CV being part of the 30% superior under a normal distribution were considered. Only two properties were selected as sensitive. These were: total chlorophyll (0,3), and phenol oxidase activity (0,38). The weights were adjusted to sum one, so the final weights were: chlorophyll=0,44, and phenol oxidase activity=0,56. Clearly this index will be closer to phenol oxidase activity than to anthocyanin content; thus, an FQI should have these properties with the proposed weights given a proper sign (Table 5.23).

The selected variables and their corresponding weights, with the proper signs, would allow obtaining quality indices for compost (CQI), soil (SQI), and fruit (FQI), which should be validated against an independent data set. From these results it is clear that most probably the SQIs would be site-specific and would have different properties on them. The present work developed a framework to select properties to be potentially included a quality index. However, the validation of the developed indices was out of the scope of this research.

Hussain *et al.* (1999) adapted a SQI in order to assess the effect of three management systems (no-till, chisel plow and moldboard plow) on SQ under maize and soybean crops. They concluded that the methodology was sensitive to detect changes on SQ caused by soil management. Glover el al. (2000) used a similar methodology to assess SQ in apple orchards and observed higher SQI in integrated management than in conventional or organic managements. In Brazil, Melo Filho el al. (2007), assessed a SQI under natural forest, observing a low value of SQI mostly because they oriented its SQI to crop production.

CHAPTER VI

General Conclusions

The use of organic matter is an agronomic practice steadily growing in the world's horticulture. Under technified irrigation systems, the use of solid organic amendments is difficult and new ways of incorporating organic matter must be explored and their effects determined; among them the use of liquid humus is a technology of great potential. In this section, the conclusions of this work are matched with the established hypotheses.

HYPOTHESIS 1: *It is possible to define maturity of compost, in terms of content of humic substances, including functional groups of hydrolytic microorganisms, hydrolytic enzymes, C and N characteristics.*

Organic Wastes from the pisco industry in Chile, represent a significant amount of organic matter, that can be processed by aerobic processes, like composting. The compost obtained under the proposed mixes in this study, started maturity process from day 60 of composting showing an increase of the concentration of humic substances (humic and fulvic acids). The final product, showed characteristics consistent with the Chilean Official Standard 2880 (2004), European Union (2010) and Compost Quality Council (2008), standards. It was possible to select some variables that could be part of a maturity index for compost, measured in terms of HS content, including: AcP, Amilolytic and total bacteria, and C/N ratio.

Additionally, if it is true that national and international quality standards for compost include microbial groups as indicators, only consider human pathogens and indicators of fecal contamination. Some hydrolytic enzymes producing microorganisms, as well as beneficial microorganisms such as phosphate solubilizer bacteria, should be consider as compost quality indicator, in relation to the positive effect of compost once applied to soil as organic amendment.

Finally, properties directly related to the major components of organic matter, such as C and N, must be included to evaluate quality of compost. However, the relationship $N\text{-}NH_4/N.\text{-}NO_3$ is a good indicator, especially in the case of mature compost or organic amendments.

The features observed regarding humic and fulvic acids contents as well as the extraction ratio defined, (compost: extractant), allowed the use of material obtained in the extraction of humic substances and the production of "liquid humus".

HYPOTHESIS 2: *Applying liquid humus can be equivalent or better than compost as organic amendment in terms of its effects on soil and fruit properties.*

Liquid humus application resulted to be equivalent to compost as organic amendment, in terms of water-soluble carbon and microbial activity. However, only compost had a possitive effect on root density, which was improved by the addition of a microbial inoculant.

Applications of compost and liquid humus resulted in significant changes in soil properties and plant. Water-soluble carbon showed increases in both cases, but the rate of increase was greater with liquid humus applications. Compost positively affect enzymatic activities associated with the hydrolysis of organic matter and the density and root mass. However, orthogonal contrasts analysis showed significant differences in root density with application of a microbial inoculant obtained from the same compost.

