The work developed in this thesis aimed to develop and implement novel strategies for the qualitative and quantitative detection of multiple (pathogenic) micro-organisms in a single assay. Such approaches are of potential use for studying the ecology and epidemiology of plant pathogens. With increasing global food demands, the ability to detect the presence and/or quantity of a particular pathogen in a crop, greenhouse recirculation water or soil, before disease symptoms appear, is of utmost importance to help minimize preventive pesticide spraying and yield loses. Also, these technologies will help to set clear threshold levels of pathogen densities and to provide crucial information upon which disease management strategies can be based.

The assays described in this thesis are based on circularizable ligation probes and allow for the detection of multiple plant pathogens in a single assay without compromising the detection reliability of each individual pathogen. Pathogens could be discriminated based on only a single nucleotide difference in the targeted DNA sequence, thereby enabling the discrimination pathogens from very closely related nonpathogenic organisms. All developed assays were able to detect multiple pathogens over concentration ranges of more than 1:10^4. In chapter 3, an assay was developed for the qualitative analysis of multiple plant pathogens in greenhouse-recirculation water systems. The assay allowed sensitive and background-free screening, making it extremely suitable for detection of quarantine organisms. In chapter 4, an assay was developed for the accurate detection and quantification of more than 10 different pathogens simultaneously, using a state-of-the art high-throughput nanoliter volume qPCR platform. The assay provided accurate and highly reproducible quantification of all the targeted pathogens over a wide range of concentrations. In chapter 5, the technology developed in the previous chapter was applied for the quantification of Organohalide Respiring Bacteria in contaminated soils. In chapter 6, the quantitative assay was further developed to compensate for the loss of sensitivity due to the nano-liter qPCR amplification volumes, and the assay was tested using a range of soil samples. Finally, in chapter 7, an internal amplification control (IAC) strategy was developed in order to monitor qPCR inhibition in soils samples analyzed in a nanoliter volume qPCR platform. With the developed IAC, more reliable error correction for qPCR inhibitions was achieved, facilitating more accurate microbial quantification in soil samples.

The assays described in this thesis may also serve as a model for the routine detection and identification of a wide range of micro-organisms in diverse biological systems where
multiplex target detection is required. Additionally, proper correction for assay inhibition due to a variety of sample matrix related factor adds further to the reliability of the quantification. The developed assays presented here add to the toolbox for effective detection of plant pathogens and other microbial agents and could also be applied in various fields outside the agricultural sector in the future.