Results suggest that applications of compost stimulate native microbial populations, which could not be recovered by plating methods, but that may be included in the plant growth promoting microorganisms (PGPM).

The use of liquid humus as carbon source in soil, showed significant effects on different properties of soil. These effects are demonstrated by the positive correlations between hydrolytic enzymes tested, and some groups of microorganisms selected for evaluation as indicators of soil quality. Some particular correlations were obtained between acid and alkaline phosphatases as well as the β-glucosidase, and groups of cellulolytic and proteolytic microorganisms.

Also there was a significant correlation between yeasts and organic matter content under field conditions.

Microbiological groups selected as indicators, did not show sensitivity to changes in time and management, however enzymes produced by them resulted in good indicators.

In terms of fruit quality, the polyphenoloxidase activity, measured in berries, was a good indicator. This enzymatic activity, showed negative and strong correlation with chlorophyll content in fruit.

Liquid humus in field, resulted in a significant increment of exportable harvest each kg of C increased exportable yield between 4 to 8 kg grape/ha. However as numerous authors have mentioned, increment in N rate did not result in yield increase.

HYPOTHESIS 3: *Changes in soil quality indicators, take place at different speeds and directions depending on organic matter application compared with base line soils*

Application of liquid humus improved soil quality indicators (alkaline phosphatase, pH, β glucosidase, humic substances, Olsen phosphorus and water soluble carbon), carbon, whose changes took place at different speeds compared with base line soils.

The riparian vegetation base line soil selected to compare the treatments or populations under field conditions, resulted in higher concentration of OM, organic and soluble N, more acidic and, higher enzymatic activity and microbial bacteria respect to mountain xerophilic forest and agroecosystem soil. Among the measured properties, there were more sensitive quality indicators, which changed at a higher speed and more frequently. These properties are: β glucosidase and Acid Phosphatase activity; phosphate solubilizing bacteria; $N\text{-}NH_4$, electrical conductivity, pH, and humic substances content.

Alkaline phosphatase, as well as the enzyme β-glucosidase, are seen as good indicators for soil quality under table grape. The biochemical changes in these properties occurred in 100% of the populations evaluated.

Also changes in the total content of humic substances in soil indicate the incremental effect of humic acids applications in time.

HYPOTHESIS 4: *It is possible to define a minimum data set to create composed quality indices based on a linear combination of several soil and fruit properties*

Liquid humus application, generated differences in polyphenol oxydase and chlorophyll content, and these two properties could be suggested as a part of minimum data set for fruit quality for table grape.

The regression of each standardized variable on time in each treatment determined that the soil properties changing most frequently and with larger weights (>0,1) were acid phosphatase, humic substances, and β-Glucosidase which can be part of the mimimum data set to create composed.

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ANNEX 1

Table 5.24. Foliar analysis 2d. year of production

T	Cha mg*g^{-1} fw	Chb mg*g^{-1} fw	Total Chl mg*g^{-1} fw	Nreductase UNR (µg NO_2/g*h)	N %	N-NH_4 ppm	N-NO_3 ppm	Nmet %	C %	C/N
1	1577,3±195,4	311,1±25,4	1888,4±220,9	6,1±5,6	2,9±0,2	480±46,8	99,1±27,9	98,8±1,2	48,4±0,6	16,9±1,1
2	1563,3±133,6	265,4±29,0	1582±162,7	6,2±6,6	3,0±0,4	546,9±5,4	55,1±3,8	98,9±1,2	49,1±0,0	16,6±2,0
3	1683,7±227,1	337,1±63,2	2020,8±290,4	7,0±7,4	2,9±0,1	523,8±74,5	65,9±26,8	98,8±1,2	48,40,5	16,70,4
4	1518,7±133,3	297,9±8,7	1816,6±142,1	7,9±3,3	2,8±0,3	536,6±136,8	49,7±11,5	98,9±1,2	48,8±0,1	17,2±1,9
5	1884,1±106,6	415,2±92,0	2299,3±198,6	8,0±8,3	2,9±0,0	568,3±106,6	65,93,8	98,8±1,4	48,1±0,7	16,6±0,3
6	1687,6±29,05	339,1±18,2	2026,8±47,3	10,4±3,1	2,8±0,0	630,5±169,1	93,0±19,1	98,5±1,7	48,0,4	17,5±0,2
7	1370±120,8	272,7±30,0	1643,7±150,9	6,8±3,4	2,9±0,1	607,1±90,2	57,8±0,0	98,8±1,4	48,5±0,8	16,50,9
8	1485,3±6,45	285,8±0,7	1771,2±5,7	11,8±6,9	2,9±0,3	560,3±170,0	63,2±22,9	98,9±1,2	48,8±0,4	16,8±1,7
9	1545,2±139,6	321,7±39,1	1867,7±178,8	8,9±5,6	2,90,1	486,6±55,3	74,0±7,6	98,9±1,1	48,9±0,5	16,7±0,6
10	1192,8±63,5	245,7±30,2	1438,5±93,7	12,0±12,0	2,6±0,0	446,0±70,9	58,116,4	98,9±1,1	48,3±0,4	18,7±0,4
11	1349,3±315,2	279,5±76,4	1628,5±391,6	12,21±8,3	2,9±0,0	516,0±38,6	40,7±8,2	98,9±1,3	48,6±0,9	16,9±0,4
12	1389,8±61,4	279,5±12,7	1676±74,1	11,8±9,3	2,9±0,0	682,7±74,0	50,7±27,0	98,7±1,7	48,2±0,5	16,6±1,0
13	1455,4±332,4	297,6±68,1	1753,6±400,6	8,2±5,6	3,1±0,2	655,5±37,5	58,1±16,4	98,7±1,6	48,4±0,2	15,8±0,2
14	1455,4±332,4	297,6±68,1	1753,1±400,6	7,2±6,1	2,9±0,0	676,8±76,1	105,5±55	98,5±1,5	47,7±0,1	16,4±0,1
15	1297,8±194,4	266,4±31,1	1564,3±225,6	8,8±6.9	2,8±0,0	325,2±21,0	77,8±15,7	99,1±0,9	48,1±0,7	17,4±0,0
16	1196,6±279,5	270,3±47,2	1466,9±326,8	15,3±10,7	3,0±0,2	616,2±126,0	89,4±33,8	98,7±1,5	47,9±0,9	16,2±1,3

ANNEX 2

Table 5.22. Pearson Correlation among all properties measured in field experiment.

	C	N	spad1	Chla1	Chlb1	TChl1	NR1	M1	L1	XL1	H1	Exp	TH	%EXP	TChlo	Poly	Anto	Brix	pH	AT	UPPO	ACP	AlkP	U	BGlu	F	Y	Cellu	Port	Amil	HS	OM	WSC	pH	EC	N	NH4	NO3	C	CN	Ols P	B	Zn	Fe	Cu	Mn	Ca	Na	Mg	P	K	C	N	SPAD	Chla	Chlb	TChl	NRed
C	1																																																									
N	,000	1																																																										
spad1	-,214	,193	1																																																								
Chla1	-,287	**-,364**	,261	1																																																								
Chlb1	-,296	-,225	,299	**,931**	1																																																								
TChl1	-,292	-,342	,272	**,998**	**,954**	1																																																							
NR2	,231	,174	**-,440**	**-,501**	**-,501**	**-,507**	1																																																						
M2	-,116	,028	,222	**,376**	,341	**,373**	-,241	1																																																					
L1	-,106	,191	,208	,127	,147	,132	**-,616**	,229	1																																																				
XL1	,299	-,016	-,014	,085	,101	,089	-,323	-,077	,318	1																																																		
H1	-,070	,197	,244	,240	,256	,246	**-,628**	**,479**	**,941**	**,406**	1																																																	
Export	,126	,062	**,412**	-,216	-,220	-,219	-,136	,092	,282	-,049	,224	1																																														
H	,063	,068	,353	-,298	-,289	-,299	-,200	-,013	**,388**	,056	,294	**,917**	1																																														
%EXP	,186	-,078	,301	,197	,153	,190	,001	,253	-,070	-,160	-,030	**,457**	,087	1																																												
chlo	,048	**,417**	,367	,041	,165	,065	**-,453**	,322	**,462**	**,352**	**,555**	,134	,166	,001	1																																											
Poly	-,214	**-,406**	-,033	-,075	-,158	-,091	,080	-,069	-,221	**-,391**	-,270	,078	,072	-,016	-,278	1																																											
Antho	-,107	,075	-,036	,056	,051	,056	**-,395**	,026	,297	,048	,330	,050	,016	-,045	,077	,123	1																																											
Brix	,237	,258	-,100	-,303	-,181	-,283	-,248	,008	,343	**,419**	,347	,154	,213	-,070	,314	-,108	,238	1																																											
pH	,158	,201	**-,426**	**-,494**	**-,454**	**-,492**	**,656**	-,296	**-,460**	-,291	**-,529**	,046	-,007	,082	-,291	-,034	**-,370**	,061	1																																										
AT	-,079	-,268	,073	,041	,071	,047	-,349	,132	,086	,004	,077	-,033	,004	,001	,047	,133	,023	,000	**-,502**	1																																									
UPPO	,057	-,290	,128	,336	**,407**	**,353**	**-,575**	,181	,093	,308	,182	,007	-,051	,190	,265	-,066	,223	,338	**-,382**	**,374**	1																																									
AcP	,069	,043	-,057	-,161	-,199	-,170	**,492**	,249	-,217	-,131	-,131	,032	,025	,016	,001	-,085	**-,515**	-,197	**,438**	**-,464**	**-,474**	1																																									
AlkP	**-,535**	**,393**	-,197	-,262	-,238	-,260	,331	-,240	-,163	**-,351**	-,269	,019	,077	-,192	-,124	-,032	-,254	-,201	**,480**	-,250	**-,538**	**,361**	1																																									
U	,254	**-,493**	-,333	-,017	-,189	-,050	,200	-,134	**-,350**	-,009	-,327	,021	,046	-,067	**-,509**	,173	-,032	-,282	,107	-,014	-,130	,134	-,207	1																																									
BGlu	**-,464**	,224	-,315	,020	-,046	,008	,309	-,181	-,106	-,242	-,163	**-,451**	**-,380**	-,330	-,146	-,110	-,144	**-,422**	,174	**-,351**	**-,542**	**,353**	**,700**	-,133	1																																								
F	-,228	-,110	-,004	-,148	-,217	-,163	,333	,087	-,196	-,342	-,172	-,112	-,045	-,234	-,239	,156	-,104	-,177	,245	-,177	-,071	,278	,138	,086	,181	1																																							
Y	-,007	,211	-,312	-,100	-,056	-,092	,097	-,266	-,024	,033	-,042	-,332	-,253	-,310	-,097	-,331	,098	-,065	-,033	,105	-,028	-,139	,179	-,033	**,368**	,178	1																																					
Cellu	**-,508**	-,084	,128	,123	,142	,128	,126	,011	-,054	-,104	-,054	,005	,068	-,168	-,085	,084	-,223	-,326	,073	-,028	-,214	,306	**,463**	-,006	,342	,177	,186	1																																				
Proteo	-,005	-,031	,039	-,033	,049	-,018	-,091	,101	,016	-,043	,035	-,106	-,131	,084	,198	-,241	-,171	,056	,039	,262	,265	-,057	-,111	-,180	-,119	,110	**,430**	,147	1																																		
Amilo	-,295	,041	-,012	,325	,255	,316	-,170	**,372**	,145	-,324	,195	,114	-,016	,301	-,113	,121	,203	-,113	,002	-,078	-,079	-,078	,108	-,064	,070	,156	-,032	-,094	-,104	1																																	
HS	-,241	-,268	-,255	-,050	-,078	-,056	,254	-,024	-,214	-,177	-,186	-,108	-,089	-,162	**-,380**	,188	,183	**-,363**	,141	-,292	-,231	,260	,208	,127	,270	,247	-,037	,181	-,109	,066	1																															
OM	-,029	,011	**-,413**	-,145	-,203	-,157	,105	-,043	-,009	-,343	-,072	,006	-,036	,040	-,253	-,024	,065	,026	,234	,006	-,119	,138	**,385**	-,170	,278	,192	**,403**	,207	,150	,110	,231	1																															
WSC	,285	-,185	-,037	-,226	-,248	-,232	-,209	-,147	,271	,335	,170	,342	**,578**	-,292	,182	-,015	-,312	,247	,001	,168	,046	-,016	-,135	,164	-,316	-,062	-,177	-,072	-,101	-,225	-,262	-,141	1																															
pH	**,692**	**-,384**	-,010	-,076	-,153	-,092	,015	-,002	-,091	,214	-,070	,001	-,041	,165	-,006	-,052	-,109	,075	-,163	,272	,272	-,111	**-,683**	,227	**-,456**	,025	-,167	**-,484**	-,058	-,237	-,278	-,153	,316	1																															
EC	-,118	,341	-,124	,024	,152	,048	,110	-,074	-,046	-,006	-,017	,020	-,002	-,033	-,016	-,087	,160	,114	,224	-,258	-,190	,093	,336	-,174	,168	-,255	**,361**	,331	,195	-,019	,125	,252	-,273	**-,684**	1																														
N	-,204	**-,399**	-,194	,084	-,011	,067	,013	-,228	-,196	-,239	-,284	,141	,050	,272	**-,523**	,183	,059	-,145	,306	-,068	-,063	,036	,247	,240	,164	,004	-,049	,127	,049	,293	,233	,279	-,197	-,138	,101	1																													
NH_4	-,263	,200	,286	,314	**,497**	**,351**	**-,489**	,289	**,383**	,180	**,428**	,031	,106	-,162	**,532**	-,121	,043	,265	**-,407**	,102	,299	-,154	-,056	**-,485**	-,163	-,308	-,085	,156	,223	,025	-,147	-,237	,044	-,303	,209	**-,357**	1																												
NO_3	-,161	**,382**	-,184	,057	,131	,072	-,007	,068	,104	-,061	,143	-,188	-,112	-,293	,181	-,276	,116	-,124	-,032	-,244	-,063	,207	,308	-,154	**,416**	,010	**,380**	,249	,101	,039	,187	,189	-,168	**-,518**	**,447**	-,125	,310	1																												
C	-,029	,011	**-,413**	-,145	-,203	-,157	,105	-,043	-,009	-,343	-,072	,006	-,036	,040	-,253	-,024	,065	,026	,234	,006	-,119	,138	**,385**	-,170	,278	,192	**,403**	,207	,150	,110	,231	**1,000**	-,141	-,153	,252	,279	-,237	,189	1																												
CN	,159	,264	-,252	-,316	-,308	-,318	,270	-,014	-,105	-,202	-,112	-,211	-,121	-,349	,066	-,126	-,079	,134	,100	-,067	-,173	,181	,122	-,101	,170	,333	,329	-,007	,041	-,331	-,069	**,431**	,083	,095	,015	**-,544**	-,129	,110	**,431**	1																											
Ols-P	-,165	-,098	,049	-,053	-,049	-,053	-,127	-,136	,150	,082	,088	-,241	-,128	-,195	,010	-,196	-,061	-,051	,031	,132	,046	-,080	,047	-,050	,180	,141	,218	,288	,274	-,142	,056	,137	,014	,117	-,302	,227	-,109	-,081	,137	-,098	1																										
B	,191	-,059	-,076	-,142	-,053	-,127	,115	,130	-,086	,037	-,071	-,119	-,058	-,091	,198	,042	**-,463**	,099	,096	,114	,207	,257	-,120	-,221	-,104	-,044	-,056	,088	,249	**-,485**	-,078	-,009	,299	,133	,026	**-,390**	,176	,152	-,009	,309	-,023	1																									
Zn	,315	,114	,139	-,267	-,173	-,252	,155	,021	,006	-,142	-,063	,231	,213	,130	,207	,032	**-,361**	,018	,206	-,050	-,079	,169	-,104	-,147	-,216	-,204	-,260	,017	,162	**-,360**	-,171	-,096	,317	,115	,044	-,293	,158	,177	-,096	,181	-,104	**,703**	1																									
Fe	,068	,099	-,196	**-,460**	**-,461**	**-,465**	**,572**	,101	**-,390**	**-,430**	**-,359**	,079	,010	,074	**-,353**	,152	-,028	-,015	**,444**	-,116	-,216	,166	,114	,204	-,045	**,354**	,042	,125	,127	-,078	,298	,269	-,089	-,096	,165	-,042	-,326	,011	,269	**,371**	-,080	,110	,207	1																									
Cu	,007	-,163	-,085	-,182	-,209	-,189	,010	**,352**	-,069	**-,495**	-,033	,296	,182	,262	-,094	,187	,235	-,048	,070	,107	,084	,095	-,090	,226	-,149	,204	-,109	-,132	,131	,089	,224	,301	-,068	,037	-,044	,172	-,252	,080	,301	,183	-,084	,042	,237	**,564**	1																								
Mn	,127	**,449**	-,133	-,092	,039	-,069	-,296	,159	,307	,121	**,367**	,170	,210	-,168	**,537**	-,332	,329	,348	-,150	,037	,243	-,201	,019	-,300	-,134	-,073	,299	-,184	,155	,095	-,135	,172	,009	-,062	,092	-,346	**,468**	,342	,172	,216	,003	-,111	-,041	-,161	,065	1																								
Ca	-,042	,034	-,264	,028	,021	,027	,060	,031	-,134	-,276	-,115	,171	,165	-,112	-,181	,067	,148	-,133	,124	-,038	-,230	,036	,283	,177	,052	,118	,043	-,086	-,221	,305	,158	,259	-,159	-,122	,088	,189	-,065	-,022	,259	,078	-,225	**-,460**	-,280	-,101	,160	**,436**	1																								
Na	,104	-,084	,059	,116	,097	,113	-,235	,159	,171	-,137	,178	-,108	-,117	-,015	,140	,237	,116	-,027	-,104	-,110	,079	,085	-,185	-,115	-,003	-,178	-,159	-,004	-,015	,024	,025	,090	-,013	-,017	,058	-,033	,115	**,383**	,090	,037	-,054	**,439**	**,510**	-,130	,177	-,074	-,214	1																								
Mg	,087	-,096	,021	,228	,256	,236	-,347	-,044	,138	,068	,139	,189	,205	-,056	,033	-,001	,214	-,061	-,154	,068	,137	-,219	-,092	,080	-,230	-,107	-,013	-,288	-,091	,189	,026	,002	-,004	-,014	,076	,103	,077	,002	,002	-,152	-,270	-,276	-,125	**-,476**	,014	**,370**	**,734**	,074	1																								
P	-,069	,195	-,280	,025	,160	,050	,111	,142	-,210	,269	-,049	-,311	-,289	-,217	,301	-,205	-,092	,315	,034	-,017	,269	,170	,180	-,230	,117	,077	,280	,214	,111	-,065	-,014	,085	-,089	-,178	,218	-,327	,343	,305	,085	,276	-,130	,195	-,202	,034	-,218	**,396**	,000	-,190	-,191	1																								
K	,107	,039	,192	-,216	-,173	-,210	,094	,052	,083	,100	,063	,078	,017	,309	,308	,053	**-,362**	,178	,043	,214	,070	,172	-,043	**-,371**	-,046	-,152	-,021	,130	,298	-,257	-,293	,009	,180	,147	-,006	-,145	-,046	-,206	,009	,088	,092	**,578**	**,350**	,050	-,089	-,315	**-,674**	,152	**-,555**	,121	1																								
C	-,136	-,136	,001	-,097	-,131	-,104	,185	,029	-,205	-,291	-,181	,062	,066	-,125	-,265	,097	,173	-,109	,171	-,348	-,037	,235	,026	**,392**	,085	**,439**	,032	,242	,060	,094	,236	-,025	-,152	-,097	,007	,239	-,028	,131	-,025	,006	-,031	-,269	-,184	**,378**	,298	-,088	,026	-,063	-,205	-,003	-,332	1																								
N	-,037	,044	,015	-,308	**-,389**	-,326	,246	-,007	-,267	-,284	-,236	,171	,200	-,157	-,262	,146	,139	-,251	,105	-,018	**-,509**	,292	,333	**,397**	,194	,052	,139	,247	-,216	,109	,277	,249	-,100	-,108	,080	,168	-,293	-,025	,249	,204	,000	-,335	-,230	,242	,202	,028	**,441**	-,057	,038	-,045	-,214	,315	1																								
SPAD	-,157	-,068	**-,495**	,149	,236	,167	-,087	-,093	-,065	,207	-,002	**-,442**	-,323	**-,467**	-,028	-,197	,145	,036	-,115	,071	,187	-,151	,070	,105	,216	,117	**,547**	,146	,150	,141	,138	,003	-,106	-,112	,059	-,031	,309	,303	,003	-,051	,216	-,183	**-,432**	-,198	-,253	**,471**	,243	-,232	,113	**,557**	**-,405**	,186	,052	1																								
Chla	-,134	-,182	**-,439**	-,317	**-,355**	-,328	**,535**	-,108	**-,386**	**-,438**	**-,413**	,031	,018	-,027	**-,368**	**,435**	-,235	-,266	,296	,179	-,285	,287	**,389**	,229	,241	**,433**	,279	**,351**	,094	,095	,235	**,427**	-,042	-,148	,103	,215	**-,423**	-,004	**,427**	,210	-,086	,013	-,115	**,429**	,287	-,168	,235	-,219	-,118	,203	,166	,120	,347	,055	1																								
Chlb	-,203	-,302	-,158	,171	,315	,200	,023	,017	-,173	-,103	-,146	-,054	-,031	-,106	-,001	,279	-,057	,001	-,094	,330	,120	-,004	,039	-,012	-,121	,032	,140	**,436**	,222	,075	,041	,024	,031	-,154	,272	,022	**,395**	,087	,024	-,071	-,159	,087	-,036	-,021	-,067	,106	,142	-,126	,013	**,486**	,060	,087	,026	**,442**	**,442**	1																								
TChl	-,161	-,224	**-,431**	-,249	-,254	-,253	**,483**	-,094	**-,381**	**-,412**	**-,399**	,017	,010	-,046	-,330	**,445**	-,221	-,238	,246	,227	-,231	,256	**,355**	,203	,191	**,393**	,278	**,402**	,129	,101	,218	**,387**	-,031	-,163	,147	,197	-,298	,014	**,387**	,174	-,109	,029	-,110	**,380**	,243	-,129	,238	-,221	-,103	,280	,160	,125	,315	,139	**,983**	**,597**	1																								
Nred	,328	-,025	,009	-,137	-,079	-,127	**,423**	,007	-,216	-,212	-,221	,155	,038	,338	,008	,086	-,346	-,151	**,542**	-,305	-,132	,275	-,105	,022	-,134	,069	-,216	,039	,148	-,062	-,008	-,152	,123	,112	,127	,025	-,257	-,047	-,152	-,080	-,068	**,425**	**,600**	,349	,179	-,321	-,292	,269	-,216	-,178	**,402**	-,015	-,253	**-,396**	,126	,004	,113	1